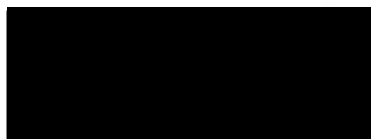


**HIGHER DEGREE THESIS
AUTHOR'S CONSENT
(DOCTORAL)**

This is to certify that I, ALEXANDER KENNETH LEIGH NEWMAN
being a candidate for the degree of Doctor of PHILOSOPHY
am aware of the policy of the University relating to the retention and use of
higher degree theses as contained in the University's Doctoral Rules
generally, and in particular Rule 7(10).

In the light of this policy and the policy of the above Rules, I agree to allow
a copy of my thesis to be deposited in the University Library for
consultation, loan and photocopying forthwith.

M. Denwood
Signature of Witness



Dated this SIXTH day of SEPTEMBER 2004



The Academic Senate on 22 November 2005 resolved that Mr Alexander Newman had
satisfied the requirements for admission to the degree of Doctor of Philosophy.

This thesis represents a major part of the prescribed program of study.

**The biology of *Mycopsylla fici* Tryon on its sole host,
Ficus macrophylla Desf. ex Pers.**

by

**Alexander Kenneth Leigh Newman
B. Mus (Adel.), B. Ag. Sc. (Hons) (Adel.)**

**A thesis submitted in the Department of Biological and Earth Sciences,
Macquarie University, NSW, Australia,
as partial fulfillment of the requirement
for the degree of Doctor of Philosophy**

31 August 2004.

To Kathleen and Sarah.

My love, respect and thanks for your boundless support.

Table of Contents

Chapters and Sections	iii
List of Tables	xii
List of Figures	xiv
List of Abbreviations	xvi
Thesis Abstract	xviii
Signed Statement	xxi
Acknowledgements	xxii

Chapters and Sections

Chapter 1. Introduction	1
1.1 Overview of the Project	1
1.2 The Moreton Bay fig	2
1.2.1 Brief overview of the systematics of the <i>Ficus macrophylla</i> complex	2
1.2.2 Distribution of <i>F. macrophylla</i> subspecies	3
1.2.3 Growth Characteristics of <i>F. macrophylla</i>	3
1.3 The fig psyllid and fig tree defoliation: an aesthetic and practical problem	7
1.4 Plant-insect interactions: evolutionary and practical consequences for the participants	10
1.4.1 Plant apparency, selection and coevolutionary 'arms race' theory	10
1.4.2 Nitrogen 'tug-of-war' between plants and insects	13
1.5 The Moreton Bay fig psyllid	14
1.5.1 The psyllid in the context of research on members of families Homotomidae Heslop-Harrison and Psyllidae L.	14
1.5.2 Brief comments on the taxonomy of <i>M. fici</i>	18
1.5.3 The effects of the activities of <i>M. fici</i> on <i>F. macrophylla</i>	22
1.5.4 Existing studies and research on <i>M. fici</i>	22
1.5.5 <i>M. fici</i> in India and Asia	24
1.6 Aim and objectives of the project	25
1.6.1 Structure of the thesis	26

Chapter 2. Survey of the Range of <i>Mycopsylla fici</i> Tryon on the Eastern Australian Seaboard	27
2.1 Introduction	27
2.2 Materials and Methods	28
2.3 Results	28
2.3.1 Locations of <i>F. macrophylla</i> specimens	30
2.3.2 Trees observed in the Sydney region	34
2.3.3 Other records and observations	35
2.4 Discussion	35
2.5 Conclusions	36
 Chapter 3. Attempts at rearing <i>Mycopsylla fici</i> under various conditions	 37
3.1 Introduction	37
3.2 Materials and Methods	38
3.2.1 Rearing <i>M. fici</i> on potted <i>F. macrophylla</i> saplings directly on its host	38
3.2.2 Rearing <i>M. fici</i> on potted <i>F. macrophylla</i> saplings in a glasshouse	38
3.2.3 Rearing <i>M. fici</i> in leaf cages directly on its host	39
3.3 Results	44
3.3.1 Rearing <i>M. fici</i> on potted <i>F. macrophylla</i> saplings directly on its host	44
3.3.2 Rearing <i>M. fici</i> on potted <i>F. macrophylla</i> saplings in a glasshouse	44
3.3.3 Rearing <i>M. fici</i> in leaf cages directly on its host	44
3.4 Discussion	45
 Chapter 4. Arthropod fauna in the <i>Ficus macrophylla</i> canopy, determined by insecticidal fogging	 46
4.1 Introduction	46
4.2 Materials and methods	48
4.2.1 Fogging survey	48
4.2.2 Collecting sheet colour effect comparison experiment	50
4.3 Results	51
4.3.1. Main survey results	51
4.3.2 Main and colour comparison surveys	57
4.3.2.1 Colour comparison	57
4.3.2.2 Main and colour comparison surveys	57
4.3.3 Fogging sample guild structures	57

	v
4.4 Discussion	62
4.4.1 Sheet colour effect comparison experiment	62
4.4.1.1 Colour comparison	62
4.4.1.2 Differences between main and colour comparison surveys	62
4.4.2 Main survey results	63
4.4.3 The comparatively small representation of <i>M. fici</i> in the fogging samples	64
4.4.4 Intra-niche competitors of <i>M. fici</i>	64
4.4.5 Guild representation and trophic levels in the fogging sample	66
4.4.6 <i>M. fici</i> within the <i>F. macrophylla</i> canopy guild structure	66
4.5 Conclusions	67
 Chapter 5. Distribution of <i>M. fici</i> within the <i>F. macrophylla</i> canopy	 68
5.1 Introduction and Aims	68
5.2 Materials and Methods	70
5.2.1 Experimental designs and choice of tree	70
5.2.1.1 Tree choice	70
5.2.1.2 Adult stratification	70
5.2.1.3 Design modification: Egg and lerp stages	70
5.2.2 Sampling stage 1: collection of adults	70
5.2.3 Sampling stage two: collection of preadult forms	72
5.3 Results	72
5.3.1 Stratification analysis	72
5.3.1.1 Eggs	72
5.3.1.2 Lerps	75
5.3.1.3 Adult stage	75
5.3.1.3.1 Overall stratification	75
5.3.1.3.2 <i>M. fici</i> sex ratios and their stratification	79
5.4 Discussion	79
5.4.1 General observations on adult numbers and egg laying	79
5.4.2 Stratification	80
5.4.2.1 Eggs	80
5.4.2.2 Lerps	80
5.4.2.3 Adults	80
5.4.2.4 Stratification with respect to sex and sex ratios	81
5.5 Conclusions	81

	vi
Chapter 6: Phenology of <i>Mycopsylla fici</i>	82
6.1 General introduction to Chapters 6 and 7	82
6.2.1 General materials and methods	83
6.2.1.1 Selection of field study trees, including replacement of a defunct study sample	83
6.2.1.2 Egg-laying studies	87
6.2.1.3 Juvenile instar studies	87
6.2.1.3.1 Growth and Development of <i>M. fici</i> juvenile instars	87
6.2.1.3.2 Stadial duration of <i>M. fici</i> juvenile instars	88
6.2.2 General sampling methods	89
6.2.2.1 Equipment	89
6.2.2.2 Sampling and tagging	90
6.2.2.3 Climate data	90
6.2.3 Materials and methods for egg laying and hatching studies	90
6.2.3.1 Materials and methods for stadial duration of juvenile <i>M. fici</i> instars	92
6.2.4 <i>In situ</i> and <i>ex situ</i> observations of <i>Psyllaephagus</i> sp.	93
6.3 Results	94
6.3.1 Egg laying and hatching dynamics of <i>M. fici</i>	94
6.3.1.1 Egg laying by <i>M. fici</i>	94
6.3.1.2 Hatchling ‘migration’ and fates	98
6.3.2 Growth and development of <i>M. fici</i> juvenile instars	99
6.3.2.1 Lerp components and structure	99
6.3.2.1.1 Notes on lerp states	99
6.3.2.1.2 Initial (lerp-forming) stages	100
6.3.2.2 <i>M. fici</i> life cycles stages within the lerp	103
6.3.2.3 Stadial duration of juvenile <i>M. fici</i> instars	105
6.3.3 Observations on the encyrtid parasitoid, <i>Psyllaephagus</i> sp.	109
6.3.4 Reactions of lerps to relative humidity	112
6.3.4.1 The effect of leaf loss on <i>M. fici</i> and <i>Psyllaephagus</i> sp. survival	115
6.4 Discussion	116
6.4.1 Egg laying and hatching dynamics of <i>M. fici</i>	116
6.4.1.1 Egg laying by <i>M. fici</i>	116
6.4.1.2 Hatchling ‘migration’ and fates	117
6.4.2 Growth and development of <i>M. fici</i> juvenile instars	118
6.4.2.1 Initial (lerp-forming) stages	118
6.4.2.2 The age structure of the lerp population	120

6.4.2.3	Stadial duration of juvenile <i>M. fici</i> instars	122
6.4.3	Observations on the encyrtid parasitoid, <i>Psyllaephagus</i> sp.	122
6.4.3.1	Possible polyembryony in <i>Psyllaephagus</i> sp.	123
6.4.3.2	Possible reasons for the observed size-dimorphism of <i>Psyllaephagus</i> sp. females, including polyembryony	125
6.4.3.3	Mortality estimates for <i>Psyllaephagus</i> sp.	126
6.4.4	Reactions of lerps to relative humidity	127
6.4.4.1	The effect of leaf loss on <i>M. fici</i> and <i>Psyllaephagus</i> sp. survival	129
6.5	Summary of <i>M. fici</i> Phenology	130
Chapter 7.	Population Dynamics of <i>Mycopsylla fici</i>	131
7.1	Introduction	131
7.1.1	Survival analysis interpretations of psylloid population dynamics	131
7.1.2	Degree days models for <i>M. fici</i>	133
7.1.3	Comparisons of psylloid activity on five study trees	134
7.2	Materials and Methods	135
7.2.1	General materials and methods	135
7.2.1.1	Survival analysis interpretations of psylloid population dynamics	137
7.2.1.1.1	<i>M. fici</i> survival analysis baseline: MFG 1998-99 egg survey data	137
7.2.1.1.2	Age-specific life-tables for <i>M. fici</i> : AGS field study data set manipulation	137
7.2.1.2	Degree days models for <i>M. fici</i>	138
7.2.1.2.1	Degree days estimates for <i>M. fici</i> : two degree days models	138
7.2.1.2.2	Degree days estimates for <i>M. fici</i> : comparing geographical locations	139
7.2.1.3	Comparisons of psylloid activity on five study trees	139
7.3	Results	143
7.3.1	Survival analysis interpretations of psylloid population dynamics	143
7.3.1.1	<i>M. fici</i> survival analysis baseline: use of the MFG 1998-99 egg study results	143
7.3.1.2	Age-specific life-tables for <i>M. fici</i>	143
7.3.1.2.1	Specific causes of mortality for <i>M. fici</i>	144
7.3.1.3	Survival analysis of lerp and leaf data	146
7.3.2	Degree days models	151
7.3.2.1	Degree days estimates for <i>M. fici</i> : two degree days models	151
7.3.2.2	Application of the two temperature models to other geographical locations	151
7.3.2.3	Location summaries	152
7.3.2.3.1	Melbourne	152

7.3.2.3.2 Adelaide	153
7.3.2.3.3 Wollongong	153
7.3.2.3.4 Sydney	153
7.3.2.3.5 Parramatta	153
7.3.2.3.6 Port Macquarie	153
7.3.2.3.7 Grafton	154
7.3.2.3.8 Brisbane	154
7.3.3 Comparisons of psyllid activity on five study trees	159
7.3.3.1 Comparisons of psyllid activity with respect to leaves	159
7.3.3.2 Psyllid activity with respect to eggs on leaves	159
7.3.3.3 Psyllid activity with respect to lerps on leaves	160
7.3.3.4 Other observations on psyllid activity with respect to both eggs and lerps	160
7.3.3.5 Comparisons of psyllid activity	161
7.4 Discussion	164
7.4.1 Survival analysis interpretations of psyllid population dynamics	164
7.4.1.1 <i>M. fici</i> survival analysis baseline: MFG 1998-99 egg survey data	164
7.4.1.2 Age-specific life-tables for <i>M. fici</i>	164
7.4.1.2.1 Specific causes of mortality for <i>M. fici</i>	164
7.4.1.3 Survival analysis of lerp and leaf data	167
7.4.1.3.1 Lerp model	167
7.4.1.3.2 Leaf model	168
7.4.1.3.3 Leaf loss with respect to side of tree (<i>TREESIDE</i> covariate)	169
7.4.2 Degree days models	169
7.4.2.1 Degree days estimates for <i>M. fici</i> : two degree days models	169
7.4.2.2 Application of the two temperature models to other geographical locations	171
7.4.2.3 Location summaries	172
7.4.2.3.1 Melbourne	172
7.4.2.3.2 Adelaide	172
7.4.2.3.3 Wollongong	172
7.4.2.3.4 Sydney	172
7.4.2.3.5 Parramatta	172
7.4.2.3.6 Port Macquarie	173
7.4.2.3.7 Grafton	173
7.4.2.3.8 Brisbane	173
7.4.2.4 Degree days estimates: possible effects of the <i>El Niño</i> phenomenon	174

7.4.3 Comparisons of psylloid activity with respect to trees and dates	175
7.4.3.1 Psylloid activity with respect to eggs	175
7.4.3.2 Psylloid activity with respect to lerps	176
7.4.3.3 Other observations on psylloid activity	176
7.5 Summary of Chapters 6 and 7	177

Chapter 8: Using fast chlorophyll fluorescence kinetics in determining stress in mature

<i>Ficus macrophylla</i> (Desf. ex Pers.) trees	180
8.1 Introduction	180
8.1.1 Chlorophyll fluorescence	180
8.1.1.1 Fast Chlorophyll Fluorescence Kinetics: Theory and Application	181
8.1.1.1.1 Photosystems within the photosynthesis ‘light’ reaction pathways	181
8.1.1.1.2 Chlorophyll fluorescence mechanisms in photosynthesis pathways	182
8.1.1.1.3 Stages in the chlorophyll fluorescence mechanism	183
8.1.1.1.4 Use of fast chlorophyll fluorescence kinetics in the detection of plant stress	185
8.2 Materials and Methods	187
8.3 Results	190
8.3.1 Results for Sydney population of <i>F. macrophylla</i>	190
8.3.1.1 Preliminary analysis	190
8.3.1.1.1 Relationship of F_V/F_M and $t_{0.5}$	190
8.3.1.1.1.1 General overview of study data	192
8.3.1.1.1.2 <i>Lerp</i> (+/-) vs <i>Date</i>	192
8.3.1.1.1.3 <i>Lerp</i> (+/-) vs <i>Tree</i>	192
8.3.1.2 Exploratory ANOVAs - complete data set	197
8.3.1.2.1 F_V/F_M with respect to <i>Tree</i>	197
8.3.1.2.2 F_V/F_M with respect to <i>Date</i>	197
8.3.1.3 First major alteration to data set	199
8.3.1.3.1 Removal of redundant variables	199
8.3.1.3.2 Balancing the design - removal of <i>BTC</i> and <i>AS97</i> data	199
8.3.1.4 Second major alteration to data set – <i>Non-plague/Plague</i> variable partitioning	199
8.3.1.4.1 One-way ANOVA of <i>Plague</i> status	201
8.3.1.5 Regression models of F_V/F_M	202
8.3.1.5.1 <i>Non-plague</i> regression model	202
8.3.1.5.2 <i>Plague</i> regression model	202

8.3.2 Chlorophyll fluorescence study of five trees from the Adelaide population of <i>F. macrophylla</i>	204
8.3.3 Comparisons of <i>F. macrophylla</i> specimens at different locations, with respect to F_V/F_M psyllid presence and environmental conditions	204
8.4 Discussion	211
8.4.1 Sydney main study	211
8.4.1.1 Preliminary analysis	211
8.4.1.1.1 Overview of F_V/F_M and $t_{0.5}$ results (all trees and all dates)	211
8.4.1.1.1.1 Relationship of F_V/F_M and $t_{0.5}$	211
8.4.1.1.1.2 Summary F_V/F_M statistics	211
8.4.1.1.1.3 <i>Lerp</i> (+/-) variable with respect to <i>Date</i>	211
8.4.1.1.1.4 <i>Lerp</i> (+/-) variable with respect to <i>Tree</i>	212
8.4.1.2 Exploratory ANOVAs – complete data set	212
8.4.1.2.1 ANOVA: tests of underlying assumptions	212
8.4.1.2.2 ANOVA – F_V/F_M with respect to <i>Tree</i>	212
8.4.1.2.3 F_V/F_M with respect to <i>Date</i>	213
8.4.1.3 Alterations to data set	214
8.4.1.3.1 First alteration: removal of data	214
8.4.1.4 Second major alteration to data set: <i>Plague/Non-plague</i> variable partitioning	215
8.4.1.4.1 One-way ANOVA of F_V/F_M with respect to <i>Plague</i> status	215
8.4.1.5 Three-way ANCOVA models of <i>Plague</i> and <i>Non-plague</i> data sets	215
8.4.1.5.1 <i>Non-plague</i> data	215
8.4.1.5.2 <i>Plague</i> data	216
8.4.1.5.3 Comparison of <i>Non-plague</i> and <i>Plague</i> ANCOVA models	216
8.4.1.6 Multiple regression analysis of <i>Non-plague</i> and <i>Plague</i> data sets	217
8.4.1.6.1 Multiple Linear Regression models	217
8.4.3 Chlorophyll fluorescence study of five trees from the Adelaide population of <i>F. macrophylla</i> : differences between the Adelaide and Sydney populations	219
8.4.4 Comparisons of <i>F. macrophylla</i> specimens at different locations, with respect to F_V/F_M , psyllid presence and environmental conditions	221
8.5 Conclusions	224

Chapter 9. Conclusion	225
9.1 Major outcomes of the studies with respect to <i>Mycopsylla fici</i>	225
9.2 Major outcomes of the studies with respect to natural enemies of <i>M. fici</i> .	228
9.3 Major outcomes of the studies with respect to <i>Ficus macrophylla</i>	229
9.4 Implications of research project findings on the future of <i>F. macrophylla</i> in an urban environment	231
9.5 Suggested further avenues of research	233
9.6 Closing remarks	235
References	237

List of Tables

Table 1.1: A brief phylogeny of the Psylloidea	19
Table 2.1: Summary of the occurrence of <i>M. fici</i> in a range of geographical locations	29
Table 4.1: Numbers of specimens in arthropod orders appearing in fogging sample	52
Table 4.2: Sample numbers per order between fogging and colour comparison experiment	54
Table 4.3: Colour comparison experiment (1)	55
Table 4.4: Colour comparison experiment (2)	56
Table 4.5: <i>F. macrophylla</i> canopy guild structure	60
Table 4.6: Summary of <i>F. macrophylla</i> canopy guild structure	61
Table 5.1: ANOVA table, Egg strata	73
Table 5.2: Table of means, Egg strata	73
Table 5.3: ANOVA table, lerp strata	74
Table 5.4: Means for quadrant stratum, lerp strata	74
Table 5.5: Table of means, quadrant \times height 2-way interaction for lerp strata	74
Table 5.6 ANOVA table for adult strata analysis	76
Table 5.7: Table of means, quadrant \times height 2-way interaction for adult strata	76
Table 5.8 ANOVA table for male and female strata analysis	77
Table 5.9: Table of means, quadrant \times height 2-way interaction for male and female strata	77
Table 5.10: ANOVA table for sex ratio strata analysis	78
Table 5.11: Table of means, quadrant \times height 2-way interaction for sex ratio strata	78
Table 6.1: <i>MFG</i> egg laying survey results, 1997 and 1998–99	96
Table 6.2: <i>MFG</i> and <i>AGS</i> life history: basic results	97
Table 6.3: Summary of <i>M. fici</i> instar stadial durations	106
Table 7.1: <i>MFG</i> egg study results	140
Table 7.2: <i>AGS</i> and <i>MFG</i> life history study result	141
Table 7.3: Survival analysis for <i>MFG</i> and <i>AGS</i> study cohorts	142
Table 7.4: Lerp survival analysis	148
Table 7.5: Key to variable names used in lerp censoring analysis	148
Table 7.6: Leaf survival analysis	149
Table 7.7: Key to variable names used in leaf survival analysis	149
Table 7.8: ‘Single Temperature’ and ‘Long term mean temperature’ degree days models	155
Table 7.9: Degree days for pre-adult stages and numbers of generations per year	155
Table 7.10: Latitudes and longitudes for the eight geographical locations	156
Table 7.11: Percentage difference in generation time wrt Sydney, long term model	156

Table 7.12: Comparison of <i>M. fici</i> stages using two temperature models	157
Table 7.13: Summer generation degree days and generations per year for eight locations	158
Table 7.14: Mean proportion of leaves with eggs for the five main study trees	162
Table 7.15: Mean proportion of leaves with lerps for the five main study trees	162
Table 7.16: Comparing five Sydney study trees: psyllid load and environmental factors	163
Table 8.1: <i>FCFK</i> study variables	189
Table 8.2: Overview of the first pass over the data	193
Table 8.3: ANOVA table for <i>Lerp</i> (+/-) with respect to <i>Date</i>	193
Table 8.4: ANOVA table for <i>Lerp</i> (+/-) with respect to <i>Tree</i>	193
Table 8.5: ANOVA table for F_V/F_M with respect to <i>Tree</i>	193
Table 8.6: Mean F_V/F_M value for five <i>F. macrophylla</i> trees for five survey periods	193
Table 8.7: ANOVA table for F_V/F_M by <i>Date</i>	198
Table 8.8: Mean F_V/F_M values for five survey periods (<i>Date</i>)	198
Table 8.9: Planned Comparison of F_V/F_M with respect to <i>Date</i> and <i>Tree</i>	198
Table 8.10: ANOVA results for F_V/F_M with respect to <i>Plague</i> status	198
Table 8.11: Summary of means for <i>Non-plague/Plague</i> analysis	198
Table 8.12: <i>Non-plague</i> and <i>Plague</i> ANCOVA models	200
Table 8.13: <i>Non-plague</i> and <i>Plague</i> multiple linear regression models	203
Table 8.14: ANOVA table for F_V/F_M of the five <i>CF</i> study trees in Adelaide	206
Table 8.15: Mean F_V/F_M values for the five <i>CF</i> study trees in Adelaide	206
Table 8.16: Comparison of mean F_V/F_M values for the Adelaide and Sydney study trees	206
Table 8.17: Five Sydney study trees: F_V/F_M and proportions of eggs, lerps on leaves 1–5	208
Table 8.18: RBG Tree comparisons: F_V/F_M and various environmental conditions	209
Table 8.19: Adelaide Botanic Gardens: <i>F. macrophylla</i> comparison	210

List of Figures

Figure 1.1: <i>F. macrophylla</i> growing as a strangler in northern New South Wales	5
Figure 1.2: <i>F. macrophylla</i> in the Royal Botanic Gardens, Sydney	6
Figure 1.3: Severely defoliated <i>F. macrophylla</i> in Sydney Domain	8
Figure 1.4: Consequences of severe defoliation for <i>F. macrophylla</i> .	8
Figure 1.5: Defoliated branchlet of <i>F. macrophylla</i> in Sydney Domain	9
Figure 1.6: Carpet of leaves	9
Figure 3.1: Potted <i>F. macrophylla</i> sapling	43
Figure 3.2: Clear leaf cage without side vents	43
Figure 4.1: <i>Parabaliotrips newmani</i> damage to <i>F. macrophylla</i> leaves	65
Figure 4.2: <i>Parabaliotrips newmani</i> damage to <i>F. macrophylla</i> leaves	65
Figure 5.1a Stratification sampling design, side view	69
Figure 5.1b: Stratification sampling design, plan view	69
Figure 6.1: <i>F. macrophylla</i> study tree MFG during field work, February 1999	85
Figure 6.2: <i>F. macrophylla</i> study tree AGS during field work, February 1999	86
Figure 6.3: SEM of <i>M. fici</i> eggs (photo: E Turak, RBGS)	95
Figure 6.4: <i>M. fici</i> egg masses around the leaf margin	95
Figure 6.5: <i>M. fici</i> egg masses over entire abaxial surface of leaf	95
Figure 6.6: SEM of psylloid egg, viewed from pedicel end	95
Figure 6.7: Settled <i>M. fici</i> hatchlings producing wax tubes and honeydew	101
Figure 6.8: Developing matrix of wax tubes	101
Figure 6.9: <i>M. fici</i> wax pore structure	101
Figure 6.10: Compound wax tube disintegrating within honeydew	101
Figure 6.11: lerp closure	102
Figure 6.12: <i>M. fici</i> lerp at initial stage	102
Figure 6.13: Recent recruits at lerp periphery	102
Figure 6.14: 2 nd and 3 rd instars	104
Figure 6.15: 5 th instar	104
Figure 6.16: Pharate adult ('H5 th ')	104
Figure 6.17: Mature <i>M. fici</i> lerp with final-moult exuviae	104
Figure 6.18: Adult female <i>M. fici</i> (photo: E Turak, RBGS)	104
Figure 6.19: Relationship of <i>M. fici</i> head capsule widths to lengths	107
Figure 6.20: Seven mean <i>M. fici</i> head capsule width groups	108
Figure 6.21: <i>Psyllaephagus</i> sp. stinging into lerp	110

Figure 6.22: The two size forms of <i>Psyllaephagus</i> sp. females	110
Figure 6.23: <i>Psyllaephagus</i> sp. female eating lerp components	110
Figure 6.24: Psylloid larva with dark inclusion and thread exiting body	111
Figure 6.25: <i>Psyllaephagus</i> sp. mummy, dorsal surface	111
Figure 6.26: <i>Psyllaephagus</i> sp. mummy, ventral surface	111
Figure 6.27: <i>Psyllaephagus</i> sp. pharate adult	111
Figure 6.28: Lerp flow, induced by very humid conditions	113
Figure 6.29: Lerp “explosion” and honeydew “rain”	113
Figure 6.30: Lerp “explosion”	113
Figure 6.31: “Midvein lerp reformation” (<i>MVLR</i>)	113
Figure 6.32: AGS lerp reactions to RH and temperature	114
Figure 6.33: MFG lerp reactions to RH and temperature	114
Figure 6.34: <i>F. macrophylla</i> leaf abaxial surface	119
Figure 6.35: <i>F. macrophylla</i> leaf abaxial surface, portion of leaf hairs removed	119
Figure 7.1: Kaplan-Meier cumulative proportion of lerps surviving	150
Figure 7.2: Kaplan-Meier cumulative proportion of leaves surviving	150
Figure 8.1: The Kautsky or ‘ <i>OIPSMT</i> ’ Curve	184
Figure 8.2: The relationship between F_V/F_M and $t_{0.5}$	191
Figure 8.3: Lerp presence and absence over time	191
Figure 8.4: Mean F_V/F_M values for the five study trees, grouped by <i>CF</i> sampling period	194
Figure 8.5: Mean F_V/F_M values for the five sampling periods, grouped by tree	195
Figure 8.6: Mean F_V/F_M values for one year, for sampling periods and trees (two graphs)	196
Figure 8.7: Mean F_V/F_M values for the five Sydney <i>CF</i> study trees over one year	207
Figure 8.8: <i>RT</i> ‘tree + environment condition’ indices for the five Sydney <i>CF</i> study trees	207
Figure 8.9: <i>BP1</i> : one of the four <i>CF</i> study trees sampled in Botanic Park, Adelaide	220
Figure 8.10: Extensive leaf carpet under <i>BP1</i>	220

List of Abbreviations

Sydney trees

<i>AGS</i>	<i>F. macrophylla</i> – Art Gallery South, Domain
<i>MFG</i>	<i>F. macrophylla</i> – Morshead Fountain Gate, RBGS
<i>L44</i>	<i>F. macrophylla</i> – Lawn 44, RBGS
<i>BTC</i>	<i>F. macrophylla</i> – Bus Turning Circle, Domain
<i>AGN</i>	<i>F. macrophylla</i> – Art Gallery North, Domain
<i>DMN</i>	<i>F. macrophylla</i> – Sydney Domain proper

Adelaide trees

<i>BP1</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 1
<i>BP2</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 2
<i>BP3</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 3
<i>BP4</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 4
<i>ABG1</i>	<i>F. macrophylla</i> – Adelaide Botanic Gardens: Tree 1

Psylloid phenology

<i>MVLR</i>	Midvein lerp reformation
<i>H5th</i>	Pharate adult psylloid – discoid stage
<i>1° PA</i>	Pharate adult – emergent stage
<i>2° PA</i>	Pharate adult – terete stage
ANCOVA	Analysis of covariance
MANCOVA	Multivariate analysis of covariance
GLIM	Generalised linear model

Degree days

$^{\circ}D$	Degree days
<i>D</i>	Development length in days
<i>T</i>	Mean daily temperature for development period
<i>t</i>	Developmental threshold temperature
<i>Z</i>	Thermal constant
<i>V</i>	Development velocity

Note: Pages xvi and xvii printed both on sides for ease of reference when consulting in latter parts of thesis

List of Abbreviations

Sydney trees

<i>AGS</i>	<i>F. macrophylla</i> – Art Gallery South, Domain
<i>MFG</i>	<i>F. macrophylla</i> – Morshead Fountain Gate, RBGS
<i>L44</i>	<i>F. macrophylla</i> – Lawn 44, RBGS
<i>BTC</i>	<i>F. macrophylla</i> – Bus Turning Circle, Domain
<i>AGN</i>	<i>F. macrophylla</i> – Art Gallery North, Domain
<i>DMN</i>	<i>F. macrophylla</i> – Sydney Domain proper

Adelaide trees

<i>BP1</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 1
<i>BP2</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 2
<i>BP3</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 3
<i>BP4</i>	<i>F. macrophylla</i> – Botanic Park, Adelaide: Tree 4
<i>ABG1</i>	<i>F. macrophylla</i> – Adelaide Botanic Gardens: Tree 1

Psylloid phenology

<i>MVLR</i>	Midvein lerp reformation
<i>H5th</i>	Pharate adult psylloid – discoid stage
<i>1° PA</i>	Pharate adult – emergent stage
<i>2° PA</i>	Pharate adult – terete stage
ANCOVA	Analysis of covariance
MANCOVA	Multivariate analysis of covariance
GLIM	Generalised linear model

Degree days

$^{\circ}D$	Degree days
<i>D</i>	Development length in days
<i>T</i>	Mean daily temperature for development period
<i>t</i>	Developmental threshold temperature
<i>Z</i>	Thermal constant
<i>V</i>	Development velocity

Note: Pages xvi and xvii printed both on sides for ease of reference when consulting in latter parts of thesis

Survival analysis: Lerp data

<i>LERPICEN</i>	date of lerp censoring event
<i>LMTEMP</i>	Temperature at “lerp merge” event
<i>LMRH</i>	Relative humidity at “lerp merge” event
<i>TLFTEMP</i>	Temperature at occurrence of “Total lerp flow” event
<i>TLFRH</i>	Relative humidity at occurrence of “Total lerp flow” event
<i>MVLRTMP</i>	Temperature at occurrence of “midvein lerp reformation” event
<i>MVLRRH</i>	Relative humidity at occurrence of “midvein lerp reformation” event
<i>ABSLPTMP</i>	Temperature at occurrence of fall of study leaf from tree
<i>ABSLPRH</i>	Relative humidity at occurrence of leaf abscission event

Survival analysis: Leaf data

<i>ABSLFCEN</i>	Date of leaf abscission censoring event (leaf data)
<i>TLFLFTEMP</i>	Temperature at occurrence of “Total lerp flow” event (leaf data)
<i>TLFLFRH</i>	Relative humidity at occurrence of “Total lerp flow” event (leaf data)
<i>MVLRLFTE</i>	Temperature at occurrence of “midvein lerp reformation” event (leaf data)
<i>MVLRLFRH</i>	Relative humidity at occurrence of “midvein lerp reformation” event (leaf data)
<i>ABSLFTEMP</i>	Temperature at occurrence of fall of study leaf from tree (leaf data)
<i>ABSLFRH</i>	Relative humidity at occurrence of leaf abscission event (leaf data)

Chlorophyll Fluorescence

<i>FCFK</i>	Fast chlorophyll kinetics kinetics
<i>F₀</i>	Fluorescence value of dark-adapted photosystems
<i>F_v/F_M</i>	Variable fluorescence to maximum fluorescence ratio (1° <i>FCFK</i> test value)
<i>t_{0.5}</i>	Half raise time (half time taken for fluorescence to rise from <i>F₀</i> to <i>F_M</i>)
<i>SO96</i>	September-October 1996
<i>ON96</i>	October-November 1996
<i>DEC96</i>	December 1996
<i>JUN97</i>	June 1997
<i>AS97</i>	August-September 1997
<i>RT</i>	Tree conditions + environmental conditions index

Note: Pages xvi and xvii printed both on sides for ease of reference when consulting in latter parts of thesis

Survival analysis: Lerp data

<i>LERP1CEN</i>	date of lerp censoring event
<i>LMTEMP</i>	Temperature at “lerp merge” event
<i>LMRH</i>	Relative humidity at “lerp merge” event
<i>TLFTEMP</i>	Temperature at occurrence of “Total lerp flow” event
<i>TLFRH</i>	Relative humidity at occurrence of “Total lerp flow” event
<i>MVLRTMP</i>	Temperature at occurrence of “midvein lerp reformation” event
<i>MVLRRH</i>	Relative humidity at occurrence of “midvein lerp reformation” event
<i>ABSLPTMP</i>	Temperature at occurrence of fall of study leaf from tree
<i>ABSLPRH</i>	Relative humidity at occurrence of leaf abscission event

Survival analysis: Leaf data

<i>ABSLFCEN</i>	Date of leaf abscission censoring event (leaf data)
<i>TLFLTEMP</i>	Temperature at occurrence of “Total lerp flow” event (leaf data)
<i>TLFLFRH</i>	Relative humidity at occurrence of “Total lerp flow” event (leaf data)
<i>MVLRLFTE</i>	Temperature at occurrence of “midvein lerp reformation” event (leaf data)
<i>MVLRLFRH</i>	Relative humidity at occurrence of “midvein lerp reformation” event (leaf data)
<i>ABSLFTEMP</i>	Temperature at occurrence of fall of study leaf from tree (leaf data)
<i>ABSLFRH</i>	Relative humidity at occurrence of leaf abscission event (leaf data)

Chlorophyll Fluorescence

<i>FCFK</i>	Fast chlorophyll kinetics kinetics
F_0	Fluorescence value of dark-adapted photosystems
F_v/F_M	Variable fluorescence to maximum fluorescence ratio (1° <i>FCFK</i> test value)
$t_{0.5}$	Half raise time (half time taken for fluorescence to rise from F_0 to F_M)
<i>SO96</i>	September-October 1996
<i>ON96</i>	October-November 1996
<i>DEC96</i>	December 1996
<i>JUN97</i>	June 1997
<i>AS97</i>	August-September 1997
<i>RT</i>	Tree conditions + environmental conditions index

Note: Pages xvi and xvii printed both on sides for ease of reference when consulting in latter parts of thesis

Thesis Abstract

Mycopsylla fici Tryon, the Moreton Bay fig psyllid, is a small hemipteran in the family Homotomidae, in the suborder Psylloidea. Its sole host is *Ficus macrophylla* Desf. ex Pers., known as the Moreton Bay fig. Both species are endemic to eastern Australia. *M. fici* has become notorious for apparently being the causative agent for the complete periodic defoliation of its host.

The general aims of this thesis were: to investigate and provide good understanding of the biology of *M. fici*; and to determine, if possible, whether the insect is indeed primarily responsible for the defoliation of its host. The thesis includes an introduction to the psyllid, its host, and some general ideas about plant-insect interactions (in order to place the psyllid and the fig in the broader context of the literature on this topic); a geographical survey of the psyllid and its host within the host's known range; a description of attempts to breed the psyllid under laboratory conditions; a description of the distribution of the psyllid in the host's canopy with specific reference to the necessity for studying the psyllid *in situ* (i.e., directly on its host); a survey of the fauna within the *F. macrophylla* canopy; detailed *in situ* studies of the phenology and population dynamics of the psyllid, including observations of its single known parasitoid, the encyrtid wasp *Psyllaephagus* sp.; and studies of *F. macrophylla* health inferred from a number of Fast Chlorophyll Fluorescence Kinetics (FCFK) analyses of the photosynthetic systems in host leaves.

Various urban, semi-rural and rainforest sites were visited along the eastern seaboard and hinterland of Australia, and in Adelaide, where *F. macrophylla* grows either as a cultivated specimen, or naturally. Although there were fewer "naturally-occurring" *F. macrophylla* specimens observed than in urban and semi-rural conditions, the psyllid only seemed apparent when the host was growing in the latter types of environment.

The psyllid was found to avoid ovipositing on *F. macrophylla* plants younger than approximately three years of age, or under 1.5 m in height, and to be selective as to choice of leaf age (irrespective of plant age) when laying eggs. Oviposition was observed to occur, usually, on the third to fifth leaves back from the first fully unfurled leaf on a branchlet. Attempts to breed psyllids on saplings in sufficient quantities for detailed life history studies

in the laboratory proved to be impractical, which meant that all studies of the psyllid were made in the field.

Results of an experiment to determine the distribution of *M. fici* in the host canopy indicated that the psyllid showed no distinct preference for the particular regions of the tree with respect to height, and therefore that sampling studies could be made from the ground.

A study to determine arthropod fauna/functional groups, and the relationship that they might have with the psyllid, and the host, identified no parasitoids of the psyllid other than the already-known *Psyllaephagus* sp. wasp. An undescribed species of thrips was discovered on *F. macrophylla*, and this insect may be an intra-niche competitor of the psyllid.

Results from the life history studies indicated that a psyllid had an approximately 3.75% chance of surviving from oviposition to adulthood. Major causes of mortality for the pre-adult stages of the psyllid were identified as leaf fall from the tree, egg predation by coleopterans (by inference), predation of psyllid juveniles (mobile hatchlings and older lerp dwellers) by lacewing larvae, and parasitism by *Psyllaephagus* sp. *Psyllaephagus* sp. appears to be the single most effective biological control agent. A second, smaller, form of the *Psyllaephagus* sp. female was discovered, as was the occurrence of multiple parasitisation of individual psyllids. This latter observation suggests that *Psyllaephagus* sp. may be polyembryonic (a common trait amongst encyrtids), and that this in turn might influence its effectiveness as a biological control agent. Degree-day models for the psyllid were developed, and are discussed in relation to various urban sites included in the geographical survey.

Fast Chlorophyll Fluorescence Kinetics (FCFK) analysis of Photosystem II (PSII) within sample leaf laminae of five *F. macrophylla* specimens growing in the Royal Botanic Gardens and Domain in Sydney indicated that photoinhibition in *F. macrophylla* leaves was more pronounced in periods of high psyllid density than in periods of low psyllid density. Other significant factors in *F. macrophylla* photoinhibition were attributable to the variability between individual trees (in both low and high psyllid “plague” density periods). Sunlight appeared to be the major influence on photoinhibition during periods of low psyllid density. Trees showing the best overall health and the least tendency to be affected by the psyllid were those growing in good soil conditions and (especially) those that had access to adequate water.

These observations were supported by a comparison *FCFK* study and observations of growing conditions of five *F. macrophylla* specimens in and adjacent to the Adelaide Botanic Gardens, the psyllid not being present in Adelaide.

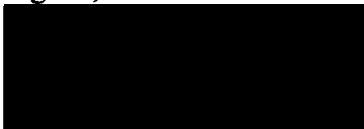
It was ultimately concluded that the psyllid was producing a detectable affect on the health of the trees, but was not the sole, or even main, factor involved. It seems most likely that the amount of available water and, to a lesser degree, nutrients are also major, if not limiting, factors influencing *F. macrophylla* health and defoliation events. The increase in resources allocated to maintaining *F. macrophylla* specimens growing in public recreational areas is strongly recommended.

Signed Statement

I, Alexander Kenneth Leigh Newman, do hereby declare that this thesis, 'The biology of *Mycopsylla fici* Tryon on its sole host, *Ficus macrophylla* Desf, ex Pers.,' has not been submitted to any other University or Institution for consideration to admission to any higher degree.

Furthermore, all work herein is the results of my own original research and endeavours, except where acknowledged in the Acknowledgements, and other portions of the thesis where appropriate.

Signed,

A solid black rectangular box used to redact the signature of the author.

AKL Newman

31 August 2004

Acknowledgements

The author wishes to acknowledge the assistance of the following people and institutions: for their support (financial and otherwise), cooperation, assistance, and goodwill throughout, or during part of, the project.

Firstly, for their patient direction, my supervisors: Drs Dinah Hales, Alan Clift and Brett Summerell, all of whom made significant investments of time and resources; the Royal Botanic Gardens Sydney and Domain Trust for employing me while undertaking my research; and the Environmental Trusts for funding not directly used in the PhD project but which enabled full-time research on the psyllid. Dr Hales provided overarching supervision for the thesis work, and much patience in editing and advising. Dr Clift provided a continual source of advice on numerical methods used in field ecological study. Dr Summerell provided the employment which enabled the research project, as well as support, encouragement, and guidance throughout the life of the project.

Mr Eren Turak, NSW Environmental Protection Authority (formerly at the Royal Botanic Gardens, Sydney), imparted much essential information from his research on *Mycopsylla fici*, enabling the project to begin with a solid foundation.

The British Library and the Waite Library at the University of Adelaide are gratefully acknowledged for providing the author with copies of rare journal articles. I thank Dr Heather Adamson, Senior Research Fellow, Department of Biological Sciences, Macquarie University, for introducing me to fast chlorophyll fluorescence techniques, for advice, the loan of equipment and review of the pertinent chapter in the manuscript. Dr Christine Stone, Forest Research and Development Division, State Forests NSW, Beecroft, also reviewed the chlorophyll fluorescence chapter, and provided useful reading material.

Associate Professor Andy Austin, Department of Applied and Molecular Ecology, Waite Campus, University of Adelaide, provided identifications of Hymenoptera, and detailed information about their likely feeding guild membership. Mr Peter Gillespie, Agricultural Scientific Collections Unit, NSW Agriculture, Orange, and Dr Laurence Mound, CSIRO

Entomology, Black Mountain, Canberra, provided identifications of Thysanoptera. Dr Mound is also thanked for the honour of naming a thrips species, newly-discovered by Mr Gillespie, after the author. Ms Danuta Knihinicki, of the Agricultural Scientific Collections Unit, NSW Agriculture, Orange, identified acarine samples and provided detailed information about their likely feeding guild membership. Dr Grahame Milledge, Australian Museum Sydney, identified spider samples. Dr Dan Bickel, Entomology Department, Australian Museum, Sydney, provided identifications for dipteran samples. Dr Courtenay Smithers, Department of Entomology, Australian Museum, provided identifications of psocopteran samples. Dr Gary Gibson, Visiting Fellow at CSIRO Entomology, Black Mountain, Canberra, confirmed the identity of the encyrtid parasitoid of the psyllid

Advice on large-scale arthropod sampling methods was given by Professor Andrew Beattie, Department of Biological Sciences, Macquarie University, Dr Alan Bishop, NSW Department of Agriculture, Gosford, Dr Nigel Stork, James Cook University, Queensland, and Professor Barry Richardson, University of Western Sydney. Dr James Cook, Silwood Park, Imperial College London, and Mr Daniel Bean, are thanked for their interest in and insights into the hymenopteran fauna associated with *Ficus macrophylla*, and their general support and encouragement.

Professor Mark Westoby, Department of Biological Sciences, Macquarie University, is thanked for imparting insights into numerical aspects of ecological research. Dr Max Moulds, Department of Entomology, Australian Museum, provided encouragement, support, advice and equipment, throughout the project. Dr Gerry Cassis, Department of Entomology, Australian Museum, provided the author with useful discussion on ecological aspects of sternorrhyncan biology. Dr Ian Naumann, Australian National Insect Division of Entomology, CSIRO Canberra is thanked for providing the author with access to the encyrtid portion within the Collection.

Airscape P/L, Central Coast kindly made the loan of a 'Golden Eagle DynaJet' fogger and its instruction manual, and also donated a container of fogging carrier, for use in a survey of the arthropod fauna in the *F. macrophylla* canopy. Mr Andy Ryland is thanked for his interest in the project, sharing some of his wealth of knowledge of, and experience in, biological control, and the opportunity to observe some specimens of *F. macrophylla* which had been subjected to

severe and unusual stresses. Personnel at Hastings Data Loggers, Port Macquarie, NSW, gave much-appreciated technical expertise, advice and support on environmental monitoring techniques. Mr Patrick Honan, formerly of the Keith Turnbull Research Institute, Victorian Department of Agriculture invited the author to visit him in Melbourne and discuss his own research into the effects of *M. fici* on *F. macrophylla* growing in the municipal parks and gardens in Melbourne's city centre.

Ms Suzanne Bullock, RBG Plant Pathology Unit, provided much valuable time and photographic expertise in assisting the author with photographing the psyllid. Mr Paul Nicholson, RBG Gardens Services, assisted the author with a survey of *M. fici* along the eastern coast of Australia and its hinterland. Mr David Bidwell, RBG Gardens Services' arborist, and his assistant, Mr Geoffrey Goodwin, provided essential and valued assistance in the sampling of the upper reaches of the canopies of various *F. macrophylla* specimens growing in the Royal Botanic Gardens and Sydney Domain, and elsewhere. Other staff, too numerous to mention individually, in RBG Gardens Services, provided care for the study trees, and horticultural assistance and advice when it was needed. Staff of the Olympic Coordination Authority are thanked for allowing access to the Homebush Bay Olympic Venue during its construction and landscaping phases in order to observe and advise on the population of *F. macrophylla* at the site.

Mrs Margaret Harwood, Higher Degree Project Coordinator at the Higher Degree Research Unit, Macquarie University, for her patience and understanding in the final weeks of the thesis.

Mr Tony Martin of the Royal Botanic Gardens Sydney, for his patience with my long absence from work.

Finally, my largest debt of gratitude is to my family, including my mother, Barbara (EB) Newman, who made many trips from Adelaide to Sydney to help the family, and my mother-and father in-law, Drs Bill and Sally Nelson, for their long-suffering support of me, as well as their continued interest and encouragement, throughout the project. Without their good graces, this project would not have been possible, but most of all my gratitude to my wife Dr Kathleen E Nelson, and my young daughter Sarah Newman, who had to put up with the worst of it, and still remained supportive to the bitter end, and to whom this thesis is dedicated.

Chapter 1. Introduction

1.1 Overview of the Project

Ficus macrophylla Desfontaines ex. Persoon (Urticales: Moraceae; common name ‘Moreton Bay fig’) is a tropical, subtropical and warm temperate rainforest species endemic to the eastern seaboard of Australia (Harden 1990). It has been planted as a major parkland tree throughout much of inhabited Australia since European settlement, and has been a significant part of the Australian urban landscape for over one hundred and fifty years. In an urban park setting, *F. macrophylla* is an architecturally attractive tree because of its spreading habit and the large buttress roots which support the trunk (Figures 1.1 and 1.2). Mature specimens cast a large amount of shade, and its large dark green shiny leaves are also admired. The tree, when growing in an urban environment, however, periodically suffers complete defoliation, which is apparently caused by massive outbreaks of a small gregarious hemipteran insect, *Mycopsylla fici* Tryon (Hemiptera: Sternorrhyncha: Psylloidea: Homotomidae: Macrohomotominae; common name ‘fig psyllid’) (Taylor and Carver, 1991; Hollis and Broomfield, 1989). The commonly applied term ‘psyllid’ is in fact technically inaccurate with respect to this insect: it is actually a *psylloid*, since it belongs within family Homotomidae, not within family Psyllidae. ‘Psylloid’, literally, ‘psyllid-like’, is used to refer generally to members of all families within the superfamily Psylloidea, and, of course includes the ‘true’ psyllids (i.e., members of family Psyllidae). There are seven distinct families currently recognised as existing in Australia (Taylor and Carver, 1991) – or eight, with Spondyliaspidae elevated from a subfamily within Psyllidae (Spondyliaspidinae) to full family status. Although the term ‘fig psyllid’ is in common use, the decision was made to use the correct taxonomic terminology within this thesis, and therefore ‘homotomid’ is used for the general term for the family in which *M. fici* is placed, and henceforth the terms ‘psylloid’, ‘Moreton Bay fig psylloid’ and ‘fig psylloid’ are used instead of ‘psyllid’, ‘Moreton Bay fig psyllid’ and ‘fig psyllid’, respectively (see also Section 1.5.2 below). Where the term ‘psyllid’ appears, it should be assumed that the insect being referred to is a member of family Psyllidae (i.e., a ‘true’ psyllid).

Homotomids appear almost without exception to feed exclusively on members of genus *Ficus*, and to be largely host-specific (Hollis and Broomfield, 1989). The fig psylloid feeds on leaf intracellular fluids (Taylor and Carver, 1991) and produces sticky coverings or ‘lerps’ (Froggatt, 1923; Taylor and Carver, 1991). The term ‘lerp’ is in fact a modification of the word

'laap', transliterated from an Australian Aboriginal language (Takagi and Miyatake, 1993). Fallen Moreton Bay fig leaves can often be coated with lerp residue during periods of high psyllid population levels, and cause a nuisance in public places such as parks, other open civic places and roadways by sticking to people's feet and to vehicle tyres. The aim of this research project is to investigate the demography of the fig psyllid and the effects of this insect on its host plant, with a view to the long-term protection and management of the fig as a major urban environmental asset.

1.2 The Moreton Bay fig

1.2.1 Brief overview of the systematics of the *Ficus macrophylla* complex

Two subspecies of the Moreton Bay fig have long been recognised: *F. macrophylla* subsp. *macrophylla* Desf. ex Pers., and *F. macrophylla* subsp. *columnaris* C. Moore. *F. macrophylla* subsp. *macrophylla* is the more common of the two subspecies, with a larger endemic range, and it is this subspecies that is commonly known as the Moreton Bay fig. *F. macrophylla* subsp. *columnaris* is endemically restricted to Lord Howe Island, a volcanic remnant which lies 500 km east off the coast of New South Wales. This form is commonly known as the Lord Howe Island fig and the Banyan fig. The systematics of some of the Australian *Ficus* species were recently revised (Dixon, 2001a; Dixon 2001b; Dixon *et al.*, 2001; Dixon, 2002) and these two former *F. macrophylla* subspecies have been formally reclassified as *formae*. The former names are used in this thesis to avoid confusion, as the name change is quite recent. The association of the two forms with the seed parasitic wasp *Pleistodontes froggatti* Mayr was used as an operational taxonomic character to assist in placing the two figs in a broader taxonomic context (Dixon 2001b; Dixon *et al.*, 2001). Fig wasps parasitise the embryonic seeds within fig synconia, and, like many other parasitoids, are host-specific (Memmott *et al.*, 1994). *F. macrophylla* is classified as one of the group known as strangler figs (Harden, 1990), which include *F. watkinsiana*, to which *F. macrophylla* is structurally similar, although the shapes of fruit and leaves differ, as do the abaxial surfaces of the leaves of the two species. The fruit of *F. macrophylla* is smaller and more spherical than that of *F. watkinsiana*, the fruit of the latter being larger and a pronounce nipple at the distal end. The abaxial surface of the leaf of *F. macrophylla* is covered with a layer of rufous hairs, while that of *F. watkinsiana* is glabrous.

1.2.2 Distribution of *F. macrophylla* subspecies

The natural range of *F. macrophylla* is bounded in the north by Torres Strait and by the Shoalhaven river in New South Wales in the south (Harden, 1990). The western side of its range is the eastern side of the Great Dividing Range. The eastern limit of *F. macrophylla* subsp. *macrophylla*, however, is the eastern mainland coast, while *F. macrophylla* subsp. *columnaris* is endemically restricted to Lord Howe Island off the coast of New South Wales, in the Pacific Ocean (Harden, 1990). *F. macrophylla* subsp. *columnaris* now occurs in places on the mainland as a result of human activity. Neither *F. macrophylla* subsp. *macrophylla* nor *F. macrophylla* subsp. *columnaris* is endemic to the Sydney Basin, having been introduced some time after European settlement. The exact dates of these introductions is not clear. *F. macrophylla* occurs naturally in rainforest areas, but now has widespread distribution in many inhabited areas throughout Australia as a result of human cultivation. It has been widely planted in coastal and inland regions because of its large spreading canopy. This can reach up to 40 m in diameter and 20 m in height when growing in open spaces (Figure 1.2), and the tree will grow well even in areas of relatively low rainfall (e.g., an average rainfall of no more than 600 mm per year). Of the two subspecies, *F. macrophylla* subsp. *macrophylla* is far more prevalent than *F. macrophylla* subsp. *columnaris*.

1.2.3 Growth Characteristics of *F. macrophylla*

In rainforests, its natural habitat, *F. macrophylla* subsp. *macrophylla* usually germinates as an epiphyte. Seeds, carried by flying foxes and birds which feed on the ripe figs, germinate in the canopies of emergent trees (including *F. macrophylla* itself). The roots of a successful seedling grow downwards, enlarging and wrapping around the trunk of the host plant as they do so (Figure 1.1). Once the roots reach the ground and are able to start transporting nutrients into the plant from the soil, the tree's growth rate increases rapidly.

The roots eventually crush the host tree's trunk, restricting its vascular elements and killing it. The entire trunk of the host is eventually engulfed by the fig. *F. macrophylla* subsp. *macrophylla* does not internally parasitise its hosts in the manner of the mistletoe. The tree is an emergent species when growing in these conditions, reaching heights of between 15 and 20 m but not spreading as widely as those specimens growing in open spaces, and having less leaf cover (Figure 1.1).

F. macrophylla subsp. *columnaris* differs from *F. macrophylla* subsp. *macrophylla* in being somewhat smaller in diameter and height. It also differs markedly from *F. macrophylla* subsp. *macrophylla* with its habit of sending down curtains of aerial roots from lateral branches, which form new trunks once they reach the ground. Thus the Banyan fig becomes a multistemmed tree, whereas the Moreton Bay fig is a single-stemmed species. *F. macrophylla* subsp. *macrophylla*, although it also initiates aerial roots, does not do so with the enormous proliferation of aerially-formed buttresses that distinctively marks *F. macrophylla* subsp. *columnaris*. The Banyan fig has been much less widely planted as a civic ornament. *F. macrophylla* subsp. *macrophylla* was used exclusively in this study, and unless otherwise stated, *F. macrophylla* refers to *F. macrophylla* subsp. *macrophylla* in this thesis.



Figure 1.1: *F. macrophylla* growing as a strangler in rain forest in Bruxner National Park, near Coffs Harbour, northern New South Wales



**Figure 1.2: *F. macrophylla* growing in the Royal Botanic Gardens, Sydney:
project tree code *MFG***

1.3 The fig psyllid and fig tree defoliation: an aesthetic and practical problem

M. fici is approximately 16 mm long from frons to folded forewing tip (approximately 20 mm from antenna tip to forewing tip) at rest in its adult form. Juveniles are gregarious and sessile on the underside of mature fig leaves. The juveniles produce and live underneath a protective sticky covering known as a lerp, which is usually around 30 mm in diameter. Peaks of outbreking populations occur between mid-spring and late summer. This insect has been implicated in major defoliation events occurring on *F. macrophylla*, with severe defoliation events occurring in mid-spring, and minor defoliation events in summer. *M. fici* is found only on *F. macrophylla*. A closely related species, *M. proxima*, lives exclusively on *F. rubiginosa*, the Port Jackson fig. Feeding activity of the fig psyllid has been implicated in causing the periodic defoliation of Moreton Bay figs, and the association between insect and host has been reported in the literature since the early part of the 20th century (Froggatt, 1901a; Froggatt, 1901b; Froggatt, 1907; Froggatt, 1923; Anonymous, 1913; Anonymous, 1924). Badly affected trees can lose between 70% and 100% of their usual leaf cover (Figures 1.3 and 1.4). The bare trees (Figure 1.5) and resultant carpet of fallen sticky leaves and lerps (Figure 1.6) create a difficult problem for urban horticulturists. Bare trees attract much comment from the general public, and the sticky leaves fasten themselves firmly to pedestrians' feet. The sticky carpet severely degrades the amenity of public spaces used for recreational activities. Other side effects are likely, such as UV irradiation (sunburn) of previously covered branches, borer and fungal invasion, and the further weakening of trees that are already stressed by soil compaction and lack of water (both lack of rainfall and lack of water penetration through pavements, etc.). Pedestrian activity over the root zone of trees can severely compact the soil, and *F. macrophylla* appears to be particularly susceptible to this (BA Summerell, pers. comm.). General public concern over the fig defoliation issue has been sufficient that the Royal Botanic Gardens and Domain Trust and The Environmental Trusts both funded parts of this research on the psyllid.

Section 1.4 aims to place the fig-psyllid system within the appropriate theoretical framework of plant-insect interactions. Fig-psyllid investigations can then be based on and interpreted within this framework.



Figure 1.3: Severely defoliated *F. macrophylla* in Sydney Domain, during the 1996 defoliation event.



Figure 1.4: The consequences of repeated defoliation and soil compaction for an *F. macrophylla* specimen in the Sydney Domain. Photo taken during the 1996 defoliation event.



Figure 1.5: Defoliated branchlet, *F. macrophylla* specimen in Sydney Domain, 1996 defoliation event.



Figure 1.6: Carpet of lerp-covered leaves under *MFG*, Royal Botanic Gardens, Sydney.

1.4 Plant-insect interactions: evolutionary and practical consequences for the participants

A number of strategies of plant defence and insect attack has been evolved over time. Plants have evolved various strategies to avoid attack by herbivores, depending upon the overall selection strategy (i.e., *r*-selected or *K*-selected (Macarthur and Wilson, 1970; Pianka, 1970; Pianka, 1972)) adopted by the particular species. The utilisation of different families of plant secondary chemicals as part of defence mechanisms is dependent on the overall selection strategy adopted by the plant (Feeny, 1976).

1.4.1 Plant apparency, selection and coevolutionary 'arms race' theory

Plant *apparency* is a term coined by Paul Feeny to describe the visibility of a plant to herbivores of various descriptions (Feeny, 1976). *r*-selected plants, those with high turnover rates, ephemerals, etc., tend to come under the category of *unapparent* plants, and attract mostly generalist 'flush feeding' herbivores. *K*-selected plants, on the other hand, which are more persistent in the environment than unapparent plants, attract mostly specialist 'senescence' feeders (Feeny, 1976). New leaves of *K*-selected species are logically treated by Feeny as *r*-selected unapparent organisms, owing to the relatively short time that leaves spend reaching maturity compared with the relatively long time spent in maturity and senescence phases.

The unapparent *r*-selected and apparent *K*-selected groups have quite distinct biochemical defence strategies. *r*-selected plants use a *qualitative* 'knock-down' strategy (Bowers and Puttick, 1988), while *K*-selected plants employ a *quantitative* 'slow-down' strategy. The *r*-selected qualitative strategists have evolved a range of secondary metabolic compounds – phytoalexins or allelopathic chemicals (Smith, 1996) – directly injurious to herbivores' survival and therefore fitness (Ahmed *et al.*, 1991; Argandona, 1980; Barria *et al.*, 1992; Copaja *et al.*, 1991; Dupré *et al.*, 1991; Feeny 1976; Fuentes-Contreras and Niemeyer, 1988; Gianoli and Niemeyer, 1997; Gianoli *et al.*, 1999; Gigovich and Niemeyer, 1995; Gonzales *et al.*, 2002; Moreno-Murillo *et al.*, 1995; Niemeyer, 1988; Niemeyer, 1992; Niemeyer, 1995; Niemeyer *et al.*, 1992; Ramirez and Niemeyer, 1999; Russell *et al.*, 2000; Weibull and Niemeyer, 1995; Zdero *et al.*, 1990a; Zdero *et al.*, 1990b; Zdero *et al.*, 1991). A plant that does not have long to reach maturity and reproduce cannot afford to lose valuable resources to herbivores if it is to be reproductively successful (i.e., have a high degree of fitness). A method

of avoiding herbivory that is correspondingly fast-acting is probably most beneficial (Feeny, 1976), and can thus allow the utilising plant to escape herbivores (Bowers and Puttick, 1988). The defensive secondary metabolites produced by the unapparent plant species have correspondingly evolved against attack by generalists, and are cyanogenic and cardiac glycosides (cardenolides), alkaloids, glucosinolates, quinone derivatives, coumarin (Gibbs, 1974; Gilbert, 1975; Feeny, 1976), and flavonoids (Russell *et al.*, 2000). These classes of fast-acting phytoalexin toxins are generally associated with their 'own' specific plant families. For example, members of family Brassicaceae can produce unpalatable and/or toxic glucosinolates; those in family Solanaceae may produce toxic and psychotropic alkaloids (e.g., *Datura* spp.); while members of families Apocynaceae and Asclepiadaceae may produce glycosides (including cardenolides) and alkaloids (e.g., *Asclepias* spp. (Malcolm & Zalucki, 1996; Malcolm *et al.*, 1989)). Exceptions do exist (Feeny, 1976) however, with members of some plant families utilising compound classes usually associated with an entirely different family (Feeny, 1976), and with further biochemical characterisation of hitherto unstudied or poorly-studied plants, the list of exceptions is likely to lengthen. As a result of ongoing selective pressure produced by plant biochemical defences, a relatively small number of species of herbivores have carved out an evolutionary niche by becoming, in turn, adapted to specialise on highly toxic unapparent plants. These herbivores can either metabolise the toxins and/or sequester the compounds in various ways to serve in their own defence against predators, one of the most well known being the monarch butterfly, *Danaus plexippus* L. (Kelley *et al.*, 1987; Malcolm *et al.*, 1989; Martin and Lynch, 1988; Nelson, 1993). By definition, the specialist herbivores feeding on *r*-selected plants are themselves *r*-selected.

Conversely, *apparent* plants are those species whose individuals are more obvious to generalist herbivores because they are present in the landscape for longer periods of time than the individuals of species of unapparent plants (Feeny, 1976). Apparent plants thus also have the general characteristic of being *K*-selected organisms, whose selection has taken place under environmental conditions of relative stability and longer-term favourable carrying capacity than those conditions which influence the adaptations of *r*-selected plants (Macarthur and Wilson, 1970; Pianka, 1970). Plants species exhibiting apparency have quite different defences from herbivores than those of unapparent species. Generally of larger structure than unapparent species, apparent plants have greater reserves of nutrients (including nitrogen), and are often present in the environment effectively as patches or islands of a few or separate individuals within a heterogeneous matrix (Janzen, 1968; Macarthur and Wilson, 1970). This latter

strategy is mediated in part by the production of plant growth inhibitory (allelochemical) substances such as gallotannins. Gallotannins are synthesised by plants *via* the secondary metabolic shikimic acid pathway, the same pathway that also produces the aromatic amino acids tryptophan, tyrosine and phenylalanine (Bentley, 1990; Diaz *et al.*, 1997; Facchini *et al.*, 2000; Floss, 1985; Herrmann *et al.*, 1992; Herrmann, 1995a; Herrmann, 1995b; Herrmann and Weaver, 1999; Ossipov *et al.*, 2003; Ruuhola *et al.*, 2001; Schmid and Amrhein, 1995; Schoch *et al.*, 2001; Weaver and Herrmann, 1997; Weiss, 1985; Wilson *et al.*, 1998). Allelochemy reduces resource competition from other individuals, including its own offspring, for organisms invoking it (An *et al.*, 1996; Chase *et al.*, 1991; Chou *et al.*, 1995; Hegazy *et al.*, 1991; Kato-Noguchi *et al.*, 1994a; Kato-Noguchi *et al.*, 1994b; Kato-Noguchi, 2001; Klein and Blum, 1995; Muller, 1986; Satoh *et al.*, 1996; Srivastava *et al.*, 1998; Tang and Zhang, 1986; Vance and Francko, 1997; Zeng *et al.*, 2001), and in turn allows for adaptation to greater longevity of those organisms owing to the resultant increased carrying capacity of the environment in which they are growing (as well as their own slower growth rate caused by the same allelopathic substances). The relatively sparser density of apparent plants assists them in escaping from herbivores, while herbivores feeding on such plants consequently may become adapted to more highly-tuned strategies to cope with this. One such strategy is to time feeding with the production of new growth – that is, before the plant has had sufficient time to build up defensive concentrations of gallotannin-protein complexes (Whittaker and Feeny, 1971).

Since apparent plants represent a longer-term resource to herbivores, the main biochemical defence employed by many of these species is the phytoalexin application of the same gallotannins used in allelopathic defence from crowding by other plants. This strategy is mediated by the gallotannic sequestration of nitrogen stored in proteins. The toxic effects of phytoalexin tannin-protein complexes on herbivores are not as acute as those of knock-down poisons typically employed by unapparent species, and are *quantitative or dosage-dependent* (Feeny, 1976). This ultimately results in *toleration* by the plant of damage by herbivores (Levitt, 1972; Levitt, 1980). The effects of apparent plant allelochemicals usually manifest symptomatically as the retardation of growth rates in both specialist and generalist herbivores which feed on this type of plant; and also on the growth rates of the plants producing them (Appel and Martin, 1992; Berenbaum and Zangerl, 1993; Bowers and Puttick, 1988; Carisey and Bause, 1997; Denno and Benrey, 1997; Leszczynski *et al.*, 1989; Mansour, 1984; Mulrooney *et al.*, 1985; Schroeder, 1986; Stamp, 1994; Stamp and Osier, 1997; Stamp and Yang, 1996; Timmins and Reynolds, 1992). Thus, apparent-feeding herbivores tend to have

longer generation times than those feeding on unapparent plants and therefore fewer generations per year or per season (Feeny, 1976). There is very little literature on qualitative phytoalexins of *Ficus* spp. (or other Moraceae members) as such, and those papers are the results of investigations into glycoside- (galacturonide-) mediated anti-microbial responses in *F. awkeotsang* Makino (Moraceae) (Shirata and Takahashi, 1982) and in *Morus alba* L., *F. carica* L. (Moraceae) and others (Komae *et al.*, 1990). Alkaloids and glucosides, amongst a number of chemical families, were isolated from *Ficus glomerata* Roxb. (Singahl and Saharia, 1980), although the emphasis in this instance was on human pharmacology. There has been a number investigations into quantitative tannin/polyphenol based reactions of vertebrate herbivores where in general greater quantities of phenolics, tannins and derivatives were shown to correlate with reduced preference in favour of species containing lower quantities of these types of biochemicals. Groups studied were mostly ruminants (Apori *et al.*, 1998; Gupta and Balaraman, 1992; Khatta *et al.*, 1999; Singh, 1977; Singh and Roy, 1981; Vaithiyanathan and Kumar, 1993; Wood *et al.*, 1994), fruit bats (Wendeln *et al.* 2000) and chimpanzees (Reynolds *et al.*, 1998). Tannins in leaves of *Ficus* sp. used as a crop plant for humans were assayed by Misirli *et al.* (1998). Alkaloids and tannins were recovered from *F. benghalensis* L. aerial roots in (human) homeopathic studies (Varma *et al.*, 1993). There is no apparent comparable literature concerning allelochemicals and either sucking or chewing insects on *Ficus* spp., although there is a paper on the consumption of leaves of *F. macrophylla* by the chewing herbivore, *Gecaroidea natalis* (a terrestrial crab) (Greenaway and Linton, 1995), which indicated that these leaves were a significant source of nutrition for the animal.

1.4.2 Nitrogen ‘tug-of-war’ between plants and insects

The limiting essential chemical element in the diet of herbivores is nitrogen (White, 1993). Herbivores, including insects, need to consume relatively large quantities of plant foliage to acquire sufficient nitrogen in the form of proteins. Nitrogen, on the other hand, is also a major limiting resource for plants, and as outlined above, plants have developed a number of strategies to hang on to their hard-won proteins. There is a consequent ‘tug-of-war’ between herbivores and plants for nitrogen, with the so-called ‘(co-)evolutionary arms race’ a result (Anderson *et al.*, 1990; Berenbaum 1988; Berenbaum and Feeny, 1981; Berenbaum and Zangerl, 1988; Berenbaum and Zangerl, 1993; Chew and Courtney, 1991; Matusda and Abrams, 1994; Hilker and Dicke, 2003; Honda and Hayashi, 1995; Zucker, 1983). The latter two articles are specifically concerned with the allelochemical coevolutionary ‘arms race’.

Sucking insects have even less access to nitrogen, than chewing herbivores, since they are not ingesting the contents of a variety of whole cells. They are reliant on a much more dilute fraction obtained directly from plant sap instead. Some sucking insects feed on phloem, some on mesophyll and some on xylem; in the case of psylloids, feeding occurs on phloem vessels. This is one of the major reasons for the production of copious amounts of honeydew from sucking insects: very large quantities of plant sap must be imbibed to provide the necessary amount of scarce nitrogen. The insect needs, therefore, to expel a proportionately large quantity of excess materials, i.e., water and undigested plant sugars. Sucking insects often employ their own means of inducing plants to yield nitrogen. One method is to take advantage of the tendency of plants under stress (e.g., drought, high temperatures) to mobilise nitrogen in an attempt to counteract stresses on various organs within the plant (White, 1984). Another method employed by sap sucking insects (and fungi) is to induce localized (and often eventually systemic) stress in the plant tissue themselves, by injecting digestive enzymes, or other secondary chemicals that signal the plant to start digesting itself (if in only small amounts at first). These are very complex feedback processes, not yet fully investigated at the biochemical level.

1.5 The Moreton Bay fig psyllid

1.5.1 The psyllid in the context of research on members of families Homotomidae Heslop-Harrison and Psyllidae L.

M. fici is a part of the superfamily Psylloidea (Carver and Taylor, 1991; Hollis and Broomfield 1989) that contains a number of species that have attained important economic pest status in various crops. A large body of research from Hawaii exists on the Cuban leucaena psyllid, *Heteropsylla cubana* Crawford (Sternorrhyncha: Psyllidae), which is a severe economic pest in the tropics as it attacks the important legume crop tree, *Leucaena leucocephala* (Lam.) Dewit (Fabaceae). This plant is an important fodder crop and shade tree (Hodkinson, 1988), and is badly affected by leucaena psyllid feeding damage (Noyes, 1990). *H. cubana* is a widespread tropical pest species with a relatively wide host range, and has received considerable attention (Beardsley and Uchida, 1990; Joshi, 1991; Nakahara and Funasaki, 1987; Nagamine *et al.*, 1987; Noyes, 1990; Uchida *et al.*, 1992). Research concerning *H. cubana* has been directed, amongst other things, at the efficacy of encyrtid parasitoids of the leucaena psyllid in genus *Psyllaephagus* (Hymenoptera: Encyrtidae) (Joshi 1991; Noyes, 1990; Beardsley and Uchida,

1990; Uchida *et al.*, 1992; Nagamine *et al.*, 1987; Nakahara and Funasaki, 1987). Grove and Ghosh (1914) gave an extensive report on the life history of *Psylla isitis* Buckt.).

Watmough (1968) made an extensive long-term study of the life histories of two species of nearctic psyllids, *Arytaina spartiophila* Förster (Sternorrhyncha: Psyllidae) and *A. genistae* Latreille feeding on *Sarothamnus scoparius* (L.) Wimmer (Leguminosae), (Scotch or common broom (currently named as *Cytisus scoparius* (L.) Link (Fabaceae)). Watmough constructed life tables from his survey data of the psyllid populations and analysed the effects of competition (overcrowding on food plants), predation and the age of food plants .

Pear-feeding psyllids (*Cacopsylla pyri* (Sternorrhyncha: Psyllidae), *Cac. pyricola*, and *Psylla chinensis* (Sternorrhyncha: Psyllidae)) have been investigated by a number of researchers. (Wilde, 1962). Wilde (1962) gave the life history and natural enemies of *Cac. pyricola* in pear orchards and domestic gardens in British Columbia from 1960 to 1962, and matched data collected against cultivation techniques and climatic data. Wilde (1962) noted that weather conditions appear important in severe outbreaks of *P. pyricola*. In this instance it appeared that heavy rain reduced the survival rate of psyllid nymphs in the field. Predators discovered were *Chrysopa* spp. (Neuroptera: Chrysopidae), *Sphaerophoria* sp. (Diptera: Syrphidae), *Anthocorus* sp. (Auchenorrhyncha: Anthocoridae) and several coccinellid genera (Coleoptera: Coccinellidae). *Cac. pyri* has recently been the focus of research in Europe as a potential vector of a phytoplasma causing an economically serious decline in pear trees (Avinent *et al.*, 1997; Baldassari *et al.*, 1996; Bues *et al.*, 1994; Bues *et al.*, 2000; Carraro *et al.*, 2000; Carraro *et al.* 2001; Kapatos and Stratopoulou, 1996; Milevoj and Kroselj, 1995; Souliotis and Broumas, 1998). *Cac. pyricola*, a direct pest of pear (*Pyrus* spp.) has also been studied extensively, both in Europe and North America (Blomquist and Kirkpatrick, 1997; Blomquist and Kirkpatrick, 2002; Booth and Riedl, 1996; Davies and Eyre, 1996; Horton *et al.*, 1998; Horton and Lewis, 1996; Lee *et al.*, 1999; Puterka 1997). Yang and Li, (1981) discovered and described seven new species of *Psylla* (*P. chinensis*, *P. betulaefoliae*, *P. heterobetulaefoliae*, *P. changli*, *P. phaeocarpae*, *P. liaoli* and *P. jiangli*) as the result of a survey conducted in eight northern Chinese provinces for pear-feeding psyllids. Yang and Li (1982) also described five new species of *Celtisaspis*. Li *et al.* (1992) studied the biology of *Psylla chinensis* and its effects on its host (*Pyrus* sp.) over a four year period with a view to implementing biological control of

this insect, and the same authors (Li *et al.*, 1994) describe control measures and thresholds arising from the same four-year survey.

In Australia, at least, psyllids and psylloids appear on relatively few plant species of current economic importance. Many psyllids and psylloids occurring endemically to Australia have hosts among *Eucalyptus*, *Acacia* and *Boronia* species and some of these psylloid species can occur in sufficient numbers to cause problems to forestry and volatile plant oils industries. As a result, there is a relatively large body of work that has been published on the biology of eucalypt psyllids. Clark (1962) undertook extensive studies on the biology of *Cardiaspina albitextura* (Sternorrhyncha: Psyllidae) on *E. blakeleyi* in the ACT and South Eastern NSW. *C. albitextura* can become a severe pest of eucalypts under suitable conditions causing defoliation in a similar way to *M. fici*, and as such can be a severe forestry pest. Clark investigated population dynamics and life cycle aspects of the psylloid, and the effects that both weather and parasitisation have on them. He also made use of locally available meteorological data such as temperature, wind speed and direction, and rainfall. Campbell (1972) studied the biology and population ecology of *Cardiaspina* spp. on *E. grandis*.

Amongst other subjects, Taylor made a life's work of describing species of 'true' psyllids, as opposed to psylloids, including some within genus *Cardiaspina* (Taylor, 1987b; Taylor, 1989), and other psyllid species (Taylor, 1976; Taylor, 1984a; Taylor, 1984b; Taylor, 1985b; Taylor, 1985b; Taylor, 1987a; Taylor, 1987b; Taylor, 1987c; Taylor, 1990a; Taylor, 1990b; Taylor, 1992a; Taylor, 1992b; Taylor, 1997a; Taylor, 1997b).

Campbell (1992) studied outbreaking populations of *Cardiaspina fiscella* Taylor (Psyllidae) and *Car. maniformis* Taylor on *Eucalyptus grandis* Maiden & Hill, including weather effects. Crawford and Wilkens (1996) made ultra-structural studies of leaf tissue from *Eucalyptus camaldulensis* that had been fed upon by *Car. retator*. White (1969) made correlations between climate records and outbreaks of *Car. densitexta* on *Eucalyptus fasciculosa*, and studied the life history and ecology of *Car. densitexta* on *E. fasciculosa* (White, 1970a and 1970b). He also studied the activities and effects of *Car. densitexta* feeding on *E. camaldulensis* (White, 1971), as well as aerial dispersal by the insect (White, 1973), and reported on amylose in the faeces of *Car. densitexta* feeding on *E. camaldulensis* (White, 1972). White (1978) gave a method for sampling *Car. densitexta* populations on *E. fasciculosa*.

Unlike many other psyllids, *Car. densitexta* has a relatively wide host range consisting of at least eight known eucalypt species (Morgan, 1984). Psyllid dispersal was reported to be restricted in summer- and autumn-emerging adults, with adult females remaining in their area of origin, i.e., their 'home tree'. Spring-emerging adults were found to disperse actively from their 'home trees'. Oviposition behaviour and oviposition distribution patterns for *C. densitexta* were reported in great detail. Leaf age and actual spatial distribution with respect to the twig and time of year were also investigated.

Taylor investigated courtship behaviour including calling (stridulation) and mating behaviour of gall-forming psyllids on *Eucalyptus* spp. (Taylor, 1984; Taylor 1985a).

The boronia psyllid, *Ctenarytaina thysanura* Ferris and Klyver (Sternorrhyncha: Psyllidae), another 'true' psyllid member of the Australian Psylloidea assemblage, is a significant pest in oil production from *Boronia megastigma* Nees. ex Bartl. (Rutaceae) (Mensah and Madden, 1991a; Mensah and Madden, 1991b; Mensah and Madden, 1992; Mensah and Madden, 1993a; Mensah and Madden, 1993b; Mensah and Madden, 1994). The authors tested psyllid response to yellow-coloured sticky-traps (see also Chapter 6, Phenology of *Mycopsylla fici*), with the longer-term view of collecting conclusive data about the biology of this insect (population dynamics, migration, dispersal etc.). The responses of the psyllid's natural enemies to the sticky-traps were also noted, with a consequent caution about the wide-scale use of such traps in boronia psyllid control.

Recent forestry-related studies of or including psylloids, principally on eucalypts, have been made by Stone, Stone and coauthors, and others (Brandon and Shelton, 1997; Collett, 2001; Madden and Stone, 1984; Mullen *et al.*, 1998; Mullen *et al.*, 2003a; Mullen *et al.*, 2003b; Mullen and Shelton, 2003; Mullen *et al.*, 2003; Mullen *et al.*, 2003; Stone, 1996; Stone, 1999; Stone, 1993; Stone and Bacon, 1994; Woinarski and Cullen, 1984). Chlorophyll fluorescence studies of eucalypts attacked by psyllids and psylloids (Stone, 1996) provided the idea and the basis for using the same technique on *F. macrophylla* in the studies for this thesis (Chapter 8), to attempt to determine whether psyllid-induced stress was detectable and quantifiable.

1.5.2 Brief comments on the taxonomy of *M. fici*

The most recent review of the systematics of *Ficus*-feeding psylloids from the Psylloidea was produced by Hollis and Broomfield (1989). In this review the authors constructed a taxonomy of these psylloids according to current zoological nomenclature. Keys were presented for described taxa to species level with the basis for construction of the taxonomic hierarchy being a 35-character cladogram. Hollis and Broomfield (1989) also provided some very general taxonomy of the family Moraceae, and the authors speculated on the unlikelihood of plant-insect stepwise coevolution between the psylloids and *Ficus* spp. given present data, although they suggested that evidence points towards coevolution between the synconium-dwelling agaonid wasps and members of genus *Ficus*.

The authors distinguish three families of psylloids within superfamily Psylloidea (Psyllidae, Triozidae and Homotomidae) feeding on five genera within family Moraceae (*Antiaris* Lesch., *Artocarpus* J. R. Forster & G. Forster, *Ficus* L., *Milicia* Sim and *Morus* L.). Genus *Ficus* is stated by Hollis and Broomfield as being the most represented genus of those within family Moraceae which are hosts to (Moraceae-feeding) psylloids (Hollis and Broomfield, 1989). *M. fici* is placed within family Homotomidae (not Psyllidae), with other *Ficus*-feeding psylloids. The relationships of the homotomids with respect to other families within superfamily Psylloidea as presented by Hollis and Broomfield are shown here, somewhat simplified, in Table 1.1. *M. fici* is labelled in red. The tree was simplified partly because of the conflicting viewpoints that still exist with respect to the phylogeny of the Psylloidea. To reiterate the statement made in Section 1.1 at the beginning of this chapter regarding the taxonomic nomenclature relating to *M. fici*, the term 'psylloid' is used throughout this thesis instead of 'psyllid', reflecting its current taxonomic placing.

Table 1.1: An overview of the PSYLLOIDEA, with emphasis on Family HOMOTOMIDAE

Suborder	Superfamily	Family	Subfamily	Genus	species	host	Country/region
STERNORRHYNCHA	PSYLLOIDEA	PSYLLIDAE	Paurocephalinae	<i>Paurocephala</i>			Australia
			Aphalarinae	<i>Phytoloma</i>			Australia
			Anomoneurinae	<i>Anomoneura</i>			Australia
			Spondyliaspinae	<i>Australopsylla</i>			Australia
				<i>Cardiaspina</i>	<i>C. albitextura</i>	<i>Eucalyptus spp.</i>	Australia
					<i>C. densitexta</i>	<i>Eucalyptus spp.</i>	Australia
					<i>C. retator</i>	<i>Eucalyptus spp.</i>	Australia
				<i>Cnetaryaina</i>	<i>Cnetaryaina. sp.</i>		Australia
				<i>Glycapsis</i>			Australia
				<i>Laslopsylla</i>	<i>L. punctipennis</i>	<i>Eucalyptus spp</i>	Australia
				<i>Spondyliapsis</i>	<i>Spondyliapsis sp.</i>		
		TRIOZIDAE	Trioziinae	<i>Pauropsylla</i>	<i>Pauropsylla sp.</i>	<i>Ficus sp.</i>	
				<i>Ceropsylla</i>	<i>Ceropsylla sp.</i>	<i>Ficus sp.</i>	
				<i>Trioza</i>	<i>Trioza sp.</i>	<i>Ficus sp.</i>	
				<i>Trioza</i>	<i>Trioza sp.</i>	<i>Allocasuarina verticillata</i>	Australia
				<i>Schedotrioza</i>	<i>Schedotrioza sp.</i>	<i>Eucalyptus spp.</i>	Australia
		HOMOTOMIDAE	Dynopsyllinae	<i>Diceraopsylla</i>	<i>D. brunettii</i>	<i>F. elastica</i>	India (W. Bengal)
				<i>Dynopsylla</i>	<i>Dy. cornuta</i>	<i>F. nervosa</i>	Southeast Asia
					<i>Dy. grandis</i>	<i>F. nervosa</i>	India (Kerala)
					<i>Dy. pinnatifida</i>	<i>F. nervosa</i>	Taiwan
				<i>Austrodynopsylla</i>	<i>A. encala</i>	<i>Ficus sp.</i>	New Caledonia
				<i>Triozamia</i>	<i>Triozamia lamborni</i>	<i>Antiaris toxicaria</i>	Central Africa
					<i>Triozamia usambarensis</i>	<i>Antiaris toxicaria</i>	Tanzania
				<i>Afrodynopsylla</i>	<i>Af. gigantea</i>	Unknown	Central Africa

Table 1.1: An overview of the PSYLLOIDEA, with emphasis on Family HOMOTOMIDAE

Suborder	Superfamily	Family	Subfamily	Genus	species	host	Country/region
STERNORRHYNCHA	PSYLLOIDEA	HOMOTOMIDAE	Macrohomotominae	<i>Mycopsylla</i>	<i>M. gardensis</i>	<i>F. mollis</i> , <i>F. tsjahela</i>	India
					<i>M. mathuriana</i>	<i>F. religiosa</i>	India (Tamil Nadu)
					<i>M. fici</i>	<i>F. macrophylla</i>	Eastern Australia
					<i>M. proxima</i>	<i>F. rubiginosa</i>	Eastern Australia
					<i>M. kina</i>	<i>Ficus</i> sp.	Papua New Guinea
					<i>M. obliqua</i>	<i>F. obliqua</i>	New Caledonia
					<i>M. propinqua</i>	Unknown	Loyalty Is.
				<i>Macrohomotoma</i>	<i>Ma. apsyloides</i>	<i>F. microcarpa</i>	SE Asia, Hawaii
					<i>Ma. gladiata</i>	<i>F. microcarpa</i>	Japan, Taiwan
					<i>Ma. robusta</i>	<i>F. benjamina</i>	Taiwan
					<i>Ma. sinica</i>	<i>F. microcarpa</i> , <i>F. microphylla</i>	China (Fujian)
					<i>Ma. striata</i>	<i>Ficus</i> sp.	India, Japan
					<i>Ma. williamsi</i>	<i>F. crassiramea</i>	Philippines
					<i>Ma. yunana</i>	<i>Ficus</i> sp.	China (Yunnan)
			<i>Pseudoseriopsylla</i>	<i>Ps. nyasae</i>	<i>F. thonningii</i> , <i>F. scassellatii</i>	Central, S. Africa	
				<i>Ps. laingi</i>	<i>F. thonningii</i> , <i>F. natalensis</i>	Central Africa	
				<i>Ps. carvalhoi</i>	<i>F. ovata</i>	Angola, Nigeria, Zaire	
				<i>Ps. kenyae</i>	<i>Ficus</i> sp.	Kenya	
				<i>Ps. etiennei</i>	<i>Ficus</i> sp.	Senegal	
			Homotominae	<i>Homotoma</i>	<i>H. ficus</i>	<i>F. carica</i>	UK, Mediterranean
					<i>H. viridis</i>	<i>F. carica</i>	W Europe, Balkans
					<i>H. angolensis</i>	<i>F. thonningii</i> , <i>F. mutandifolia</i>	Angola
					<i>H. chlamydodora</i>	<i>F. natalensis</i> , <i>F. thonningii</i>	Central, S Africa
					<i>H. eastopi</i>	Unknown sp.	Cameroon (Bamenda)

Table 1.1: An overview of the PSYLLOIDEA, with emphasis on Family HOMOTOMIDAE

Suborder	Superfamily	Family	Subfamily	Genus	species	host	Country/region
STERNORRHYNCHA	PSYLLOIDEA	HOMOTOMIDAE	Homotominae	Homotoma	H. altissimae	F. altissima	China (Yunnan)
					H. anneslae	Anneslea fragrans (Theaceae)	China (Yunnan)
					H. benjaminae	F. benjamina	China (Lincang)
					H. distincta	F. religiosa	India
					H. indica	F. macrocarpa/F. microcarpa	India (Uttar Pradesh)
					H. maculata	F. erecta var. beechyana	Taiwan
					H. pyriformiscola	F. pyriformis	China (Yunnan)
					H. radiata	F. erecta, F. caulocarpa.	Northeast Asia
					H. bakeri	F. benjamina var. nuda	Philippines, W Malaysia
				Synoja	Sy. cornutiventris	Ficus sp.	Colombia, Panama, Peru
					Sy. floccosa	Ficus sp.	Mexico
					Sy. pulchra	Unknown	Mexico, Panama
		CARSIDARIDAE	Carsidarinae	Protyora	Pr. sterculiae	Brachychilton sp.	Australia
APHIDOIDEA (Aphids)							
ALEYRODOIDEA (Whiteflies)							
COCCOIDEA (“Mealybugs” and scale insects)							
AUCHENORRHYNCHA (Cicadas, “planthoppers”)							
HETEROPTERA (“true” bugs: assassin bugs, mirids, pentatomids)							

1.5.3 The effects of the activities of *M. fici* on *F. macrophylla*

The fig psyllid is a severe pest of the Moreton Bay fig tree from social and urban horticultural points of view rather than a large scale economic one. Literature specifically on *M. fici* is relatively scarce, however, and is restricted mostly to taxonomic studies (Froggatt, 1901a; Froggatt, 1901b; Froggatt, 1903; Froggatt, 1909; Tuthill and Taylor, 1955; Hollis and Broomfield, 1989) with some study of the psyllid's biology (Froggatt, 1907; Froggatt, 1923).

The gregarious lifestyle of the juveniles is quite unlike that of most other psyllids, which tend either to dwell in a solitary state under small, delicately-spun lerps (Clark, 1962; Clark and Dallwitz, 1975; White, 70a; White, 1970b; White, 1971; White, 1972), or to be free-living (Li *et al.*, 1992; Li *et al.*, 1994; Mensah and Madden, 1993a; Uchida *et al.*, 1992). The lerps of many other psyllids, especially *Cardiaspina* spp. feeding on eucalypts, have a precisely woven netlike non-soluble structure composed largely of amylose (a starch) (White, 1972) which is synthesised by the psyllids: the lerps produced by *M. fici*, however, are an amorphous mass composed of soluble honeydew and wax filaments. The *M. fici* lerp components are described in more detail below. The juveniles live under this structure until eclosion.

1.5.4 Existing studies and research on *M. fici*

Walter W. Froggatt, NSW Government Entomologist and one of the founders of modern entomology in Australia (Marks, 1991), described the fig psyllid in various books and monographs at the beginning of this century. He produced a comprehensive review of known Australian members of what was at the time considered as family Psyllidae (but which are now variously placed in other families within Psylloidea, including in family Homotomidae (Hollis and Broomfield, 1989)) in three monographs (Froggatt 1901a; Froggatt 1901b; Froggatt 1903). Shorter descriptions by Froggatt of the psyllid's life cycle and in particular the damage caused to its host plant appeared in his *Australian Insects* (Froggatt, 1907) and *Forest Insects of Australia* (Froggatt, 1923). Froggatt made specific mention in both of these latter publications that the damage done to the leaves of its host was a direct result of the psyllid feeding. Froggatt described the psyllid accurately, with one major exception: the material from which the lerp is constructed. Froggatt misinterpreted the viscous lerp material to be made from fig 'latex', liberated from the leaf tissue as a result of psyllid stylet damage (Froggatt, 1907). Froggatt believed that in puncturing the leaf tissue while feeding, the psyllid caused leaf sap

to flow from the damaged tissue, thus producing a sticky mass known as a 'lerp', which protects the juvenile insect instars. Despite being incorrect, the error has been repeated *seriatim* in the literature referring to *M. fici* (Taylor and Carver, 1991; Tuthill and Taylor, 1955). The author was told emphatically that the lerp was made of latex (KL Taylor, pers. comm.).

Taylor and Carver (1991) gave an introduction to the hemipteran superfamily Psylloidea in Australia, with a key to known families within the superfamily. The authors mentioned, in passing, the two known Australian fig psyllids, the other being *M. proxima*, the 'Port Jackson fig psyllid' (Taylor and Carver, 1991), a homotomid closely related to *M. fici*. Little specific fig psyllid information was provided here except to repeat, without reference, Froggatt's misinterpretation (see also below) of lerp components with respect to the supposed fig latex.

It was not until Eren Turak commenced study of *M. fici* at the Royal Botanic Gardens Sydney in 1993 that this mistake was corrected in Australia, although the true nature of similar lerps was described four years previously in a short paper by Jayaraj *et al.* (1989), and in the same year by Takagi and Miyatake (1993) (see also below). It is not certain that the insect studied was *M. fici*, since the psyllid is supposedly host specific (Hodkinson, 1974; Hollis and Broomfield, 1989), and the psyllid that Jayaraj *et al.* (1989) described was feeding on a different species of fig, *F. amplissima*.

Turak (1994, pers. comm.) was puzzled by the fact that the lerp appeared more labile and susceptible to atmospheric moisture than a supposedly hydrophobic latex structure should be, and started investigating the lerp structure in close detail. He found that the lerp was water soluble, and also contained white threadlike structures. Observation of psyllid juveniles led to the discovery that the main lerp component was honeydew excreted as psyllid waste products, and that the white threadlike material was wax filaments extruded from a pair of ringed glandular structures on the terminal abdominal segment of all psyllid juvenile instars. The wax filaments served as a reinforcing matrix in which the honeydew became trapped and formed a dense, thick protective 'roof' (lerp) over the juvenile psyllids. The lerps of *Lasiopsylla rotundipennis* Froggatt (Sternorrhyncha: Psyllidae) were examined by Gilby *et al.* (1976), and the lerps bear an interesting resemblance to those of *Celtisaspis usubai* Miyatake (Spondyliaspidae). The lerps of *L. rotundipennis* and *Ce. usubai* in turn also bear similarities in appearance and structure with those of homotomid *M. fici*, but differ in the composition of

their main component, which is a starch, rather than the smaller, simple sugars that constitute the main component of the *M. fici* lerp.

Turak also made a connection between the lerp components and the fate of the leaves on which the psylloids and their lerps resided. He observed that under conditions of very high relative humidity, the lerp in its early stages of development would absorb water to the point that the honeydew became a thin solution that was capable of flowing over the leaf abaxial surface. This could presumably prevent gas exchange between leaf tissue and the leaf boundary layer. Turak then inferred that this might be the cause of the massive defoliation of the host plant by initiating leaf abscission in some way (E Turak, pers. comm.). It was to determine the cause of these defoliation events that Turak was employed at the Royal Botanic Gardens Sydney, funded by a research grant from the Royal Botanic Gardens and Domain Trust which enabled him to work half-time on the project. Turak left the RBG to take up full time employment at the Environmental Protection Authority at the end of 1993. The author was employed in mid 1994 at the Royal Botanic Gardens Sydney to continue Turak's work on the psylloid and its apparent effects on its host. The studies became the basis for this PhD thesis in mid 1995, with the successful application for a grant from the Environmental Trusts.

1.5.5 *M. fici* in India and Asia

Jayaraj *et al.*, in a short note (1989), mentioned the activities of a psylloid (or psyllid) recorded as *M. fici* on *Ficus amplissima* Smith in the southern Indian state of Tamil Nadu. Heavy infestations of *M. fici* associated with massive leaf damage and defoliation were noted. The fig psylloid had previously been thought to be host-specific (and is still considered so in Australia). The authors reported in a reasonable amount of detail on the life history of this psylloid, including the number of juvenile instars (Froggatt did not elucidate the number of juvenile instars within a life cycle). The note also gave a description of the lerp components (honeydew and wax) and indicated that these components are produced by the psylloid itself. The details on the lerp components in the Indian report differ markedly from Froggatt's observations concerning the lerp's components. Jayaraj *et al.* (1989) made no mention that fig latex forms any part of the lerp, and their observations coincide with the independent discovery of the lerp's constituents by Turak. Jayaraj *et al.* may have misidentified another species of homotomid as *M. fici*; Hollis and Broomfield (1989) have since added new species of fig-

feeding homotomids. Singh (1996) reported on a *Psyllaephagus* sp. parasitoid of *Mycopsylla* sp. that was attacking *Ficus religiosa* in Mizoram, India.

A highly detailed study (Takagi and Miyatake, 1993) of the lerp components of two species of Japanese psyllids and psylloids, *Ce. usubai* (Psylloidea: Psyllidae) (Yang and Li, (1982), who erected the genus), but is now placed in Psylloidea: Spondyliaspidae (Miyatake, 1994)) and *Macrohomotoma* sp. (Psylloidea: Homotomidae), revealed a very similar structure to the lerp of *M. fici*, both in terms of overall morphology, and of components (i.e., wax and honeydew). Takagi and Miyatake (1993) suggest that the wax-secreting mechanisms of psylloids could be employed as a taxonomic characteristic, based on the differences between the types of glands secreting wax filaments and the structure of the filaments themselves. Jayaraj *et al.* may have mistaken another species of large communal lerp-producing psylloid for *M. fici* on the basis of the lerp structure.

1.6 Aim and objectives of the project

There are obvious gaps in our understanding of fig psylloid biology. For example, there has been no detailed study of the life cycle, or any attempt to determine the detailed effects of the psylloid on its host. The goal of the project was to therefore to address these deficiencies by studying the demographics of the fig psylloid, and the psylloid's effect on the Moreton Bay fig. Individual objectives are specified below. It is hoped that this information will aid in establishing a practical long-term integrated management plan for the fig tree.

1.6.1 Structure of the thesis

The project was divided up into two main components: arthropod studies, and studies of the effect of the psyllid on the tree. The structure of the thesis is as follows. (Note: there is no Literature Survey chapter; literature on the psyllid and the theoretical framework of plant-insect relationships has been discussed above, and literature on methodologies is discussed in the relevant chapters.)

- A survey was conducted of tree condition and psyllid presence throughout the range of the tree in natural and cultivated conditions. This was undertaken to compare the psyllid's prevalence in cultivation and in the wild (Chapter 2);
- Attempts were made to rear *M. fici* in artificial conditions and in large numbers to facilitate life cycle investigations (Chapter 3);
- An insecticidal fogging survey was conducted to ascertain the free-living arthropod fauna within the *F. macrophylla* canopy that would affect the psyllid by predation or competition for resources (Chapter 4);
- The distribution of the psyllid within the canopy was assessed (Chapter 5);
- Field studies were carried out to obtain life-table and other life cycle data for the psyllid, and to construct degree days models for the psyllid (Chapters 6 and 7);
- A study of tree health was made using the application of Fast Chlorophyll Fluorescence Kinetics, and correlations with psyllid load and tree health were made (Chapter 8);
- A concluding chapter draws together the outcomes of the work and shows how they can be applied to management of the Moreton Bay fig in urban situations (Chapter 9).

Chapter 2. Survey of the Range of *Mycopsylla fici* Tryon on the Eastern Australian Seaboard

2.1 Introduction

During background research at the inception of the project, anecdotal evidence was acquired that *Mycopsylla fici* was present on *Ficus macrophylla* in Melbourne, Victoria as well as in New South Wales. The pool of information indicated a wide geographical range of dispersal for the psyllid, and also a wide climatic range that it had been able to live in successfully.

Many organisms, including insects, which are introduced into regions outside their natural range and habitat(s), can often reproduce at vastly different rates than the region in which they originally evolved. Psyllid developmental rate (and that of parasitoids of psyllids) can either become faster or slower, and will be largely influenced in this respect by climatic elements, particularly temperature (Lui and Tsai, 2000; Luft *et al.*, 2001; Mcfarland and Hoy, 2001; Patil *et al.*, 1994). Atmospheric humidity (also possibly temperature) may (Das *et al.*, 1998; Luft, *et al.*, 2001; Macfarland and Hoy, 2001) or may not (Das *et al.*, 1988; Mcfarland and Hoy, 2001; Patil *et al.*, 1994) influence rate of development. Other changes in environmental conditions that influence rate of development can be the ability or inability to exploit available food sources, and a reduction in food plant quality (Cobbinah, 1996; Mensah and Madden, 1994). Other changes in food quality and abundance can occur as a result of host plant growth in a more (or less) fertile soil; or an insect species' introduction into a relatively unpopulated or even empty ecological niche where resource competition is therefore low (Price, 1997). A change of habitat may also induce a dramatic change in population numbers of a given organism as a result of the existence of fewer or no natural enemies in the new environment, this is, however, a complex and sometimes controversial area of ecological study (Mayr, 1963; Price, 1997; Southwood, 1977).

The introduction of *F. macrophylla* into an urban environment possibly presented the psyllid with an increase in resources, compared with that pertaining in its natural conditions, because of increased availability of its host (more abundant and therefore more detectable) and an increase in the actual volume of its host plant (change in general shape from a relatively narrow 'cylinder' to that of a spreading 'hemisphere' or dome). An increase in host quality in a regularly irrigated parkland situation might also be considered as beneficial to the growth and

development of the psyllid. *M. fici* periodically can reach plague proportions on its host when growing in an urban environment, resulting, in certain circumstances in complete defoliation of the tree. Circumstances pertaining in the psyllid's natural habitat may reduce the likelihood of plague outbreaks because of some other factor in that habitat. A capacity of the fig tree to defend itself from the psyllid by defoliating may have evolved in the natural habitat. It was decided to perform a transect for the psyllid in coastal and hinterland regions which were within it and the fig tree's natural range, reaching from Wollongong in New South Wales, to Brisbane in southern Queensland. Melbourne in Victoria and Adelaide in South Australia, two places outside the natural range of *F. macrophylla* and *M. fici*, were included later as places visited in search of the psyllid.

2.2 Materials and Methods

A survey of *F. macrophylla* trees growing in coastal urban and forest environments north from Sydney to North Stradbroke Island, off the coast of Brisbane in Moreton Bay, was made in early November 1994. The survey was conducted by the author, and Paul Nicholson, the latter of whom was a horticultural apprentice with the Royal Botanic Gardens, Sydney at that time. At the time that the survey was conducted, the psyllid was present in Sydney at the egg stage. Towns and national parks were visited, with the northernmost (and easternmost) place being North Stradbroke Island, in Moreton Bay, off the coast of Brisbane, Queensland. It is Moreton Bay from which *F. macrophylla* takes its common name.

2.3 Results

Results are summarised in Table 2.1.

Table 2.1: The occurrence of *M. fici* at different geographical locations on the Australian mainland (November 1994, January 1997 & January 2001)
Locations are ranked in ascending order of latitude (data via Hill *et al.*, 2004)

Location	<i>F. macrophylla</i> +/-	<i>M. fici</i> +/-	Tree condition	Latitude (° ' S)	Longitude (° ' E)	Location type	Canopy shape
Melbourne	+ ¹	+	+	37 49	145 00	Urban parkland	Dome
Adelaide	+	- ²	+	34 56	138 36	Urban parkland	Dome
Wollongong	+	+	+	34 25	150 54	Urban area + Rainforest	Dome
Sydney	+	+	+	33 53	151 12	Urban parkland	Dome
Newcastle	+	+	+	32 55	151 45	Urban parkland	Dome
Port Stephens	+	+	+	32 42	152 04	Rural township	Dome
Wingham Brush Reserve (Taree)	+	+	+	31 54	152 28	Rainforest	Cylinder
Bruxner Park (Coffs Harbour)	+	?	+	30 17	153 08	Rainforest	Cylinder
Yamba	— ³	—	—	29 27	153 22	Rural township	—
Evans Head	+	+	+	29 07	153 26	Home paddock on farm	Dome
Woodburn	—	—	—	29 05	153 26	Rural township	Dome
Ballina	+	+	+	28 52	153 34	Rural township	Dome
Binna Burra, Lamington NP	+	-	+	28 42	153 30	Rainforest	Cylinder
Byron Bay	—	—	—	28 39	153 37	Rural township	—
Mt Warning (Mt Warning NP)	+	-	+	28 24	153 16	Rainforest	Cylinder
O'Reilly's Green Mountains (LNP)	+	-	+	28 13	153 08	Rainforest	Cylinder
Tweed Heads	—	—	—	28 08	153 33	Urban	—
Burleigh Head NP (Burleigh Heads)	+	?	+	28 06	153 27	Urban national park	Dome
Surfers Paradise	—	—	—	28 00	153 26	Urban	—
Southport	—	—	—	27 58	153 24	Urban	—
Witches Falls NP (North Tamborine)	+	-	+	27 56	153 11	National park	Cylinder
North Stradbroke Island	+	+	+	27 35	153 28	Quayside near rainforest	Dome
University of Queensland, St Lucia	+	+	+	27 30	153 00	Urban parkland	Dome

¹ Presence; ² Absence; ³ No data/Not recorded

2.3.1 Locations of *F. macrophylla* specimens studied along the transect

Travelling north from Sydney, a municipal park in Newcastle on the NSW Central Coast was the first place on the journey where psylloids were observed. The trees appeared to be in fair condition, and had signs of psylloid activity in the canopy and on fallen leaves. Foliage cover was estimated to be approximately 75-80%.

Byron Bay, on the northern NSW coast was searched, but no *F. macrophylla* specimens were found.

Evans Head, another northern NSW coastal town was visited, and a *F. macrophylla* specimen was found growing in the garden of a farmhouse about 6 km from the township, on the Evans Head-Woodburn road. Lerps were observed on the tree and adult psylloids were collected. The tree was in reasonable condition, however, with at least 80% leaf cover.

In Ballina, another coastal northern NSW town, *F. macrophylla* specimens were found, and lerps were observed on trees. Foliage cover was estimated at between 60-70%.

Surfers Paradise and other points on the Gold Coast in southern Queensland were searched for *F. macrophylla* specimens, but without success. The likely reason for the absence of the trees is the heavily urbanised nature of this area, where most street trees are various species of palms. Many domestic ornamental trees in this area are conspicuously flowering tropical exotics, as well as palms.

The University of Queensland main campus at St Lucia, Brisbane, southern Queensland contains many *F. macrophylla* trees throughout the campus. Heavy infestations of psylloids were observed on all trees, which appeared in rather bad condition. Foliage cover estimates put the best examples at about 50%, with the worst being around 20%.

The northernmost and easternmost point of travel was North Stradbroke Island, east of Brisbane in Moreton Bay. A specimen of *F. macrophylla* was found growing near the mainland ferry landing-jetty. Lerps were observed on the tree, which was in a similarly poor

condition to the trees observed at the University of Queensland at around 40-50% retained leaf cover.

Lamington National Park, southeastern Queensland, about 30 km inland from the coast at Surfers Paradise, was the next site visited. *F. macrophylla* was found growing in the subtropical rainforest, but unlike the rounded, dense canopies of specimens growing in relatively open urban spaces, canopy architecture was taller, more slender and with a tendency towards being emergent. Canopy walkways and ladders had been installed by members of the O'Reilly family, which still runs their well-known guest-house (O'Reilly's Guest House) in that part of the Park. The walkways enabled us to get into the canopy of the fig trees, but no psylloids at any stage of the life cycle, including eggs, were found. A search of fallen leaves also failed to detect any signs of the psylloid as egg masses, lerps or necrotic blotches. The *F. macrophylla* specimens were in excellent condition at this site. Likewise, no psylloids were observed in the rainforest surrounding the Groom family's 'Binna Burra Mountain Lodge' complex.

Witches Falls National Park, in the Tamborine Mountain area in southeastern Queensland is approximately 30 km further north of the northern border of Lamington National Park, and was visited next. *F. macrophylla* canopy architecture was like that of the specimens seen in Lamington National Park: tall, relatively slender, and emergent. The trees were also in good condition. As a result of the tree architecture, access to the canopy was not possible and observations were made from the ground, assisted by a pair of 7 × 35 binoculars. No psylloids at any life cycle stage were detected in the canopy, or on any of the fallen leaves that were inspected.

Rainforest at the base of Mount Warning, in Mt Warning National Park, northern NSW, approximately 60 km inland by road from the coast at Brunswick Heads was visited next. *F. macrophylla* specimens were observed, with tall, slender canopy architecture in the forest configuration of the two previously visited sites. The trees appeared to be in very good condition. No psylloids of any life cycle stage were detected either in the canopy or on fallen leaves on the ground.

F. macrophylla specimens with the tall and slender canopy architecture typical of forest-growing figs were observed growing beside a track in Bruxner National Park near Coffs Harbour, northern NSW. One of the trees observed had started life as a strangler (Chapter 1, Figure 1.1). Later study of photographs taken using a 35 mm single lens reflex camera and a 135 mm telephoto lens suggested that the psyllids might have been present, because of small dark round marks on some of the leaves consistent with the presence of lerps or lerp-induced necrosis, but this evidence was not apparent when viewing the canopy from the ground using the binoculars. No evidence of the psyllid was found on the fallen fig leaves at this site. The *F. macrophylla* specimens here were in poorer condition than those at other forest locations, most likely as a result of the drier inland conditions obtaining in this region, than in those regions further north.

Wingham Brush Reserve is small patch of rainforest forest near the banks of the Manning River near Taree, inland from the Central Coast, NSW. This reserve was the final site visited on the survey. This site contained at least 15 specimens of *F. macrophylla* specimens. Nearby large islands situated in the middle of the Manning River also contained *F. macrophylla* specimens, but were inaccessible, and too distant for observation, even with binoculars. The canopy architecture was, again like the other forest sites investigated, tall and relatively narrow, with an emergent crown. No psyllids were observed on any leaves fallen to the ground from the Reserve trees. The Wingham Brush Reserve was and is the subject of an ongoing bush rehabilitation and regeneration program, and the *F. macrophylla* trees all appeared to be in good condition, although the trunks of some trees were still encumbered with *Myrsiphyllum asparagoides* (L.) Wild. (Asparagaceae) (bridal creeper).

Psyllids on *F. macrophylla* at Wollongong, and in nearby temperate rainforest at Mount Keira Scout Camp on the Wollongong Escarpment, were investigated in 1996. The canopy structures of the *F. macrophylla* specimens growing on open farmland and in the urban part of this locality were of the spreading domed shape typical of fig trees seen in other such areas. Psyllid lerps were observed on all trees, including a large, buttressed, relatively narrow-canopied, emergent specimen in the rainforest. The trees in the urban areas were in much worse condition than the one in the rainforest, the urban trees being 50% or more defoliated, and the remaining leaves were of a lighter, more yellowish, green than the colour of healthy *F. macrophylla* leaves. The *F. macrophylla* specimen growing in the rainforest on the

Wollongong Escarpment, however, was estimated to have had at least 80% of its foliage intact on the tree. The arborists were able to scale the trunk of the tree using their tree-climbing ropes and retrieve some fresh foliage from the upper part of the canopy. Psylloids were found to be present on the retrieved leaves, but were fewer in number than on the leaves of urban trees, and the leaves of this tree were also much darker and healthier-looking. Several *F. macrophylla* specimens with spreading domed canopies were observed growing in open stock-grazing paddocks on a farm north of Wollongong, but these trees also appeared to be healthy, and no psylloids were apparent.

The author travelled to Melbourne in January 1997 to observe the effects of the psylloid on the Moreton Bay fig trees. Mature *F. macrophylla* specimens are as ubiquitous in Melbourne's municipal parks and green spaces, including the Royal Botanic Gardens, Melbourne, as they are in Sydney. The Melbourne visit took place three months after a massive *F. macrophylla* defoliation event that occurred in Sydney in September 1996, and all Melbourne trees observed bore large populations of psylloids. The specimens growing in Albert Park, Melbourne, were in very bad condition, having 40% or less foliage remaining: the leaves that remained on the trees were yellowing and senescent. Those *F. macrophylla* specimens growing in the Fitzroy Gardens in the city centre, and in the Royal Botanic Gardens Melbourne bore heavy psylloid loads but appeared to be in much better condition, with darker foliage and an estimated canopy cover of between 70 and 85%. The inner-Melbourne trees received more irrigation and nutritive mulch applications than those of Albert Park, which latter were growing in dry and dusty conditions without a protective mulch layer.

A very large number of *F. macrophylla* specimens is also planted in Adelaide, in the municipal parks surrounding the city centre and in the Adelaide Botanic Gardens and Botanic Park. The author could not remember ever observing *M. fici* on the Adelaide population of *F. macrophylla* in the 35 years that he lived there, and on a trip there in December 2000, not a single psylloid was observed, nor was there any evidence of there ever having been a population of *M. fici* in Adelaide. Enquiries made of staff at the Adelaide Botanic Gardens also indicated that the psylloid had never arrived in Adelaide. No written reports, scientific or otherwise, of the insect's existence in Adelaide have been traced. (See Chapter 8 for a further discussion on the Adelaide population of trees).

2.3.2 Trees observed in the Sydney region other than those in the Royal Botanic Gardens and Sydney Domain

Two chlorophyll fluorescence surveys of a subsample of the *F. macrophylla* population growing in Cook & Phillip Park, Sydney City Centre, were conducted in 1998, as a consultancy for a major redevelopment project in that precinct. The trees were growing directly adjacent to deep excavations, but appeared to be in good health. At the time of the first survey the psyllid was present only in its egg stage. The condition of the trees improved further in the six month period between the first and second surveys, during which time an irrigation system had been installed, and a well-composted rich organic mulch applied, around the base of the trees. A cohort of psyllids was beginning to hatch and form lerps at the time of the second chlorophyll fluorescence survey, but appeared not to have had an observable or a measurable effect on the trees at that stage. The Cook and Phillip Park trees were much better in physical appearance than the *F. macrophylla* specimens in nearby Hyde Park, which were defoliating severely. The latter trees had not been given the same horticultural care as the Cook and Phillip Park specimens.

F. macrophylla specimens at the Homebush Bay Olympic site were observed from 1998 to 2000. Psyllids were present in large numbers on these trees, and the trees had only 30 to 40% foliage cover remaining at the height of the psyllid population. The trees began to recover and by the time that the Olympic Games began in September 2000, the appearance of the trees was very good. The recovery of the trees may have been aided in part by the application of pesticidal oil sprays, which were used in an attempt to control the psyllid at egg hatch, but retain the population of natural enemies intact.

Silverwater Gaol, Silverwater NSW, contains two *F. macrophylla* specimens transplanted from another part of same site. These trees were studied and reported on as a consultancy in 1998. One tree was in fair condition, and had about 50% leaf cover remaining, which remnant was a yellow-green in colour. The psyllid population on this tree was moderate at the time of observation. The other tree was in an extremely poor condition, and was growing over a reclaimed rubbish dump. It had no more than 5% of its foliage remaining. The psyllid population on the remaining leaves was very low. The prognosis for this tree was that it was likely to die: this was the only tree studied that appeared likely to do so in the short or medium term.

Three *F. macrophylla* specimens were studied, again as a consultancy, at the M5 Freeway-Eastern Distributor link project at Bexley North, Sydney, in 1999. The trees had been transplanted approximately 300 m to 500 m from their original respective positions in the proposed road bed site before the start of roadbed construction to avoid the trees' being destroyed. All trees were supporting heavy psyllid infestations: two trees had about 70% foliage remaining; the third tree had approximately 20% of its foliage remaining.

2.3.3 Other records and observations

Hobart, Tasmania: no occurrence of *M. fici* were observed on three Moreton Bay Figs growing in Hobart and trees were in excellent health.

Auckland, New Zealand: no occurrence of *M. fici* were observed in approximately 30 trees inspected in inner areas of Auckland. All trees were in extremely good condition and excellent health.

Lord Howe Island: herbarium specimens held at the National Herbarium of New South Wales collected from Lord Howe Island contain lerps of *M. fici* indicating that the insect is present on natural stands of *F. macrophylla* subsp. *columnaris* on Lord Howe Island.

2.4 Discussion

All *F. macrophylla* specimens growing in urban areas within the tree's endemic range on the eastern Australian seaboard that were encountered during the main 'transect' survey, were found to have high densities of psyllids (at least two active lerps per leaf on all visible leaves) present on them. *F. macrophylla* trees growing in or close to areas of human habitation in rural areas, including farmsteads and townships, were also found to be carrying high densities of *M. fici*. In distinct contrast, however, the *F. macrophylla* specimens encountered in rainforest locations, even those patches that had been disturbed or disrupted by human activity, revealed almost no sign of *M. fici* either on their leaves, or on fig leaves that had fallen to the ground. The only tree in this type of environment on which it could positively be established that did have an *M. fici* population in residence was the specimen growing in the patch of rainforest at the base of the Wollongong Escarpment at Mount Keira. There were indications that psyllids might be living on trees in Bruxner National Park in Northern New South Wales, based on the study of photographic evidence, but the evidence in the latter case was not conclusive.

2.5 Conclusions

An inference can be made that most urban conditions on the eastern Australian seaboard and in Melbourne in which *F. macrophylla* was observed to be growing do not appear to present an optimal environment for the reasonable or sustained health of *F. macrophylla*, although they appear to provide a better environment for the proliferation of *M. fici* than does the natural habitat such as the rainforests discussed above. This suggests in turn that the problems associated with *F. macrophylla* growing in urban areas are largely human-induced. The solution to the problem of *F. macrophylla* health may therefore be as simple as allocating more resources to the care of the trees growing in urban areas. This is discussed in more depth in Chapter 9 - Conclusions.

In the next three chapters, I describe preliminary experiments that determined the methodologies used in Chapter 6, the major study of the psylloid's phenology. These preliminary studies were:

- attempts to rear the psylloids in laboratory conditions (Chapter 3);
- establishment of the total fauna of the fig (Chapter 4);
- a study of the distributions of psylloids on a typical tree (Chapter 5).

Chapter 3. Attempts at rearing *Mycopsylla fici* under various conditions

3.1 Introduction

There are two basic approaches to the study of life processes of biological entities, including arthropods, which are complementary to each other for a broad understanding of the organism in question. These approaches are: the study of an organism in its natural biological context (i.e., in the field); and study of the organism under artificial conditions (e.g., in a laboratory, a plant nursery, etc.). The division between the two approaches becomes necessary when, for example, an environmental variable (e.g., temperature or relative humidity) needs to be held at a specific and/or constant level to determine a particular effect. Neither approach should be used to the exclusion of the other if a true understanding of an organism is to be reached. Examples of field studies from the point of view of research on *M. fici* would be, for example, the observation of parasitoids and predators of the psyllid on the tree; reactions of the psyllids' lerps to fluctuations in relative humidity around the leaf boundary layer; or the length of egg laying periods and length of adult life span. Examples of laboratory experiments in relation to the psyllid could be: the study of the exact length of separate psyllid instars at constant temperatures, or the elucidation of the psyllid's lower developmental threshold temperature and the construction of a robust degree days model that could be applied reliably in a field situation to predict psyllid outbreaks. Laboratory conditions can be manipulated to provide stable, standardised environments in which subjects can be reared under various and particular conditions as required, and in which efficiently controlled and repeatable observations and experiments can be conducted. The aim of the series of trials which are the topic of this chapter was the successful rearing of *M. fici* under laboratory or at least controllable conditions, in order to develop the robust degree days model referred to above, and other detailed studies of the psyllid's juvenile life history such as instar durations.

Laboratory study is also useful in that subjects can be studied at call, and the gross effects of weather and varying seasonal conditions can be minimised or at least reduced. The rate of study is thus generally increased over the rate at which study would occur in the field. In many instances, it is not practical or even possible to take equipment used in detailed arthropod study, such as large microscopes, behavioural study arenas, etc., into the field. This is especially the case in high-profile public locations where equipment cannot be set out in, for example, a field tent, without the equipment being complained about, interfered with or stolen.

One of the initial aims of the project was to investigate the possibility of mass rearing the encyrtid parasitoid of the psyllid, *Psyllaephagus* sp. for release as an inundative control agent. Mass rearing for pest control programs is also usually conducted under standardised laboratory conditions (Blossey *et al.*, 2000; Carpenter and Greany, 1998; Halperin, 1990; Karim *et al.*, 1998; Laing and Eden, 1990; Lym, 1992; Reis *et al.*, 2000), and is the prerequisite for pest control programs using inundative sterile release techniques for the control of such insects such as *Bactrocera tryoni* (Diptera: Tephritidae) (Queensland fruit fly) (Meats and Fay, 2000; Meats, 1983) and the screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae) (Knipling 1985; Knipling 1955; Lindquist, 1955). Specialised laboratories and conditions are used in these instances to produce large populations of a particular species, such as a parasitic wasp, or *gamma*-irradiated sterile male flies. The use of inundative release of *Psyllaephagus* sp. to control *M. fici* population sizes is particularly attractive, as 1., only the psyllid itself is targeted; and 2., no pesticidal chemical that would harm other organisms would be used. A species of encyrtid wasp, *Psyllaephagus rotundiformis* (Howard) (Hymenoptera: Encyrtidae), has been used in a release program to control the leucaena psyllid, *Heteropsylla cubana* (Nakahara and Funasaki, 1987) (Sternorrhyncha: Psyllidae). The ability to breed *M. fici* and its parasitoid *Psyllaephagus* sp. in the laboratory would therefore be essential to breed the parasitoid in the quantities required for such a control program.

3.2 Materials and Methods

3.2.1 Rearing *M. fici* on potted *F. macrophylla* saplings directly on its host

Ten potted, two-year-old, 300 mm high *F. macrophylla* plants with approximately 10 leaves on each plant, one to a 250 mm pot (Figure 3.1), were placed on branches of the *F. macrophylla* specimen growing at the Morshead Fountain Gate in the Royal Botanic Gardens, Sydney (project code *MFG*), during an adult psyllid emergence period, in an attempt to collect eggs for laboratory breeding and study. Plastic coated plant-tying wire was used to secure the plants in position. The plants were left in the tree for ten days, then removed and checked for eggs.

3.2.2 Rearing *M. fici* on potted *F. macrophylla* saplings in a glasshouse

Eight two year old *F. macrophylla* plants (as above), one to a 250 mm pot, were kept in a 2000 mm × 800 mm × 1000 mm cage covered with fine gauge gauze ('Royal' voile). The cage was installed on a 2400 mm × 1000 mm × 6 mm medium density fibreboard (MDF) sheet, in turn placed on a wire frame bench in the Plant Pathology glasshouse in the RBG plant nursery.

Supplementary lighting was not considered necessary as sufficient sunlight was available. Any gaps between the base board and the bottom wooden cage frame were sealed with 10 mm × 20 mm cross-section fine grain foam rubber door sealing strip, to prevent psylloids from escaping through any crevices or other irregularities. Caged plants were watered regularly and supplied with growth nutrients in the form of slow release fertiliser granules (general purpose ‘Osmocote®’). 50 male and 50 female *M. fici* adults collected by aspiration from the canopy of *MFG* were released into the cage once the plants were in position. Many psylloid matings were observed taking place on the plants in the cage, and it was therefore assumed that the potential number of fertilised psylloid eggs present in the cage population would not be a limiting factor in this experiment. The plants were checked daily for eggs, until the psylloid population in the cage had died out. All plants were left in the cage until the inhabitants of the two lerps produced, on only the one plant, emerged as adults.

3.2.3 Rearing *M. fici* in leaf cages directly on its host

In an ‘ideal’ situation for the fig psylloid, the absence of predators and parasitoids could well be considered to be one of the most desirable elements, along with constant mild temperatures (20 °C to 25 °C) and a continual supply of non-abscissant leaves. It has not been previously known from a quantitative point of view what effect predators and parasitoids have on fig psylloid population sizes, other than a vague idea of their existence. To date, no evaluation has been carried out in this respect. To redress this situation, a predator exclusion experiment was designed to determine the percentage of a known number of hatchlings, assumed unparasitised, reaching adulthood. Given the tendency of parasitoid populations to both appear and peak later than prey populations, an assumption was made that an apparent absence of adult parasitoid females would be likely to indicate that the group of newly hatched psylloids were parasitoid-free. During my own observations, the parasitoid was only observed to sting into closed lerps no younger than about twelve days old, and not to attack either free-wandering or *proto*-lerp-forming psylloid juveniles, so it may be that this assumption will hold true.

In order to investigate the number of psylloid larvae reaching adulthood from the egg hatch stage in a given lerp without natural enemies depleting their numbers, a set of leaf exclusion cages was placed over newly emerged groups (or ‘*proto*-lerps’) of psylloid larvae on the undersides of fig leaves on the *F. macrophylla* specimen growing at the Morshead Fountain gate at the Royal Botanic Gardens Sydney. The design of the cages was based on a type

described by Adams and van Emden (1972) and heavily modified by the author to suit the particular circumstances. The experiment was run twice, the first time in autumn 1998, and the second time in spring 1998.

The cages were made from 25 and 35 mm lengths of 25 mm and 50 mm outside diameter clear acrylic tube of three mm wall thickness. A knitted nylon fine-mesh voile was glued over one end making the cage top, to prevent ingress of natural enemies and egress of psylloids (Figure 3.2). Two different configurations of both sizes of cage were used. One type had two cutouts approximately eight mm high and 20 mm long, one on each 'side' of the cage ring, to allow a cross flow of air. The slots were covered with gauze. The other type had only the top cover, with no gauze-covered slots. The gauze was glued to the upper cage rim using organic-solvent contact cement. The bottom of the cage was sealed onto the leaf by a layer of foam rubber, which was attached to the cage by contact cement, and to the leaf by water-soluble acrylic contact cement. The cages were put in place over the proto-lerps, with 'cellotape' (clear 'sticky tape') temporarily wrapped around both leaves and cages to ensure that the cages firmly sealed onto the hairy leaf undersurfaces while the contact adhesive set. The sticky tape was then carefully removed 24 hours later. In a couple of instances in both experimental runs it was necessary to seal gaps between cage and leaf because the cage rim overhung the leaf margin by a millimetre or so. These gaps appeared because of insufficient room on leaf tips, or insufficient room between midrib and margin on small leaves when using 50 mm cages. Placing cages over midribs was avoided where possible in the first run of the caging experiments because the foam rubber seal did not appear to conform well over the midrib as the foam rubber cushion was too thin. In the second run of cages, a thicker layer of foam rubber was used, which gave a good seal over midribs, thus allowing the cages to be safely applied over midribs when necessary. Gaps between cage rim and leaf margin were sealed in the cages in the first run using a high-tack green vinyl electrical insulating tape patches: most patches remained in place for the duration of the experiment.

Acrylic contact cement was used to attach the cages to the leaves because it is insoluble in water when dry, since a preliminary test-run indicated that hydrocarbon solvent-based contact adhesive had a more immediate phytotoxic effect on the leaves than the water solvent-based contact adhesive. At the end of the first run, leaf tissue directly under the adhesive showed necrotic symptoms, but the tissue within the cages appeared normal as far as the naked eye or

stereo dissecting light microscope could determine, and lerps appeared to develop normally on the leaf tissue. The presence of parasitoids in the fig canopy was checked in part at the beginning of the experiment by direct observation and by the use of yellow sticky traps (Seabright Sticky Trap Laboratories Emeryville, CA, USA) placed in the tree near the leaf cages. Parasitoid presence was also checked at the end of the experiment by searching for parasitoids or parasitoid exit holes in lerps when the cages were collected for evaluation.

Cages were placed one to a leaf, except in one instance in the second run, where two cages were placed on separate lerps on the same leaf. This was done because of the unusually low numbers of hatching psyllids at the time that the second run of cages was deployed. Any cages that were found to have had their integrity compromised were analysed separately from the intact cages.

Each leaf was tagged with a different coloured electrical tape (Figure 3.2, top right-hand corner), according to the type of cage, for rapid location and as a visual distinguishing marker to reinforce the difference between the types. For the first run, ten cages per type were used, with the exception of the small gauze type, which was represented by 20 cages. For the second run, cage types were represented by five cages each, because of the loss of cages during the first run. Tags were numbered from 110 for the first run, and from 15 for the second in arbitrary (random) order of proto-lerp selection. In the first and second runs, the cages were placed only on the northeastern quadrant of the tree, within reach from the ground.

Several days after the second run was deployed, a third set of ten small gauze cages was placed on the western side of the tree, to spread the cages out.

The leaf cages were checked periodically to ensure that their integrity was still intact, i.e., that no holes sufficiently large to allow ingress of predators had appeared, that the cage-to-leaf surface seal was not broken, and that any electrical tape margin-seals were still in place.

All cages remaining on leaves, intact or otherwise, were collected all together by removing their leaves, when it was certain that no more adult psyllids (or adult parasitoids) would emerge from under the lerps. Cages were then carefully removed from the leaves and the

contents investigated. Interestingly, on leaves with otherwise perfectly sealed cages, psyllid final moult skins could often be found outside the cage, the pre-adult final instars having pushed their way through the foam and glue barrier to reach the outside. Mostly, though, the adult psyllids emerged into the cages, where they remained until they died. The sex ratio of each cage was also counted for comparison between cages and with other data sets (stratification and fogging).

The rationale behind employing the different cage configurations was to detect any possible cause of mortality in the psyllids due to artificially elevated temperatures within the cages. This was based on an assumption that the smaller cages would tend to be hotter, having more stagnant air within them than the larger ones, and that the slotted cages would similarly be cooler than the unslotted cages as they had better ventilation characteristics.

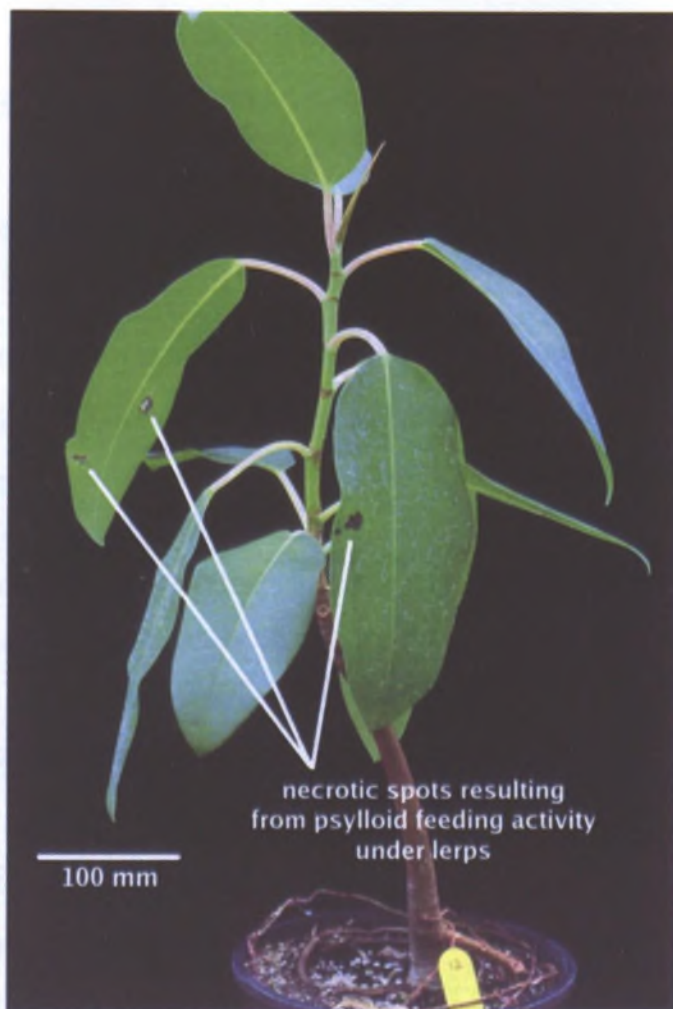


Figure 3.1: Potted *F. macrophylla* used in *M. flci*-rearing experiments: note the three necrotic spots caused by the three sole lerps of the caged psyllid egg-laying experiment

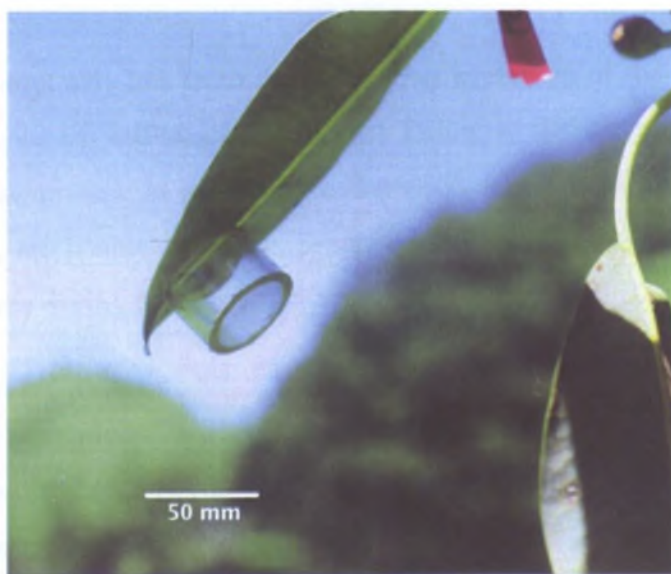


Figure 3.2: Clear 50 mm acrylic psyllid leaf cage, no side vents. Note the electrical tape leaf tag in upper right-hand corner

3.3 Results

3.3.1 Rearing *M. fici* on potted *F. macrophylla* saplings directly on its host

No eggs were found to have been laid on any of the leaves on any of the plants. Further attempts to collect eggs on saplings placed within the tree's canopy were therefore abandoned.

3.3.2 Rearing *M. fici* on potted *F. macrophylla* saplings in a glasshouse

From the 50 females placed in the caged, only two egg masses were laid, on different leaves of the same plant. The eggs of these two clutches hatched, however, and three small-sized lerps established. All three lerps went to full term, one lerp producing ten adults (assessed by counting final-moult exuviae – see also Chapter 6), the other two producing five and six adults each. Three necrotic patches can be seen on two of the leaves on the plant in question, in Figure 3.1. (Figure 6.28 in Chapter 6 presents a photograph of the largest of the three lerps, but in a different context than that being discussed here.)

3.3.3 Rearing *M. fici* in leaf cages directly on its host

Most cages that had parted from the leaf were found to have tent-spinning spiders in residence and a depressed number of adult psylloids compared with cages with intact seals. The lower psylloid numbers may also have been due to emerging adult psylloids eluding the spiders and escaping from the confines of the cages. Dead adults attacked by the spiders could also have fallen out of the cages. Dead adults were found trapped in the web tents inside the cages.

20% of cages were completely lost from the tree, either by failure of the cage seal, by being removed by passersby, or by leaf abscission. Cages falling to the ground would have either been destroyed by lawnmowers, or picked up and carried off by inquisitive humans or birds (currawongs, magpies, etc.): only one cage, separated from its leaf, was recovered from the lawn under the study tree during the first run.

Newly hatched instars were plentiful when the first run was started in February 1998, but were much more sparse when the second run was started in mid-October 1998. The psylloids from the second cohort hatched noticeably later than the corresponding cohort in 1997, on which former occasion there was also a minor outbreak of the insects. These phenomena have been noticed at other sites in the Sydney region (e.g., the Homebush Bay Olympic site).

The second run of the leaf cage experiment also failed, as the seals on almost all cages did not hold, either as a result of growth of the leaf lamina, or the failure of the glue to adhere sufficiently to the leaf surface, or pressure from the expanding lerp against the seal breaking it: or perhaps a combination of two or more of these possibilities. The breaking of the seal by lerp expansion was actually observed to have taken place in over 50% of cases. The net result was that the counting of final moult skins was made meaningless.

3.4 Discussion

Failure to collect eggs from saplings placed in a tree canopy, or to produce more than two egg masses or more than three lerps from 50 presumably mated females in the glasshouse cage, when hundreds of egg masses and similar numbers of lerps were required for laboratory experiments, meant that studying the psyllid life cycle and other aspects of its life history, including its parasitoid, would not be possible under laboratory conditions. It was not a practical proposition to bring excised branches into the laboratory itself, because of lack of space for them, and the lack of means to keep them alive and healthy for the period of time that would have been required. Unless a successful repeatable, method of breeding the psyllid in laboratory conditions can be devised, or an artificial diet developed, the mass rearing of parasitoids in the laboratory will not be possible.

The failure of the leaf cages also meant that this particular technique was not available for use in study of psyllid life history, without substantial and therefore impracticable modification, and this method was also abandoned.

A survey of the non-sessile arthropod fauna within the *F. macrophylla* canopy, as collected by insecticidal fogging, is examined in the next chapter.

Chapter 4. Arthropod fauna in the *Ficus macrophylla* canopy, determined by insecticidal fogging

4.1 Introduction

The arboreal arthropod population structure and species components of a species of tree are generally unknown (Erwin, 1983; Janzen, 1987; Kuno, 1991), usually containing many more species than are usually observable from the ground, or even from directly within the canopy, especially if the tree is large (Basset, 1992; Basset and Kitching, 1991). Amongst the multiplicity within the arboreal arthropod community (Basset and Arthington, 1992; Winchester and Ring, 1999), it is possible that there might exist arthropods which have a significant but unobserved impact on a known member. In the case of *Mycopsylla fici* on *Ficus macrophylla*, the possible existence of such significant but unknown arthropods needs to be investigated if the psyllid-fig system is to be optimally understood. One would expect to find a variety of guilds (Mayr, 1963; Root, 1973) that live in the canopy: plant feeders (phytophages) of various types, predators and parasitoids that feed on the plant feeders, detritus feeders (detritivores) and fungal feeders (fungivores). One would also, therefore, expect some sort of trophic level structure or 'trophic pyramid' (van Emden, 1995) if these guilds are present.

Another important issue is that if the psyllid is susceptible to being controlled effectively by whatever means, the resultant impact on the total microecosystem of *F. macrophylla* needs to be evaluated not only from the point of view of the maintenance of the original balance of that community, but also that such a change in guild structure in the canopy of the tree might produce a deleterious effect on the tree as a whole (Carson, 1962). Adverse effects might include, for example, the induction of secondary pests by loss of significant predators from the system (Eveleens *et al.*, 1973; Messenger *et al.*, 1976; Luck *et al.* 1977). One of the most notorious examples is the cotton pesticide treadmill where the abuse of pesticides led to a pesticide application feedback loop and a consequent cascade of effects throughout an entire ecosystem that began in 1950 and culminated in a severe ecosystem collapse in the late 1960s and early 1970s in four countries in Central America, and which had far-reaching consequences (van den Bosch, 1971; van den Bosch, 1972). Serious pesticide problems have subsequently irrupted, for example in West Africa (Anonymous, 1999a; Anonymous, 1999b; Eveleens, 1983) and in India (Kishor, 1992), and are not likely to be the last (Jayaraman,

2001; Kalra *et al.*, 1999; Kalra *et al.*, 1994; Press Trust of India, 1997). Primary pest resurgence and pesticide resistance are also components of the pesticide treadmill feedback loop and related issues (van den Bosch, 1971; van den Bosch, 1972; van den Bosch, 1980).

Two basic assumptions need to be made at this point about the nature of such hypothetical arthropods. The first assumption is that the putative psylloid-affecting arthropods are free living, and can therefore be readily retrieved using any of the standard methods of arboreal arthropod surveying. The term “free living” is used here to include all those species of arthropods which are not residing permanently within the various woody parts of the tree (stems, branches, trunks etc.) or semi-permanently attached to the bark or leaves (excluding the psylloid itself). This is because such sessile species will not be affected by any of the generally used methods of retrieval such as branch shaking or insecticidal fogging (Erwin, 1983; Morse *et al.*, 1988; Paarman and Stork, 1987; Southwood *et al.*, 1982a; Southwood *et al.*, 1982b; Stork 1987a & 1987b.; Stork, 1988; Richardson *et al.*, 1999). The second assumption, embedded within the first, is that none of the stem-dwelling arthropods actually affects the psylloid directly (as opposed to an indirect influence such as borers, which may destroy a branch or tree on which psylloids are living), and missing these in the survey will not adversely affect our knowledge of the psylloid.

For trees with inaccessibly large canopies such as *F. macrophylla*, the main technique for surveying arthropods used currently is insecticidal fogging. This technique has been used with great success by Erwin in Costa Rica and South America (1983), and by Stork in Borneo (Stork, 1987a; Stork 1987b) for large-scale arthropod fauna collection (see also Lowman *et al.*, 1996, and Lowman and Wittman, 1996). Collection of faunal rain was made by these researchers using very large purpose-made funnel-trays (Erwin, 1983) and large sheets (Stork, 1987a and 1987b). The use of other methods such as the a canopy crane (van Pelt and North, 1999; Ring and Winchester, 1996) and permanent observation structures within the canopy itself (Inoue *et al.*, 1995) were outside the scope of the project. Insecticidal fogging techniques were therefore employed to elucidate the non-sessile arthropod fauna in the *F. macrophylla* canopy.

The aims of the survey presented in this chapter were to determine: 1., the overall population structure of free-living arthropods on a large, mature *F. macrophylla*; 2., the guild structure of this general population of arthropods; and 3., what guild or guild member, if any, was most likely to be competing with or eating the psyllid.

4.2 Materials and methods

4.2.1 Fogging survey

The collecting expeditions of both Erwin and Stork referred to in the Introduction of this chapter were conducted in very dense tropical rainforest, where it was difficult to set up suitable collecting devices. Collecting from *F. macrophylla* in an urban parkland is quite different, as the study tree project code *L44* (RBG records accession no. 10112, RBG grid reference no. RBG *16s10*, growing on Lawn 44 and known popularly as the “Children’s Fig”) is far more isolated from other trees than it would be in a rain forest situation. The site posed its own potential problems, however, since the tree is located on Lawn 44, one of the more recreationally popular areas of the Gardens, and also because it is adjacent to the major pedestrian footpath along the edge of the Sea Wall. This footpath is used by joggers from early morning to early evening. This meant excluding the public from a wide area around the tree, both to prevent fog drift affecting early-morning joggers and other passers-by, and interference or disturbance by the public in the fogging process. As the fogging needed to be carried out at a time when air movement was at a minimum, early morning was chosen and the problems of public interference and joggers were thereby minimised.

The collecting apparatus consisted of a radial array of orange polyethylene sheeting (concrete underlay membrane), which was staked to the ground around the trunk of the tree to collect the faunal rain. 120 one square metre plastic sheets were arranged in 30 sets of two metre × two metre squares. These groups were arranged in three concentric rings of ten evenly spaced groups each, around the trunk. The rings were placed just inside the 40 metre diameter drip-line, around the five metre diameter tree trunk, and midway between the inner and outer rings. The collecting array was designed following verbal discussions with Dr Nigel Stork (James Cook University, Cairns and Townsville) and Professor Barry Richardson (University of Western Sydney). Each separate one square metre sheet was assigned a distinguishing code for sorting and mapping purposes. Depending on the density of the arthropod, landing position would only approximate its canopy origin, because of buoyancy and drift on air currents. The

denser the arthropod, the more likely its trajectory would approach the vertical. The survey sample was generated by blowing a knockdown pyrethroid fog into the canopy, and collected on the ground. The fogging process was started at 7:15 AM on 26 March 1996, using 1% Mavrik (active ingredient 400 g/l τ -fluvalinate) in a 1:1 polyethylene (PEG):water carrier mixture from a DYNA-FOG "Golden Eagle" pulse-jet fogger. The fogger was kindly lent to us for use in this experiment by AirScape P/L. The restriction with respect to the length of the loan, however, precluded the fogger's use when the adult psyllid emergence was under way, and also prevented a pre-experiment trial on the same or another tree. The latter was also prevented by the availability of the arborists and their equipment. The insecticidal fog was applied to the canopy of the tree from the full extension of the RBG arborists' truck-mounted hydraulic lift. The fogging was begun on the western (windward) side of the tree and carried out for approximately fifteen minutes, intermittently moving the lift platform to ensure even coverage on that side of the tree. A ten minute break in the middle of the fogging period was necessary in order to move the truck around to the northeastern side of the tree. The move was made to ensure adequate penetration of the fog throughout the entire volume of the 40 m diameter canopy. The resultant faunal rain was allowed to fall for approximately three hours, after which the sheets were carefully collected. The weather was overcast, with no appreciable air movement other than that generated by the fogger itself. Some rain had fallen during the night, and had ceased about an hour before fogging commenced. Rainwater pools were sponged from the collecting sheets before fogging commenced. Water droplets remaining on the collection sheets after sponging meant that care was needed when retrieving the sheets, so that no specimens were lost from accidental runoff. Specimens were removed from the sheets and stored: each sheet was formed into a funnel by clipping an edge onto a custom-made frame and weighting the bottom. Specimens were then carefully washed from each sheet using reverse osmosis-purified water in a five-litre garden sprayer set at a wide cone, then stored in 80% ethanol (EtOH) diluted from (nominal) 95% EtOH, after straining through a stainless steel sieve of 200 μ m mesh aperture. Each sample was stored separately in a plastic 75 ml screwtop sterile specimen container labelled with the respective sheet's code. Individual samples were then cleaned of debris (dust, grass clippings, bird-droppings, etc.) and transferred to clean EtOH. Following cleaning, the sample was sorted to arthropod order. Numbers of individuals in each order subsample was counted, the subsample then being stored separately in a small vial with 80% EtOH. Order and sample ID labels were added to each vial, the vial then being plugged with absorbent cotton wool and immersed in 80% EtOH in 400 ml screwtop glass jars with internal plastic seals, labelled respectively.

4.2.2 Collecting sheet colour effect comparison experiment

After *post hoc* deliberation on the apparent success of the collecting procedure, it was considered that a bias, induced by the orange colour of the plastic collection sheet, might have occurred during the sampling procedure. Light with wavelengths close to those reflected by the polyethylene sheet (ca 610 nm), e.g., yellow (ca 580 nm), is attractive to many insects, including psyllids and psyllids (Mensah and Madden, 1992). It was considered possible, therefore, that part of the sample may have comprised arthropods that were not associated with the tree, were unaffected by the pesticide, but that might have been attracted to the sheets by the sheets' colour and caught there by static electricity, rather than falling passively out of the air after having been poisoned by the insecticidal fog (see Section 4.4.1.2 below). A comparison, sample was therefore conducted in early April of the following year (1997), using a randomly selected subset of ten four-m² group positions from the original set of 30. Five groups were of the original orange polyethylene sheet, and five groups were of a similar plastic sheet but in black: colour was randomised with respect to four-m² group position.

The sheets were left under the tree for the same length of time as the initial fogging experiment, then collected and treated as in the initial part of the experiment. Sheet colour was analysed for faunal differences, and also against a randomly chosen subset of the original survey, using the finest gradation realistically available (family).

The following arthropod groups were sent to various taxonomists for identification:

Diptera: Dan Bickel, Australian Museum, Sydney;

Coccinellidae: Dinah Hales, Macquarie University, Sydney;

Hymenoptera: Andy Austin, University of Adelaide

Mites: Danuta Knihinicki, NSW Agriculture, Orange;

Psocoptera: Courtenay Smithers, Australian Museum, Sydney;

Spiders: Graham Milledge, Australian Museum, Sydney;

Thysanoptera: Peter Gillespie, NSW Agriculture, Orange;

4.3 Results

4.3.1. Main survey results

A total of 4961 specimens was recorded from a total catchment area of 480 m². The percentage presence of each insect order or other arthropod group found in the main sample is presented in Table 4.1 on the following page. The bulk of arthropods in the total sample comprises psocopterans, thysanopterans and hymenopterans.

Arthropods were found in the following orders (number indicates minimum number of species):

- Hymenoptera¹: 14 species: 3 fig fruit wasps, rest braconid, chalcidoid and ichneumonid parasitoids of arthropods
- Hemiptera: Heteroptera²: 3 species - possibly predatory
- Hemiptera: Sternorrhyncha: 2 species (Homotomidae)
- Coleoptera¹: 4 species (1 Bruchidae, 3 Coccinellidae)
- Neuroptera¹: 2 species
- Thysanoptera²: 3 species
- Psocoptera: at least one species
- Phthiraptera: at least 1 species
- Collembola: at least 2 species
- Spiders²: 5 spp. Araneae and Salticidae
- Mites: 3 species
- Ticks: at least one species
- Crustacea: Isopoda: at least one species

¹ Contains known parasitoid(s) or predator(s) of *M. fici*

² Likely to include predators of *M. fici*.

Table 4.1: Numbers of specimens in arthropod orders appearing in 1996 fogging sample.

Order	Σ specimens in Order	Percentage of total sample	Sheets with at least 1 specimen in Order	Sheets with no specimens in Order
Acarina	217	4.37%	60	60
Araneae	21	0.42%	14	106
Coleoptera	278	5.60%	73	47
Collembola	315	6.35%	78	42
Diptera	265	5.34%	77	43
Hemiptera	454	9.15%	90	30
Hymenoptera	1047	21.10%	94	26
Lepidoptera	16	0.32%	15	105
Neuroptera	53	1.07%	33	87
Phthiraptera	2	0.04%	2	118
Psocoptera	1020	20.56%	85	35
Salticidae	45	0.91%	28	92
Thysanoptera	1210	24.39%	93	27
Crustacea	12	0.24%	7	113
Other hexapods	6	0.12%	5	115
Grand total	4961	100.00%	120	120

A species of thrips new to science was found by Peter Gillespie at the NSW Department of Agriculture at Orange, in the thrips samples sent to him for identification. This thrips was named *Parabaliotrips newmani* by Laurence Mound at the Australian National Insect Collection, CSIRO Entomology, Canberra (Gillespie *et al.*, 2002). *P. newmani* has subsequently been found on specimens of *F. macrophylla* subsp. *columnaris* growing on Lord Howe Island (Gillespie *et al.*, 2002) 500 km off the coast of New South Wales.

None of the psocopterans was identified as a result of the poor condition of both juveniles and adults specimens within the sample.

Table 4.2: Comparison of sample numbers per order between fogging and colour comparison experiments (data from 1996 and 1997)

Order	Number in sample		
	Fogging	Orange control	Black control
Acarina	217	11	32
Araneae	21	0	1
Coleoptera	278	4	10
Collembola	315	127	1364
Diptera	265	12	8
Hemiptera	454	7	7
Hymenoptera	1047	50	46
Lepidoptera	16	2	0
Neuroptera	53	0	2
Phthiraptera	2	1	2
Psocoptera	1020	24	21
Salticidae	45	1	0
Thysanoptera	1210	5	14
Crustacea	12	1	5
Other hexapods	6	7	0
Grand total	4961	252	1512
		1764	

**Table 4.3: Comparison of the collection methods used in the *F. macrophylla* fogging survey (April 1996) and colour comparison survey (April 1997)
(2-sided *t*-tests, $\alpha = 0.05$ significance level)**

Difference between Orange and Black Controls										
Orange mean	Black mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.
15.3125	94.5	-0.930692	30	0.359442	-0.930692	15.27557	0.366489	16	16	32.47095
338.7857										
Difference between Orange and Black Controls, Collembolans removed from both sets										
Orange mean	Black mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.
7.866667	9.866667	-0.4069	28	0.687176	-0.4069	27.99683	0.687176	15	15	13.38905
13.53232										
Difference between April 1995 and 1996 Control totals										
Main/3 mean	Control mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.
103.1875	109.8125	-0.067239	30	0.946838	-0.067239	19.10915	0.94709	16	16	137.959
369.1827										
Difference between April 1995 and 1996 Control totals Collembolans removed from both sets,										
Main/3 mean	Control mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.
103.0667	17.73333	2.276499	28	0.03066	2.276499	14.93862	0.037971	15	15	142.8003
26.16013										

Table 4.4: Comparison of the collection methods used in the *F. macrophylla* fogging survey (April 1996) and colour comparison survey (April 1997): April 1996 values adjusted for differing numbers of collecting sheets used in two experiments (2-sided *t*-tests, $\alpha = 0.05$ significance level)

Difference between Orange and Black Controls											
Orange mean	Black mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.	Std dev.
17.5	108	-0.931884	26	0.35997	-0.931884	13.2335	0.368087	14	14	34.28332	361.7504

Difference between Orange and Black Controls, Collembolans removed from both sets											
Orange mean	Black mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.	Std dev.
9.076923	11.23077	-0.390442	24	0.699654	-0.390442	23.99981	0.699654	13	13	14.04434	14.084

Difference between April 1995 Fogging and April 1996 Control totals											
Main/3 mean	Control mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.	Std dev.
117.9286	125.5	-0.067676	26	0.946561	-0.067676	16.31107	0.946866	14	14	141.7346	393.8836

Difference between April 1995 Fogging and April 1996 Control totals, Collembolans removed from both sets											
Main/3 mean	Control mean	<i>t</i> -value	df	p	<i>t</i> sep. var. est.	df	p 2-sided	<i>n</i>	<i>n</i>	Std dev.	Std dev.
118.9231	20.46154	2.367474	24	0.026314	2.367474	12.81342	0.034352	13	13	147.4712	27.16498

4.3.2 Main and colour comparison surveys

4.3.2.1 Colour comparison

Table 4.2 above compares the numbers of individuals per order between the main fogging sample and the colour comparison sample. Percentages of various arthropod orders found in the two surveys are compared in Table 4.3 above. A total of 1764 specimens was recorded from 120 m² in the colour comparison experiment. The orange and black comparison experiment arrays were compared using two sided *t*-tests, to determine if there was a preference for colour, the results of which are presented in Table 4.4 above.

4.3.2.2 Main and colour comparison surveys

The results of the initial fogging survey were compared with those of the colour comparison experiment to determine any differences in number of arthropods collected, between the two experiments. The fogging survey order totals were divided by three to enable a direct comparison with respect to area to be made with the 40-sheet sample used in the comparison experiment (Table 4.4). The *t*-tests were repeated with collembolan totals deleted from both sets of order totals, and are presented in Table 4.4. This was done because the collembolans, being detritivores dwelling on larger branches would not have a direct effect on *M. fici*.

4.3.3 Fogging sample guild structures

The fogging sample contained five main feeding guilds ranked by number of members: Phytophages, Predators, Parasitoids, Detritivores/Scavengers and Mycovores (Table 4.5 below). All taxa were identified to at least order. The largest of these guilds was that of the Phytophages, with 11 members in four orders. This guild divides into two distinct groups: chewing (four members in two orders); and piercing-sucking (seven members in four orders). Predators comprised the second largest guild with 10 members in six orders. This guild contained the following groups: piercing-sucking (five members in three orders), chewing (three members in two orders), ambush predators (one member in a single order) and filter-feeding predators (one member in a single order). The third largest guild, the Parasitoids, contained six members in a single order (Table 4.5). The guild divided into Egg parasitoids (two members within a single order), Endoparasitoids (three members within a single order), and Hyperparasitoids (one member within a single order). Detritivores and Scavengers were composed of three members in three orders, and the smallest guild by member, the Mycovores,

contained two members in two orders. Table 4.6 below summarises the guild structure presented in Table 4.5.

Numbers of individuals in each guild and sub-guild were estimated by somewhat arbitrarily dividing the numbers in an order by the number of guilds or sub-guilds that the order contained. The exceptions were Thysanoptera and Acarina, where estimates were made based on numbers of individuals in relation to order recorded from the taxa in one-m² sub-samples studied by the respective taxonomists. These estimates were extrapolated back through the entire sample to produce the total individual guild representative estimates. An error caused by underestimation using this method, of the order of 10%, is likely.

In terms of estimated numbers of individuals in a guild in the fogging sample, the guilds ranked as follows:

1. Phytophages, including psylloids (1535);
2. Detritivores (958);
3. Parasitoids (825);
4. Mycovores (649);
5. Predators (486).

Table 4.6 also gives the estimated number of individuals in each sub-guild.

Table 4.5: Guild structures within the *F. macrophylla* canopy as determined from fogging survey samples

Guild	No. members	Order: Superfamily	Family: Subfamily	Genus/species	Known or suspected target
Phytophages – chewing	4	Hymenoptera: Chalcidoidea ¹	-	-	<i>F. macrophylla</i> ?
		Hymenoptera: Chalcidoidea	Agaonidae ¹	-	<i>F. macrophylla</i>
		Hymenoptera: Chalcidoidea	Agaonidae ¹	-	<i>F. macrophylla</i>
		Lepidoptera	Zygaenidae ⁷	-	<i>F. macrophylla</i>
Phytophages – sucking	7	Hemiptera: Sternorrhyncha	Aphididae ^{7*}	-	<i>F. macrophylla</i> ?
		Hemiptera: Heteroptera	Miridae ^{7*}	-	<i>F. macrophylla</i>
		Hemiptera: Sternorrhyncha	Psyllidae ⁷	<i>Mycopsylla fici</i>	<i>F. macrophylla</i>
		Acarina	Oribatidae ⁵	-	Unknown
		Thysanoptera: Tubulifera	Phlaeothripidae ³	<i>Gynaikothrips australis</i>	<i>F. macrophylla</i>
		Thysanoptera: Tubulifera	Phlaeothripidae ³	-	<i>F. macrophylla</i>
		Thysanoptera: Terebrantia	Thripidae: Thripinae ⁴	<i>Parabaliotrips newmani</i>	<i>F. macrophylla</i>
Phytophages – total	11				
Detritivores/Scavengers	3	Acarina	Oribatidae ⁵	-	Dead organic matter (various)
		Hymenoptera	Formicidae ¹	-	Various biota
		Psocoptera ⁷	-	-	Fungi
Mycovores	2	Coleoptera ⁶	-	-	Fungi
		Psocoptera ⁷	-	-	Fungi
Predators – sucking	5	Acarina	Phytoseiidae ⁵	-	Mainly tetranychid mites
		Acarina: Mesostigmata	Phytoseiidae ⁵	-	Insects and other arthropods
		Neuroptera ⁷	-	-	<i>Mycopsylla fici</i>
		Hemiptera: Heteroptera	Miridae ^{7*}	-	Insects and other arthropods
		Hemiptera: Heteroptera	Reduviidae: Emesinae ⁷	-	Insects and other arthropods

Guild	No. members	Order: Superfamily	Family: Subfamily	Genus/species	Known or suspected target
Predators – chewing	3	Coleoptera ⁷	Coccinellidae	-	<i>Mycopsylla fici</i>
		Diptera: Syrphoidea	Syrphidae ³	-	Insects and other arthropods
		Hymenoptera	Formicidae ¹	-	Various biota
Predators – ambush	1	Arachnida	Salticidae	-	Insects and other arthropods
Predators – web-builders	1	Arachnida	Aranae	-	Insects and other arthropods
<i>Predators – total</i>	10				
Egg parasitoids	2	Hymenoptera: Chalcidoidea	Mymaridae ⁷		Insect egg parasitoid
		Hymenoptera: Chalcidoidea	Scelionidae ¹	<i>Telenomus</i> sp.	Lepidopteran egg parasitoid
Endoparasitoids	3	Hymenoptera: Ichneumonoidea	Braconidae ¹	-	Insects: often Lepidoptera
		Hymenoptera: Chalcidoidea	Encyrtidae ⁷	<i>Psyllaephagus</i> sp.	<i>Mycopsylla fici</i>
		Hymenoptera: Ichneumonoidea	Ichneumonidae ⁷	-	Insects: often Lepidoptera
Hyperparasitoids	1	Hymenoptera: Chalcidoidea	Ceraphronidae ¹	-	Dipterans in leaf litter and soil
<i>Parasitoids – total</i>	6				

Identifications by:

¹ AD Austin

² D Bickel

³ PS Gillespie

⁴ PS Gillespie, LA Mound & C-L Wang: *species nova*

⁵ D Knihinicki

⁶ BA Summerell

⁷ Author

Table 4.6: Summary of arthropod guild structure in *F. macrophylla* canopy
Data collected from fogging survey April 1996

Guild	Sub-guild	No. members	No. Orders	Est. no. individuals
Phytophages	Total	11	4	1535
	Chewing	4	2	99
	Sucking	7	2	1436
Predators	Total	10	6	486
	Chewing	5	3	139
	Sucking	3	2	281
	Ambush	1	1	45
	Filter feeding	1	1	21
Parasitoids	Total	1	1	825
	Endoparasitoids	1	1	705
	Egg parasitoids	1	1	60
	Hyperparasitoids	1	1	60
Detritivores/Scavengers	—	3	3	958
Mycovores	—	2	2	649

4.4 Discussion

4.4.1 Sheet colour effect comparison experiment

4.4.1.1 Colour comparison

The first *t*-test included counts of species within order Collembola (springtails), and was not significant at the 5% error level, indicating no difference between the two colours. The second test excluded Collembola counts since inspection of the data had revealed a very large number of springtails (1364 individuals) on the black sheets. This second *t*-test was even less statistically significant than the first, so it was accepted that the orange coloured sheeting was not producing a bias in the sample caused by attractance, and that the results of the original survey were valid.

4.4.1.2 Differences between main and colour comparison surveys

The *t*-test comparing the fogging order means with the comparison set total order means was not significant at the 5% error level (Table 4.4), but the *t*-test reapplied with the collembolan means removed from both the fogging and comparison sets resulted in a significant test statistic (Table 4.4). This result indicates that the pesticidal fog was successful in producing a knockdown sample, since if the pesticide had not been effective, the sample sizes would have been similar and the test statistic not significant. It is unlikely that flying insects below the canopy of the tree would have been affected by the pesticidal fog, since the drift pattern of the fog was in an upwards direction; the fogging plume was actively directed upwards and was travelling at a relatively high velocity (east) with respect to that of the breeze; and the fog itself was at a higher temperature than the surrounding atmosphere on leaving the fogger nozzle, which would have contributed to the fog's upward movement, even though the fog would have cooled rapidly on contact with the air. The movement of the fog plume would therefore have been likely to have been up through the tree and out of the upper part of the canopy, and then driven away from the canopy in an easterly direction by the breeze. Pesticidal fallout after cooling and falling of the fog plume cooled would have occurred after it had been carried some distance (ca. 20 m) from the canopy perimeter, and therefore not affecting the samples.

The reason for the sizes of the collembolan portions of the original and comparison samples is not known. The appearance of the collembolans in both samples may have been the result of the insects leaping from the ground onto the sheets, since they would certainly exist in large numbers in the turf growing beneath the tree. Whether or not the collembolans were terrestrial

or arboreal in origin, they are not likely to have a direct influence on the psyllid as each occupies a completely different ecological niche, and the 'nutrient recycling' niche occupied by the collembolans is more likely to be beneficial to the tree (and therefore the psyllid) than not.

4.4.2 Main survey results

Once the collembolan data was removed from the sample, the statistically significant results of the two-sided *t*-test between fogging and comparison samples indicated that, all other variables being equal, the pesticidal fog was successful in generating a useful sample of arboreal or aerial insects. Nothing new or unexpected in the way of psyllid natural enemies was found in any of the samples. A single individual from a species in the predatory hemipteran family Reduviidae (most likely in subfamily Emesinae, on account of its raptorially adapted forelegs) was found on each of two sheets. Two of the mite species in the samples belonged to the predatory family Phytoseiidae, but none of these was observed to attack *M. fici* eggs or juveniles.

Three groups of taxa, ranked with respect to numbers, are apparent from Table 4.1. The first (largest) of these groups, of 1000 or more, comprised taxa in the orders Hymenoptera (parasitoids), Thysanoptera (piercing-sucking phytophages) and Psocoptera (detritivores and mycovores). The second group, of the order of several hundred, were Acarina (predators and phytophages), Coleoptera (predators and mycovores), Collembola (detritivores and mycovores), Diptera (various guilds, including ectoparasitoids and scavengers), and Hemiptera (piercing-sucking phytophages, and piercing-sucking predators). The third and smallest ranked group, containing less than 55 individuals in each order, included Araneae (web-building predators), Salticidae (ambush predators), Lepidoptera (chewing phytophages), Neuroptera (predators), Phthiraptera (bird parasites) and some crustaceans (detritivores, most likely soil-dwelling). One order that was absent from the sample was Blattodea (detritivores). This was somewhat surprising given their abundance in the region, but is of no other note other than this, as Blattodea would not have any negative impact on the psyllid population, again because of the different niches occupied.

The comparatively low numbers of predators such as neuropterans, araneids and salticids in the fogging sample may be attributable to low numbers of prey (e.g., exposed psyllid hatchlings).

The high numbers of hymenopterans, psocopterans and thysanopterans can be similarly explained by extensive availability of resources. In the case of the hymenopterans, most of which were parasitoids, a shortage of psylloids would not be of consequence, since most of the hymenopterans were parasitoids of organisms other than *M. fici* (AD Austin, pers. comm.). As far as the psocopterans and thysanopterans are concerned, the limiting factors would most likely be temperature and relative humidity.

4.4.3 The comparatively small representation of *M. fici* in the fogging samples

The relatively small numbers of *M. fici* presented in Table 4.1 are a result of the fact that the psylloid was largely present in its sessile lerp, and therefore protected, form at the time of fogging. Those stages which were susceptible to the insecticide, i.e., mobile (pre-eclosion) fifth instars (see Chapter 6, Phenology of *Mycopsylla fici*) and adults, do appear in the sample, but numbers are not high, since psylloid adult eclosion had only just begun. If the sample had been conducted, say, a week or even a few days later, the mobile fifth instar and adult psylloid numbers would have been much greater as the eclosion phase of the psylloid's life cycle began to enter its peak region (see Chapter 6, Phenology of *Mycopsylla fici*).

4.4.4 Intra-niche competitors of *M. fici*

The main competition for food and space that the psylloid is likely to suffer is that provided by the very large number of thysanopterans. The damage to fig leaves caused by thrips' feeding activity is in the nature of severe distortion of the leaf lamina, usually manifesting in *F. macrophylla* as a tight curling of the leaf around the midvein (Figure 4.1), accompanied by the sclerotisation of much of the leaf tissue that would be available to the psylloid for feeding. This has the net effect of reducing the food source and sites for lerp production. While the number of thrips in the samples was high compared with other taxa, visual inspection of *F. macrophylla* leaves throughout the life of the research programme revealed very little actual damage at any given time. There were certainly no plague outbreaks of thrips resulting in severe damage to the foliage of the trees that would compare with the outbreaks of and damage caused by the psylloid. Lepidopteran defoliators such as the so-called 'native fig moth' *Eustixis caminaea* Meyrick (Lepidoptera: Zygaenidae) were, when present on *F. macrophylla*, in very low numbers.



Figure 4.1: Damage to *F. macrophylla* leaf (MFG) by *Paraballothrips newmani*. Note the contrast between the damaged leaf and the newly- and recently-unfurled leaves



Figure 4.2: Severe callusing damage on *F. macrophylla* leaf (MFG) by *Paraballothrips newmani*

4.4.5 Guild representation and trophic levels in the fogging sample

The second-largest main guild as estimated in Table 4.6, the detritivores and scavengers, is most likely to be largely terrestrial in origin, but the nature of the collecting method, and the poor state of the psocopteran samples, disrupted by the sample storing and cleaning processes, unfortunately blurred the distinction between putative arboreal and terrestrial detritivores. Other than detritivores, the largest trophic level in this sort of system is usually the phytophages, followed by the predatory and parasitic trophic levels, with the hyperparasitic level being the last and smallest level of the trophic pyramid. If the sample predators and parasitoids (with hyperparasitoid numbers removed, since they belong to the topmost trophic level) are grouped as one trophic level, their numbers total 1251. Similarly, with detritivores and mycophages summed, the total of this trophic level (traditionally placed near the base of the trophic pyramid) is 1607. The resultant trophic levels *within the canopy*, (as far as can be determined), resemble not so much a pyramid as a 'house' (a rectangle with a triangle on top). This suggests either an inadequately sized sample, or that a large number of lower (phytophages) or upper (hyperparasitoid) trophic level organisms was not resolved by the sampling method, or that the trophic levels in the *F. macrophylla* canopy do not follow normal trophic level patterns. It also suggests that sampling should have been undertaken at several different times over a 12 month period. Other factors influencing the shape of the pyramid would also be that younger psyllid juveniles (up to fourth instar) would not have been sampled to any large degree as they are sessile until the late fifth instar stage, and that adult psyllids had not eclosed in large numbers at that time.

4.4.6 *M. fici* within the *F. macrophylla* canopy guild structure

From the fogging sample results as viewed on paper, it seems that there might be a large number of potential enemies for the psyllid, but the actual numbers of *M. fici* and its *M. fici*-specific encyrtid parasitoid were low in the sample, since the psyllids at that time were present mostly in various juvenile stages under their lerps. Competition with other phytophages (see also Section 4.4.4) for food and space does not appear to adversely affect *M. fici*. The notably low number of neuropterans indicates that the psyllids had advanced past their vulnerable stage and that the neuropteran population had either decreased through lack of food, or migrated to a more favourable patch. The diversity of arthropod fauna is not as large as might be expected, and this may be a result of the fact that the tree is not growing in its natural environment, either in terms of its endemic geographical location, or in terms of its rainforest habitat.

4.5 Conclusions

The results of the fogging survey lead to the following overall conclusions about the free-living arthropod fauna in the *F. macrophylla* canopy:

- No hitherto unexpected species were found that could be exploited and manipulated for the biological control of *M. fici*;
- *M. fici* is only one among a number of other phytophages that make a living on the tree;
- One of the more prevalent exploiters of *F. macrophylla* as a food source is a species of thrips new to science;
- The size of the thrips population was large;
- Five major guilds were identified in the fogging sample: three guilds contained four or five sub-guilds;
- Three trophic levels were identified in the fogging sample;
- Attempts to control the psyllid using pesticides have the potential to disrupt the complex ecosystem within the fig canopy, and may lead to long-term deleterious consequences both to the fig tree itself, and to the surrounding environment.

The stratification of *M. fici* within the *F. macrophylla* canopy is examined in the next chapter.

Chapter 5. Distribution of *Mycopsylla fici* within the *Ficus macrophylla* canopy

5.1 Introduction and Aims

The fact that *M. fici* could not be laboratory-reared with sufficient success (see Chapter 3) meant that all observations on the psylloid had to be made *in situ* on host trees in the field. It was vital to use the most efficient method of sampling the psylloid on the host tree to maximise the collection of a statistically appropriate amount of good quality information. If the majority of psylloids was concentrated at the top of the tree, *in situ* observations would be severely curtailed for logistical reasons. Conversely, if the psylloids were evenly distributed throughout (or concentrated towards the bottom of) the canopy then observations and collections could be made from the ground, with a minimum of effort, equipment and organisation being required. Sampling from the upper canopy would require the Gardens' hydraulic lift and arborists' time to be booked in advance: both of these resources were in short supply at the time that the experiment was to be conducted. The capacity for sampling as the need arose would also be severely restricted as a result. Less information would therefore be able to be collected if observations had to be made from the top of the tree. Even and lower-canopy distribution of psylloids, on the other hand, would allow sampling and surveying to be made from ground level without compromising the quality of information being collected.

The aim of the work described in this chapter was to determine the distribution of *M. fici* in the *F. macrophylla* canopy as expeditiously and as accurately as possible so that any necessary contingency planning with respect to *in situ* sampling methodologies could be made. Further, data on the distribution of psylloids in the canopy would allow cost-effective control strategies if necessary. A standard stratification experiment was therefore designed to determine the distribution of the psylloid in the tree.

Information from the adult sample could be extracted relating to the sex ratio of the psylloid, and this was also recorded.

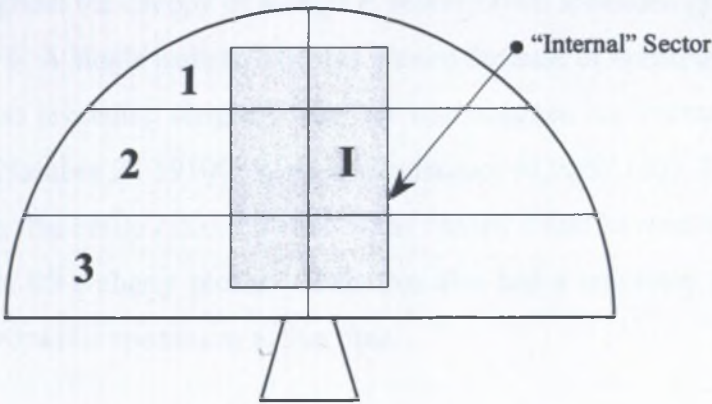


Figure 5.1a: Side view of the stratification system

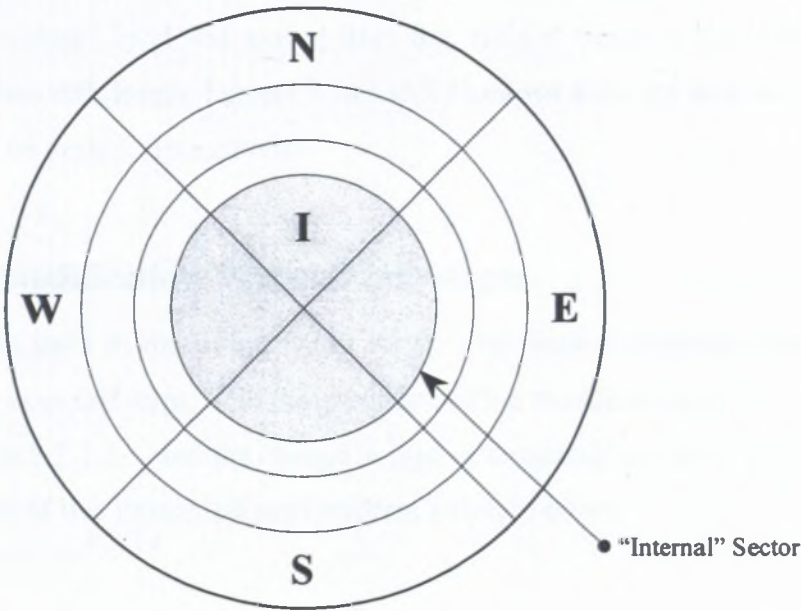


Figure 5.1b: Plan view of the stratification system

5.2 Materials and Methods

5.2.1 Experimental designs and choice of tree

5.2.1.1 Tree choice

A conventional stratification sampling study was undertaken to determine the psylloids' distribution throughout the canopy of a large *F. macrophylla* specimen growing in the Sydney Domain on Lawn 6. A single sample tree was chosen because of constraints on personnel and equipment (see also preceding chapter). The tree was assigned the internal project code *DMN* (RBG Accession Number ZI 19100, RBG grid reference *9L9q/9L10q*). The tree was selected for its accessibility: the entire circumference of the canopy could be reached from the bucket of the RBG hydraulic lift ('cherry picker'). This tree also had a relatively leafy state compared with other *F. macrophylla* specimens at that time.

5.2.1.2 Adult stratification

The canopy volume was arbitrarily divided initially into four quadrants (north, east, south, west) by four heights (low, middle and upper canopy, plus an extra 'height' designated 'internal'), producing a 4 × 4 factorial design. The placing of the internal level in the 'Height' factor was somewhat arbitrary, and in the second stratification survey, conducted on egg and lerps stages, the 'Internal' level was moved from the 'Height' factor to the 'Quadrant' factor, producing a 5 × 3 factorial design. Figures 5.1a and 5.1b above show the side and plan views of the layout of the 4 × 4 design, respectively.

5.2.1.3 Design modification: Egg and lerp stages

The same protocols used in the initial (adult stage) stratification sampling experiment were used to sample the eggs and lerps, with the exception of the modification to the factorial design described in Section 5.2.1.2.1, and the change in type of sampling unit from whole branchlet to 10 leaves from each of two branchlets per Quadrant × Height sector.

5.2.2 Sampling stage 1: collection of adults

The experiment was conducted in two separate stages in mid to late September, 1996, at the height of the psylloid plague outbreak. The first stage was the collection of adults, and was undertaken over two days a week apart. The North and East sectors ('Bottom' to 'Top') were collected during the first stage, and the South, West and 'Internal' sectors collected a week

later. As large numbers of bags containing live and rather active adult psylloids were involved, several trips to the laboratory were required so that samples could be processed expeditiously and reduce the likelihood of portions of the sample escaping through wrinkles in the necks of the bags. This procedure slowed the sampling rate down considerably more than expected, and a whole working day was required to complete the sampling of just two out of the five 'quadrants'. This meant that the first stage had to be completed a week later when the hydraulic lift became available again. The difference of seven days had an observable effect on the numbers of adult psylloids: the adults were present in far greater numbers. This represented the peak of a major psylloid outbreak, not long after which this tree and many others in the Gardens/Domain precincts defoliated almost completely.

Adult psylloids were collected from (randomly selected) branchlets of the tree in each of the defined sampling sectors. Most branchlets encountered on this tree bore between ten and twenty leaves. Ten samples were taken from each Quadrant x Height combination, with the exception of the four sectors in the topmost level. A reduction in the number of bags per sector to five in the top level was made because of the combination of the reduced collecting volume caused by the inward curve of the canopy with height, the full extension of the lift arm falling short of being able to reach up to the top of the tree, and the flimsy nature of the uppermost branches. One hundred and forty experimental units (branchlets) in total were collected. Each bag was labelled with a simple alphanumeric code defining its source from within the tree, and collection sequence. Samples were made by placing a pre-opened 75-litre black plastic garbage bag over a branchlet as quickly as possible to minimise the number of insects escaping: psylloids can jump reasonable distances as well as fly, hence the derivation of the name from its Latin root, *psylla* (lit., flea) (Froggatt, 1913). The bag's neck was held tightly around the branchlet stem and the branchlet shaken vigorously thirty times (fifteen shakes up and down and fifteen shakes from side to side) to dislodge the adult psylloids, after which the bag was removed quickly, with the branchlet pointing downwards into the bag to prevent psylloids from falling out of the bag. Bags were securely knotted to prevent escape, and stored in a domestic chest freezer to kill the psylloids for counting. The frozen samples from each bag were transferred to individual 75 ml plastic screwtop specimen containers and labelled with the bag's reference code. The psylloid contents were subsequently sorted by sex and counted. Other arthropods present in the samples were also recorded for possible use (see also Chapter 4, Arthropod fauna in the *Ficus macrophylla* canopy, determined by insecticidal fogging).

5.2.3 Sampling stage two: collection of preadult forms

The second stage design was conducted 12 months after the initial survey, during the growth cycle of the September cohort of juveniles. This part of the experiment was conducted to complete the information on the distribution of *M. fici* on *F. macrophylla*. Given that adult psylloids are winged and therefore highly mobile compared with the sedentary juvenile stages a possibility existed for a difference in canopy distribution between these two parts of the psylloid life cycle. Adult psylloids might not necessarily have been present at egg laying sites during the first stratification sampling. The 'Top' Height factor sampling unit quantity was increased to the same as that of the two lower levels, with twenty leaves collected per sample (two leaves from each of the ten randomly chosen sample branchlets per Quadrant/Height sector). Since the immobile stages of the psylloid were being collected, sampling an entire branchlet was not necessary.

The variables of interest were numbers of egg masses and lerps per leaf. Collecting of samples was completed in a single day, as the second sampling stage simply merely involved the removal of leaves after tagging with an appropriate alphanumeric identification code, and did not require the frequent lengthy interruptions to the collection process that the adult samples needed. A total of three hundred leaves was collected. Leaves were placed in a 75-litre plastic garbage bag for each sector, and stored in the -15 °C chest freezer prior to counting. Total numbers of extant lerps per leaf were counted, as were necrotic spots and blotches whose cause was directly attributable to lerp presence.

5.3 Results

5.3.1 Stratification analysis

5.3.1.1 Eggs

The egg data were analyzed using a 5 x 3 factorial analysis of variance at the 5% error level. Factors were quadrant (five levels: north, south, east, west and 'internal') and height (three levels: lower, middle and upper canopy). Table 5.1 below presents the results of the analysis, which revealed egg mass numbers to be statistically significant with respect to both Quadrant and Height. The means for each Quadrant x Height sector level are given in Table 5.2, where the Internal 'quadrant' at three heights is shown to contain the largest numbers of egg masses, on average.

Table 5.1: ANOVA results for *M. fici* egg mass stratification on *F. macrophylla*

Factor	Effect df	Effect MS	df Error	MS Error	$F_{(n, 285)}$	prob.
Quadrant	4	15838.32	285	396.4089	39.9545	0.000000
Height	2	1476.04	285	396.4089	3.72354	0.025331
Quadrant × Height	8	908.26	285	396.4089	2.29123	0.021605

Table 5.2: Mean number of egg masses per leaf per factor level, quadrant × height interaction

Level	Mean egg mass number
North Bottom	37.75
North Middle	39.05
North Top	23.50
East Bottom	24.50
East Middle	35.55
East Top	34.00
South Bottom	14.85
South Middle	14.55
South Top	7.45
West Bottom	39.75
West Middle	33.50
West Top	40.75
Internal Bottom	59.90
Internal Middle	67.05
Internal Top	46.20
Grand mean	34.56

Table 5.3: ANOVA results for *M. fici* lerp stratification on *F. macrophylla*

Factor	Effect df	Effect MS	df Error	MS Error	$F_{(n, 285)}$	prob.
Quadrant	4	1569.328	285	206.2139	7.610198	0.000008
Height	2	145.763	285	206.2139	0.706855	0.494055
Quadrant × Height	8	284.351	285	206.2139	1.378912	0.205329

Table 5.4: Mean lerp numbers per sample for 'Quadrant' stratum

Quadrant	Mean lerp numbers
North	7.6
East	2.4
South	1.7
West	9.4
Internal	14.1
Grand mean	7.0

Table 5.5: Mean number of lerps per leaf per factor level, quadrant × height interaction

Level	Mean lerp numbers
North Bottom	9.35
North Middle	7.50
North Top	5.90
East Bottom	3.30
East Middle	3.05
East Top	0.95
South Bottom	2.35
South Middle	1.95
South Top	0.90
West Bottom	6.25
West Middle	3.85
West Top	17.95
Internal Bottom	14.70
Internal Middle	12.45
Internal Top	15.10
Grand mean	7.04

5.3.1.2 Lerps

The lerp data were analyzed using a 5 × 3 factorial analysis of variance at the 5% error level, with quadrant (five levels: north, south, east, west and internal) and height (three levels: lower, middle and upper canopy) as factors. Table 5.3 above gives the results of the analysis, with Quadrant only showing significant differences in lerp numbers. Quadrant stratum means are presented in Table 5.4 above. Table 5.5 above gives the means for the interaction term for interest only as this term was not statistically significant.

5.3.1.3 Adult stage

5.3.1.3.1 Overall stratification

The adult data were analyzed using a 4 × 4 factorial analysis of variance at the 5% error level. Factors were Quadrant (four levels: north, south, east and west) and Height (four levels: lower, middle, upper and ‘internal’), with five empty values added to each of the Level 3 groups within the height factor to balance the half-sized samples at the top of the tree with respect to the rest of the levels, the latter having ten data points each. Analysis of the data with time as a covariate resulted in a null analysis, so time was removed from the subsequent analysis. Table 5.6 below presents the ANOVA table for mean adult numbers per stratum with respect to Quadrant and Height. Both strata were found to contain statistically significantly different mean adult numbers. Table 5.7 below presents the stratum means for the Quadrant × Height interaction term of the ANOVA model. Although the model is different than that used for the egg and lerp stratification, the ‘Internal’ stratum again contains relatively high mean values.

Table 5.6: ANOVA results for adult *M. fici* stratification on *F. macrophylla*

Factor	Effect df	Effect MS	Error df	Error MS	$F_{(n, 123)}$	prob.
Quadrant	3	1254.282	123	321.6083	3.90003	.010586
Height	3	5424.620	123	321.6083	16.86716	.000000
Quadrant × Height	9	874.348	123	321.6083	2.71867	.006341

Table 5.7: Mean number of *M. fici* adults per sample per factor level, quadrant x height interaction

Factor level	Mean no. adults/sample
North Bottom	8.3
North Middle	5.8
North Top	54.8
North Internal	50.8
East Bottom	5.1
East Middle	10.8
East Top	31.2
East Internal	25.8
South Bottom	18.0
South Middle	17.0
South Top	29.8
South Internal	36.4
West Bottom	27.3
West Middle	33.2
West Top	30.6
West Internal	38.9
Grand mean	26.5

Table 5.8: ANOVA table for adult *M. fici* males and females with respect to stratum

Stratum	Wilks' <i>Lambda</i>	Rao's <i>R</i>	df 1	df 2	prob.
Quadrant	0.869578	2.967273	6	246	0.008152
Height	0.682079	8.643983	6	246	0.000000
Quadrant × Height	0.773786	1.869792	18	246	0.018895

Table 5.9: Mean adult *M. fici* males and females per sample per factor level, quadrant × height interaction

Stratum level	Males	Females
North Bottom	4.7	5.7
North Middle	2.9	2.9
North Top	25.2	29.6
North Internal	22.6	28.2
East Bottom	2.5	2.6
East Middle	5.3	5.5
East Top	17.8	17.4
East Internal	10.5	16.1
South Bottom	8.3	9.7
South Middle	10.4	9.2
South Top	14.8	15
South Internal	13.4	21.8
West Bottom	13.6	13.7
West Middle	16.2	16.6
West Top	15.8	14.8
West Internal	20.6	19.3
Grand mean	12.8	14.3

Table 5.10: ANOVA table for adult *M. fici* sex ratio with respect to stratum

Stratum	Effect df	Effect MS	Error df	Error MS	$F_{(n, 124)}$	prob.
Quadrant	3	5.006289	124	2.829887	1.769077	0.156539
Height	3	5.202107	124	2.829887	1.838274	0.143669
Quadrant × Height	9	6.516153	124	2.829887	2.302619	0.019901

Table 5.11: Mean adult *M. fici* sex ratios per sample per factor level, quadrant × height interaction

Stratum	Sex ratio
North Bottom	0.91
North Middle	0.48
North Top	0.72
North Internal	0.90
East Bottom	0.98
East Middle	0.83
East Top	1.08
East Internal	0.65
South Bottom	0.90
South Middle	1.78
South Top	0.92
South Internal	0.62
West Bottom	0.99
West Middle	1.11
West Top	0.98
West Internal	1.52
Grand mean	0.96
Std. deviation	0.32

5.3.1.3.2 *M. fici* sex ratios and their stratification

The analysis of variance was significant at the 5% error level for both Quadrant and Height strata (Table 5.8 above). Table 5.9 above gives the mean values per Quadrant x Height sector for males and females. Sex ratio stratification analysis (Table 5.10 above) revealed significant differences in the interaction term, but not in the individual terms. The interaction terms appear in Table 5.11 above.

The mean number of total psylloids per sample unit (bag) was 26 (25.6); mean number of males per sample was 12 (12.08); and mean number of females per sample was 14 (13.64). In contrast, the mean number of total psylloids per stratum sector was 28 (27.1); mean number of males per sample was 13 (12.8); and mean number of females per sample was 15 (14.3). The sex of each individual psylloid was scored at the time of counting, and the overall male: female sex ratio on a per-sample basis for the total of 3,575 psylloids counted from the 124 sample branchlets was 0.88 (1678 males to 1897 females). The sex ratio on a per-sector basis, calculated by the analysis of variance (grand mean of sectors), was 0.96 male to 1 female.

5.4 Discussion

5.4.1 General observations on adult numbers and egg laying

The numbers of adult psylloids was so great at the time of their collection that almost all space for occupation by psylloids, male or female, was taken up on the entire tree. The consequent overcrowding appeared to force some females onto nearby trees, as there were observed instances of the female psylloids not only alighting and resting on nearby trees, but also laying eggs on one particular example. This adjacent tree was another species of fig (*F. nymphaeifolia* Miller; RBG accession number 19701, RBG grid location *DL6-9L9t/9M10n*). The eggs on the *F. nymphaeifolia* specimen were, however, laid in far more sparse a pattern than the closely packed egg masses typically laid on *F. macrophylla*, and it is highly unlikely that these eggs would have hatched. It is even more unlikely that any hatched crawlers would have survived longer than a few hours. *F. nymphaeifolia* is a South American species, and while it can be considered as part of the Gondwanan assemblage, its relationship to *F. macrophylla* is likely to be sufficiently distant that the already host-specific psylloid would not be able to feed successfully from this novel species. There was certainly no evidence of lerps on leaves either on the tree or underneath it. Unavailability of the hydraulic lift prevented further observations to determine the fate of the eggs on the *F. nymphaeifolia* specimen.

5.4.2 Stratification

5.4.2.1 Eggs

The results suggested a tendency for the psyllid females to avoid laying eggs on the southern part of the tree. Again, the internal part of the tree bears a greater proportion of egg masses than the rest of the tree, i.e., North, East and West (bottom to top), and is echoed by a similar concentration of lerps. Since there is no 'time delay in sampling' factor to contend with, the egg and juvenile stratification results can be considered to be more reliable than the adult stratification results.

5.4.2.2 Lerps

The 'Quadrant' stratum's statistical significance (Table 5.3) was produced by the relatively large concentration of lerps in the 'Internal' substratum (Table 5.4). This result was useful since it meant the height could be disregarded from a point of view of lerp studies, and laid the basis for a decision on the employment of a simpler, ground-based sampling methodology for the psyllid phenology studies discussed in Chapter 6.

5.4.2.3 Adults

The concentration of adults in the 'Top' height stratum, even after accounting for reduced sample numbers, and within the 'North' stratum, may be attributable to phototropism and/or overcrowding caused by a plague outbreak of psyllids. The likelihood that the air in those regions was warmer in these regions of the tree than in the lower or more southerly (shaded) regions, and thus produced more activity in the adults, can probably be discounted by the relatively large numbers of psyllids found in the middle and south of the tree canopy. In any event, the stratification of adults was considered not to be important for the purposes of the major part of subsequent life history studies.

5.4.2.4 Stratification with respect to sex and sex ratios

The analysis of variance of male to female sex ratio result (Table 5.10) was influenced by relatively large numbers of males in the 'South Middle' and 'West Internal' sectors (Table 5.11), and by relatively large numbers of females in the 'North Middle', 'East Internal' and 'South Internal' sectors. The difference between the per-sample and per-sector sex ratios reflects this, and the time delay between the first and second parts of the sampling process may be an influencing factor here. Since the adult phase was only subjected to close scrutiny from the point of view of egg-laying dynamics, the apparently uneven distribution of the adults was not considered important.

5.5 Conclusions

Results of the analysis indicate that clumping did not occur in a structure that would enforce a difficult sampling regime. It was decided, in the light of the results discussed above, that the *in situ* study of the juvenile parts of the life cycle of *M. fici* could be made from ground level, without recourse to mechanical aids such as the truck-mounted hydraulic lift. It was decided on the basis of the sector stratification to divide the tree in half on a North-South axis, to make sampling at ground level more even.

At this juncture, therefore, it was decided that all further life history studies of *M. fici* would be conducted *in situ* on the *F. macrophylla* trees themselves. This is developed further in Chapter 6, Phenology of *Mycopsylla fici*.

Chapter 6. Phenology of *Mycopsylla fici*

6.1 General introduction to Chapters 6 and 7

Practical, informed decisions about management of the fig psyllid cannot be made without detailed knowledge of the insect's phenology and population dynamics. To provide data for the study of the psyllid's phenology and population dynamics, four relatively large-scale *in situ* field studies were undertaken on the fig psyllid itself during the project:

1. First *MFG* egg laying study (November 1997 to March 1998);
2. Second *MFG* egg laying and hatching study (November 1998 to March 1999);
3. Detailed study of the life cycle of a psyllid cohort on *MFG*, from hatching through to the early and unexpected demise of most juvenile psyllids (December 1998-January 1999);
4. Detailed study of the life cycle of a psyllid cohort on *AGS*, from hatching through to the early extinction of the associated *Psyllaephagus* sp. cohort (December 1998-March 1999).

This chapter is concerned with the basic phenology, lerp structure and parasitoid of *M. fici*. Material discussed includes data collected during the two egg laying and hatching studies conducted on *MFG*, as well as the two life history studies conducted on *MFG* and *AGS*.

- Egg laying and hatching dynamics of *M. fici* including hatchling 'migration' and fates (Sections 6.3.1 and 6.4.1 and their subsections);
- Growth and development of *M. fici* juvenile instars, including lerp structure, the effects of relative humidity on lerp and leaf survival the age structure of lerp populations, stadial duration of juvenile *M. fici* instars (Sections 6.3.2 and 6.4.2 and their respective subsections)
- Observations on the encyrtid parasitoid, *Psyllaephagus* sp. (Sections 6.3.3 and 6.4.3 and their respective subsections).

Chapter 7 studies the population dynamics of the psyllid, and includes survival analysis of both cohorts, and the development of a degree days model for *M. fici*.

6.2.1 General materials and methods

6.2.1.1 Selection of field study trees, including replacement of a defunct study sample

The four field studies listed above in Section 6.1 were undertaken on two mature *Ficus macrophylla* specimens at different periods between mid-November 1997 and late March 1999. The *F. macrophylla* specimen (project code *MFG*) growing near the Morshead Fountain Gate in the Royal Botanic Gardens Sydney, close to the intersection of Macquarie Street and the Cahill Expressway (Figure 6.1), was chosen for egg laying and main life history studies. A second similar *F. macrophylla* specimen, growing adjacent to the southeastern corner of the Art Gallery of New South Wales (Figure 6.2), was also selected (project code *AGS*).

In situ field studies from egg hatch to adult eclosion were started on the *F. macrophylla* specimen project code *MFG*, on 11 December 1998. Death of early-instar psyllid juveniles resulted in this psyllid cohort becoming extinct within about six weeks, even though a cohort with sufficiently large numbers to study had hatched from the eggs. The main cause of death in this instance was attributed from observation to lacewing predation on hatchlings and some older instars, although this factor did not account for all deaths. Observations on this population, therefore, were finally terminated in mid-January 1999.

The search for a newly-hatching psyllid population to replace the *MFG* population was commenced when it became clear that the *MFG* psyllid study cohort size was going to fall below a statistically valid sample size well before the completion of the life cycle. A suitable replacement population was located on another *F. macrophylla* specimen on 29 December 1998. The chosen tree, given the project code *AGS*, (Figure 6.2) was situated in the Sydney Domain, adjacent to the southeastern corner of the Art Gallery of NSW, at the top of the slope that descends to Sir John Young Crescent in Woolloomooloo. Observations on the *AGS* psyllid population began immediately upon its discovery. Although further psyllids, possibly a new cohort, began hatching on *MFG* just before the study of the population was begun on *AGS*, it was decided not to risk another failure when there was an obviously large and apparently robust population of psyllids hatching on *AGS*. The failed-cohort study, however, produced some useful mortality and lerp dimension data. One implication of the failure of this particular cohort was the possibility that predators might be having a marked effect on psyllid populations in their early juvenile stages. The possibility of predation as a major cause of the

MFG study population decline was reinforced by results from the hatching portion of the 1998-99 *MFG* egg-laying and hatching study in February 1999, as well as observations made during the *AGS* life history study.

Observations of the hatching *MFG* and *AGS* populations were made on a daily basis throughout the duration of both studies, commencing around 7:00 am or earlier. Observations during the *MFG* study were conducted from the beginning of egg hatch until the study was finally completely abandoned on 11 January 1999. The subsequent *AGS* study that replaced that on *MFG* was begun on 29 December 1998. The disappearance of the adult *Psyllaephagus* sp. cohort from *AGS* and its environs, rather than that of the adult psylloids, was used to mark the end of the field study, as this represented the final activities associated with the studied generation of psylloids. Sticky traps were used to determine the final point at which the *Psyllaephagus* sp. (and *M. fici*) study cohorts died out, with observations being made until seven days after sticky traps ceased to yield *Psyllaephagus* sp. individuals (5 March 1999).

Using observations and photographs of psylloids on the leaf having just emerged from the egg, it was possible to make a *post hoc* determination of the age range of the psylloid juveniles on *AGS* that were observed on the first day of the field survey. It was estimated that hatching had commenced at the most two days before the start of observations, i.e., 27 December 1998. The observations made on the first day, therefore, represented a combined record of numbers hatched per day for the first three days of the post-egg cohort's life cycle (Chapter 7, Section 7.3.1). Subsequent days' observations followed the formation and growth of new lerps, as well as the growth of those lerps recorded on day 1 of the *AGS* study (see Section 6.3.2.2).



Figure 6.1: *F. macrophylla* study tree MFG during field work, February 1999



Figure 6.2: *F. macrophylla* study tree AGS during field work, February 1999

6.2.1.2 Egg-laying studies

The first of the two egg studies (November 1997 to March 1998) was restricted to daily counting of numbers of newly-laid egg masses and date of hatching only. The second egg study (November 1998 to March 1999) was a repetition of the first, with the additional recording of numbers of eggs per mass, and, at the end of the study, numbers per egg mass hatched per day. The second study was conducted after the study leaves and their identifying tags (in the first study) were apparently tampered with, before the start of the egg-hatching period in March 1998. The initial egg-laying study was successful in its first objective, however, yielding useful data on number of egg masses laid. The second egg study had suitable modifications made to the leaf tagging system (see Section 6.2.3 below) so that it was less visible to vandals, and allowed for the collection of additional information omitted in the first egg study.

6.2.1.3 Juvenile instar studies

6.2.1.3.1 Growth and Development of *M. fici* juvenile instars

The psylloids are known (so far without exception) to have five instars (Hodkinson, 1974). The aims of the experiment described in this section were:

- to detect any variation from the general psylloid plan that might have occurred in the *M. fici* life cycle;
- to determine the mean length in days for each instar for use in calculating a degree days model for separate *M. fici* instars (see Chapter 7, Sections 7.3.3 and 7.4.3 and their subsections).

The basis for the understanding of any insect includes the determination of its phenology. Without a knowledge of the number and length of juvenile stages or instars (Wigglesworth, 1973), and the number of stages within a given time span, predictions about an insect's population sizes, population growth rates, or its responses to changes in various environmental conditions, will not be as reliable or robust as those for which such information is available.

Observational and data recording techniques for the main field studies were developed during the chlorophyll fluorescence studies of fig tree health (see Chapter 8), and the two egg laying studies, and refined to their final form during the *MFG* and at the beginning of *AGS in situ* life

history studies. The *AGS* study was conducted in two simultaneous parts. The first part comprised the major detailed field study of psylloid post-hatching settlement, lerp growth and adult emergence *in situ*. This part was initially begun as the third study, on *MFG*, but became the fourth study when the *MFG* cohort died out prematurely and unexpectedly. The second part of the fourth study was conducted for the purpose of *ex situ* study of psylloid juvenile instar growth rates and entailed destructive sampling of lerps by excision from leaves, followed by measurement of psylloid head capsules under the microscope. Sampling for the *ex situ* study was conducted on a part of the psylloid population not under the direct observation of the main set of study leaves.

All field observations of the psylloids in the four studies described above (with the exception of the lerp excisions for the instar duration samples) were non-destructive and non-invasive, with their egg masses, psylloids and lerps left intact and untouched by the observer. Estimates of psylloid growth rates were made by measuring arbitrary dimensions of the lerp relative to the direction of longitudinal and lateral axes of the leaf, plus the height of the lerp from the leaf surface. The reason for using this method was the assumption that lerp size would increase proportionally with increasing psylloid size: lerp size, once recruitment and settlement had been completed, would therefore be proportional to actual growth rate of the juvenile psylloids under the lerp. The destructive lerp sampling was conducted simultaneously with the second of the two life history studies in order to check this assumption, and to provide data on the number of juvenile psylloid instars.

Observations were also made on the activities of the parasitoid of *M. fici*, *Psyllaephagus* sp., during the *in situ* field studies.

6.2.1.3.2 Stadial duration of *M. fici* juvenile instars

The length of time (Wigglesworth, 1973) that an insect spends in each instar (i.e., between moults), or *stadium*, is useful information if, for example, control measures are to be implemented for that insect. The accurate prediction of an instar which is vulnerable to, for example, chemical control, would aid in the effective timing of insecticidal sprays.

The motivating factors for this study were:

- That *M. fici* differs from many other psylloids in that it lives *communally* for its entire juvenile existence, apart from hatching and choosing a lerp site;
- WW Froggatt's misinterpretation of the constituent materials of the *M. fici* lerp (Froggatt, 1901a; Froggatt, 1901b; Froggatt, 1923).

The morphometric characters chosen to determine the length of the juvenile stadia in days were head capsule width and length, on the basis that head integument in insects changes only directly after ecdysis, and thereafter remains constant for the duration of that instar, i.e., until the completion of the next ecdysis (MacNally, 1988; Wigglesworth, 1973). The length of hind tibiae could also have been used as a comparative index, but the difficulty in measuring these structures, including artifacts produced by the mounting processes, in the early instars would have been likely to have produced unacceptably inaccurate results. Additionally, on the basis of head capsule measurements, workers would be able to determine the instar of individual psylloids by measurement using field-portable tools such as a set of dial calipers.

6.2.2 General sampling methods

6.2.2.1 Equipment

A light, portable 20 × magnification stereo field microscope with high precision optics (*Nikon 'Naturescope'*®), carried on a neck-strap, was used to count egg mass and egg numbers, egg hatchings, and psylloid settlement, and also to observe lerp production and other activity on leaves and within lerps, before the lerps became too opaque for observation. Other equipment included a clipboard for data-sheets, a 150 mm clear plastic ruler for measuring lerps, and a ball-point pen, which were each strung with thin polypropylene twine and also worn around the neck along with the microscope. Efficiency in observation and data recording was thus increased. Items supplementary to the main data acquisition array were kept in various pockets of a carpenter's tool belt worn around the waist, allowing ready access to items such as tagging tape, marking and paint pens, pencils, scissors, specimen jars, insect aspirator, sticky traps and tie-wire, and a water bottle. This 'portable field laboratory' enabled continual, efficient observations to be made throughout the day. Extra equipment taken into the field were specimen jars used for the head capsule study, and were carried in a small expanded

polystyrene foam drinks cooler or 'esky' with a frozen 'ice-brick', to prevent overheating of excised lerp samples in the hot weather which occurred throughout the study period. The 'esky' was left in a heavily shaded spot under the study tree until required.

6.2.2.2 Sampling and tagging

The same basic methods of tagging and sampling were applied in all four field studies. Leaves on both eastern and western sides of the trees were selected at random, the only restriction being that they could be reached comfortably while standing on the ground, with minimal pulling at branches. In some instances, leaves came off despite the minimal force applied: in all these cases, however, leaf abscission had occurred, and the leaf would have fallen from the tree within the next few days, even without the extra handling. Leaves were tagged using approximately 100 mm lengths of blue or white electrical tape, cut down in width while still on the roll. A tape tag was then loosely wrapped around the leaf petiole, closed on itself, and numbered using a felt-tipped marker pen. The final dimensions of the labels were approximately 6 mm × 50 mm for the first egg-laying study (but see Section 6.2.3 below).

6.2.2.3 Climate data

Daily climate data for the period 31 March 1995 to 31 August 1999 were obtained in the form of digital spreadsheet files from the Bureau of Meteorology recorded at Observatory Hill, Sydney. The environments of Observatory Hill and the Royal Botanic Gardens, Sydney are similar: the distance between Observatory Hill and the study trees is within the (approximate) range of 950 m and 1200 m; Observatory Hill and most of the trees are a similar distance from the shores of Sydney Harbour; and all are subject to prevailing winds of similar directions and strengths.

6.2.3 Materials and methods for egg laying and hatching studies

For the first egg-laying survey, 150 leaves on *MFG* were selected and tagged as described in Section 6.2.2.2, and were observed daily beginning at about 7:00 am or earlier, through the 20 × microscope. Observations commenced at the first appearance of new psylloid egg masses on 36 uncolonised or 'clean' leaves on the eastern (predominantly morning sun) and western (predominantly afternoon sun) sides of the tree, for an initial total of 72 leaves. Observations were made until seven days after the last egg mass had been laid on a study leaf. The number

of leaves was added to on a daily basis as 'clean' leaves of the appropriate age were discovered, until the number of leaves in the study reached 150. The counting process took place over a two hour period initially, with the time taken extending to the vicinity of six hours at the end of the hatching period, as the number of leaves was added to. Pre-existing egg masses on all leaves, including those added as the study progressed, were marked off with their own "pre-existing" code. Each egg mass was marked with its date-code as close as possible to it, using a black, fine ball-point pen, directly on the underside of the leaf. Counting of hatching of egg masses was to have been started in late January 1998. On returning to the tree just prior to the egg-hatching period for this cohort, however, the study leaves could not be found: either the tags had been removed from the sample leaves, or the tagged leaves had been removed from the tree. The egg-hatch part of the data collection thus could not be completed. The possibility that the leaves themselves had been removed was the more likely of the two, given that no such event occurred to tagged leaves before or after this misadventure. Horticultural staff and those directly responsible for the tree and the area around it, had been informed in advance of the research studies being made on the tree, and had been asked not to do anything to the tree without consultation, to which they had agreed. It was assumed that the tag removal was done by a member of the public. This is a disadvantage of working in a heavily-used public area.

For the second egg-laying study conducted a year later from December 1998 to March 1999, leaves were chosen in somewhat less publicly accessible regions of the canopy skirt of *MFG*. The leaf-tagging labels used were reduced in total area by over 50%, to approximately 4 mm × 30 mm, with the colour of the tape changed to a matte-finish neutral (50%) grey, in order to reduce their visibility to casual observers. Egg masses were counted in this study as for its predecessor, but the additional information of egg numbers per egg mass was also collected. Hatchings of eggs per individual egg mass were also counted on a daily basis in March 1999. This allowed comparison between numbers of eggs laid and numbers hatched, and therefore provided a greater resolution of cohort mortality. The full list of variables recorded in the second egg study is given below. The egg counts in the second egg study were made immediately before the main life history study on *MFG*, during the adult presence of the preceding (spring) generation of psylloids. The hatch counts began some weeks after the life history study on *AGS* was completed. A map of the positions of all study leaves on *MFG* was made to aid location. The map was especially useful in locating leaves when egg hatching commenced.

Data were collected for the following variables:

- existing egg masses recorded and marked where present;
- number of newly laid egg masses per leaf per day;
- date laid, recorded as code next to egg mass on leaf;
- number of newly laid eggs per egg mass per leaf per day;
- absolute position of egg masses on leaf (margin, mid-laminar, etc.);
- number of natural enemies (predators and/or parasitoids) present on leaf;
- number of eggs or egg masses eaten;
- number of eggs or egg masses unhatched by end of hatching period, assumed unfertilised or dead.

6.2.3.1 Materials and methods for *ex situ* study of *M. fici* growth and development: stadial duration of juvenile *M. fici* instars

The sample population for this study was taken from lerps excised from fifty randomly chosen and specially tagged leaves on the *AGS* study tree. All leaves chosen carried at least four lerps, and excisions were conducted at the time of the main *in situ* observational study. Two hundred and eighty sample lerps ultimately were cut from this sub-population of fifty leaves. The actual collection procedure involved excising a single lerp from each of ten leaves per day from the population of fifty, using a pair of surgical 'sharp-sharp' scissors. This leaf sample set was distinct from that used for the main study. Clippings were made on a rotational basis, with one set of ten leaves (1,...,10; 11,...,20, etc.) from the sequence 1,...,50 used on each successive day. Sampling was carried out daily for four weeks. Collection ceased when leaves had undergone abscission and either fallen from the tree or been desiccated completely on the tree, the cessation of adult emergences from all lerps had occurred (emergences were determined by the appearance of additional final exuviae on the leaf surface), or a leaf had had all lerps removed from it by the excision process. Leaf clippings were stored in a -20 °C chest freezer in 75 ml plastic specimen jars, one day's samples to a jar, until head capsule measurements were made. There were ten leaves from the original study population of fifty remaining on the tree at the end of the survey.

Before measuring, lerps were prepared by dipping in 70% ethanol for about 60 seconds to facilitate wetting, then soaked for at least 30 minutes in RO-purified water to dissolve lerp

sugars. Lerps were patted gently with a '0000' nylon-hair artist's paintbrush to express residual lerp sugars from the wax matrix. The expressed sugar solution was then removed from the lerp area with the paint brush, and facial tissues. Sands (1984) gave a method of dissolving cuticular wax using xylene, but xylene is now known to be quite an active carcinogen. 'CRC 5.56®' (CRC Industries (Aust) Pty Ltd) was tested for suitability. This solvent dissolved psyllid wax threads almost instantly even if lerp remnants were wet from the removal of the honeydew component of the lerp, and was therefore used in all preparations. The Material Safety Data Sheet (MSDS) for the product (CRC Industries (Aust) Pty Ltd, 2003) contra-indicated activity as a carcinogen. The solvent was applied using a second '0000' artist's paintbrush, leaving the juvenile psyllids intact for measurement. Ten psyllid juveniles were selected from each of two randomly selected lerps per collection day.

Measurements of each sample (forty measurements per sample per day) were made using an Olympus SZH-10 stereo dissecting microscope at $50 \times$ magnification with a 20-division eyepiece graticule, and later a Carl Zeiss Jena stereo dissecting microscope with 100-division eyepiece graticule, at the same magnification as the Olympus instrument. The eyepiece graticules on both microscopes were calibrated using a standard slide micrometer (1 mm in 100ths), at $50 \times$ magnification. Data were analysed and graphed in the Statistica 5.1 computer software package (StatSoft, 1998).

6.2.4 *In situ* and *ex situ* observations of *Psyllaephagus* sp.

Activities of adult *Psyllaephagus* sp. were observed under the $10 \times$ magnification Nikon "Naturescope®" on the leaves of the study tree during the *AGS in situ* field study. Adult *Psyllaephagus* sp. individuals were also collected in the field, using a jar-aspirator, transported to the laboratory, and were placed in a clear 90 mm diameter plastic petri dish for the purposes of observation *ex situ*. The observation arena was then viewed under an Olympus SZH-10 stereo dissecting microscope set at various magnifications between $70 \times$ and $250 \times$ inclusive. The same technique was also used to observe the activity of juvenile lacewing predation on psyllids. A small laboratory cage for determining the viability of collected pupated parasitoids was constructed from two circular cake tins of 250 mm diameter, capping a cylinder made from a piece of clear acetate sheet and resulting in a structure 300 mm in height. Small breathing holes were punched in the bottom of one of the cake tins, such that no wasp would be able to escape.

6.3 Results

6.3.1 Egg laying and hatching dynamics of *M. fici*

6.3.1.1 Egg laying by *M. fici*

M. fici females lay their eggs (Figure 6.3) in closely-packed arrays known as egg masses (Figure 6.4), usually on the abaxial margins of leaves (Figure 6.4), two or three fully unfurled leaves down from the branchlet terminal shoot. Thus, the eggs are laid on leaves that are approaching maturity, and are neither too young nor too old. Occasionally, egg masses may be laid further towards the leaf midvein (Figure 6.5), or next to the midvein itself. In very rare instances, egg masses will be laid on the adaxial surface of leaves, but this was observed to occur only if the leaf had been twisted so that the abaxial surface was uppermost. The egg masses in all of the 'inverted' cases were observed to be less closely packed than normal. Eggs were inserted into the leaf tissue via a tubular pedicel approximately $23\ \mu\text{m} \times 14\text{--}15\ \mu\text{m}$ in outside diameter, an inside diameter of $11\ \mu\text{m} \times 6\text{--}7\ \mu\text{m}$, with a wall thickness of about $5\text{--}6\ \mu\text{m}$ (Figure 6.6). Eggs were ovoid with a reticulate-textured chorion and a prominent suture (Figure 6.3), and were approximately $230\ \mu\text{m}$ in diameter and $435\ \mu\text{m}$ in length, with a depth of approximately $240\ \mu\text{m}$. The chorion immediately surrounding the suture was not reticulated and was approximately $10\text{--}15\ \mu\text{m}$ wide and $200\ \mu\text{m}$ long, and projected above approximately $5\ \mu\text{m}$ the surface of the egg. Separate egg masses were often laid contiguously, and could form arrays of up to ten or more different masses. Once a particular site had been chosen, it would continue to be exploited. The trigger that caused contiguity to be broken was not determined. In extreme (although rare) cases, however, egg masses were observed to have been laid almost without a break around the entire margin of the leaf. It is not known whether contiguous egg masses were laid by the same female, but it is considered unlikely by the author since the spacing between the eggs and the angle at which the eggs were oriented within an egg mass, varied between egg masses (Figure 6.4).

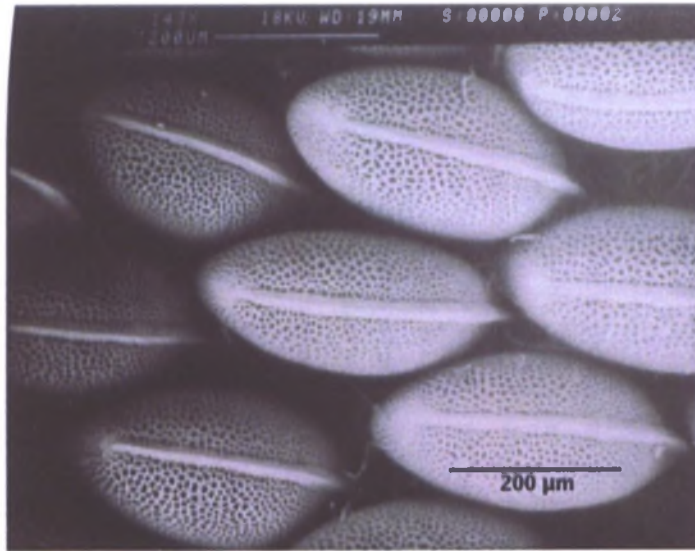


Figure 6.3: SEM of *M. fici* eggs (photo: Eren Turak, RBGS)

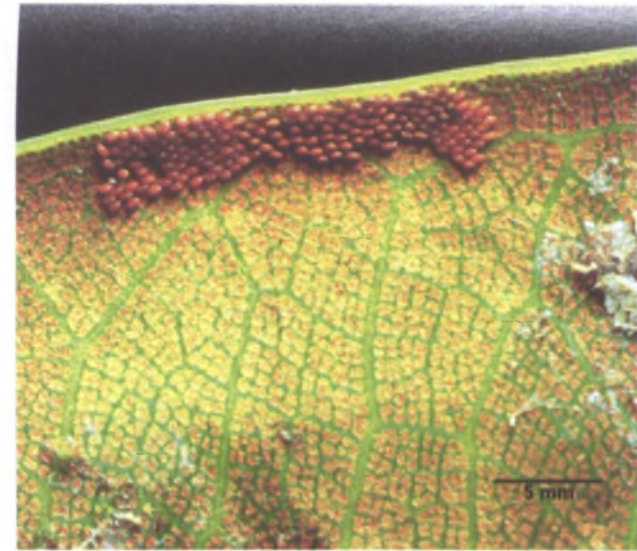


Figure 6.4: An aggregation of about eight *M. fici* egg masses (photo: RBGS)

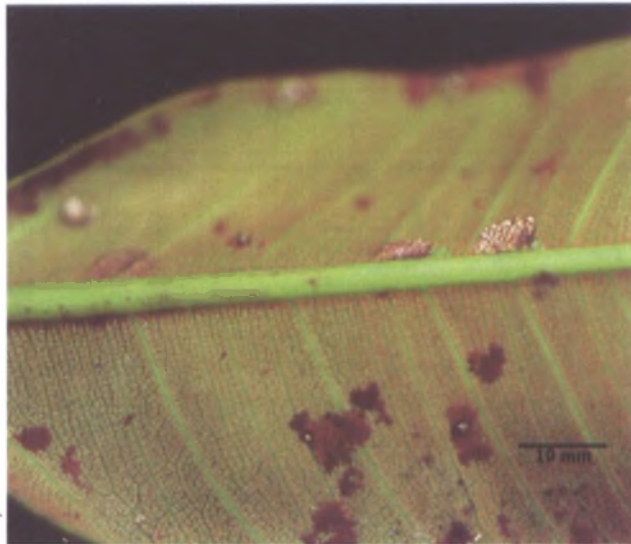


Figure 6.5: *M. fici* egg masses scattered over entire abaxial leaf surface

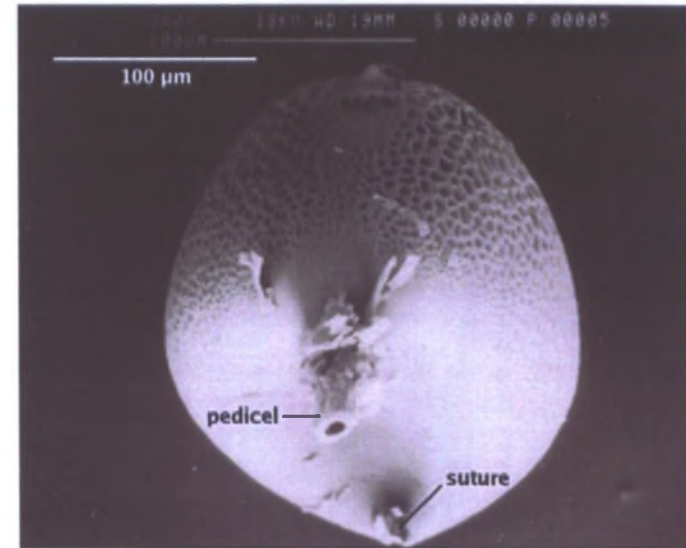


Figure 6.6: SEM of egg: pedicel is a hollow tube. (photo: Eren Turak, RBGS)

Table 6.1: *MFG* egg laying survey results, 1997 and 1998-99

Egg Survey	Variable	Numbers/means	Percentages
<i>MFG Nov. 1997</i>	Total number of leaves in survey	65	—
	Number of leaves lost from survey	65	100.00%
	Total eggs laid/counted	94640	—
	Number of days survey run where eggs were laid	39 days	—
	Raw average eggs laid/leaf	1456.00	—
	Mean eggs/leaf at egg lay \pm std error	611 \pm 24	—
<i>MFG 1998-99</i>	Total eggs laid/counted	13335	—
	Total leaves in survey	95	—
	Number of leaves lost to lawnmower ¹	2	2.11% ³
	Total leaves remaining after mowing incident	93	97.89% ³
	Number of egg masses lost to lawnmower (12+7)	19	3.79% ³
	Number of eggs lost to lawnmower (298+198)	496	3.72% ³
	Total eggs after two leaves lost ²	12839	96.28 ³
	Mean eggs laid/leaf (lost leaf data removed)	26.83 \pm 8.38	—
	Total egg laying period	33 days	—
	Mean eggs laid/leaf before leaves lost \pm std error	141.9 \pm 94.85	—
	Mean eggs/egg mass	25.8 \pm 14.45	—
	Mean days to egg hatch	56.9 \pm 0.18 days	—
	Total number hatchlings counted	8379	62.84%
	Total number settled hatchlings counted	7773	92.77%
	Number of eggs surviving to egg-hatch	9593	72.63%
	Number of counted eggs predated	3246	24.18%
	Number of counted eggs lost to lawnmower	496	3.71%
	Number of counted eggs not hatched	1973	14.80%
	Number of eggs hatching successfully	7620	62.84%
	Gross total number of eggs lost	5715	42.86%
	Total number of eggs lost minus eggs on lost leaves	5219	39.15%

¹ Branches trailing to ground: two survey leaves (*EE* and *EG*) run over by lawnmower² All values from here down are based on the data adjusted for the loss of leaves³ Percentage of original total (13335)

Table 6.2: MFG and AGS life history studies: basic results and causes of mortality

Survey	Variable	Numbers/means	Percentages
MFG 1998-99 Life History	Total number hatchlings counted	1343	—
	Total number settled hatchlings counted	1343	—
	Number of hatchlings observed removed by predation	382	28.44%
	Number of hatchlings attributed lost to predation (underestimate)	606	45.12%
	Mean number of hatchlings settled per lerp \pm std error	13.1 \pm 0.99	—
	Total number of leaves dropped from tree during survey	4	6.15%
AGS 1998-99 Life History	Total number hatchlings counted	13335	—
	Total number settled hatchlings counted	11499	86.23%
	Mean number of hatchlings settled per lerp \pm std error	51.5 \pm 2.8	—
	Number of hatchlings removed by predation	1829	13.72%
	Number of counted emerged parasitoid adult (from exit holes)	636	4.77%
	Estimated total number of parasitoids (incl. those on fallen leaves)	1431	10.73%
	Number of hatchlings stuck emerging from eggs	7	0.05%
	Number of juveniles stuck or drowned in honeydew	23	0.17%
	Number of emerging adults dead, stuck in final moult cuticles	32	0.24%
	Number of adults dead, stuck in honeydew	14	0.11%
	Number of adults successfully emergent	1951	14.63%
	Number lost to cohort, attributable to leaf drop	8498	63.73%
	Total number of leaves dropped during survey	40	55.56%
	Mean overall number of <i>M. fici</i> juveniles lost per day \pm std error	217.9 \pm 38.7	1.64% \pm 0.29%

Figure 6.6 shows that the *M. fici* egg pedicel is hollow, and it is possible that water is taken up from the leaf through this channel (Buckner *et al.*, 2002; Byrne *et al.*, 1990; Constance *et al.*, 1994). The mean time between oviposition and hatching, 56.9 ± 0.18 days (see Table 6.1 above) is discussed in Section 6.4.1.1.

Egg masses laid later in the period tended to have fewer eggs than those laid earlier (Table 6.2). Mean egg mass numbers per leaf for the two surveys, and mean numbers of eggs per egg mass for the second *MFG* egg study are also presented in Table 6.1 (see above). Mortality for the second study is also given in this table. Although no predator was observed eating the eggs, the damage caused was consistent with that caused by a predator. The lack of observations of predators eating eggs suggests that they were mostly active at night. The damage to the eggs exhibited itself in the form of egg cases being truncated sharply just above the junction of the pedicel and the shell (vitelline envelope and chorion). Other possible causes of egg mortality may have been weakly inserted eggs dropping out of the leaf, or weakly inserted eggs being brushed from leaf surfaces by leaves rubbing against each other or branchlets in high winds.

6.3.1.2 Hatchling ‘migration’ and fates

Psyllid hatchlings were found to be highly mobile: study leaves without any egg masses at all were found to be colonised by psyllid hatchlings, and in sufficient numbers to produce several lerps of average size. This meant that the hatchlings must have been either actively migrating (i.e., walking) from other leaves, or dropping from higher leaves and managing to land on and hang on to those below. The tenacity with which the juvenile psyllids appeared to be able to survive disruptive events such as the loss of their protective coverings (see Section 6.3.2.1.1 below), and their capacity to recover from such events, was quite remarkable. Once the hatchling first instars have found a suitable site on the leaf, they settle down and begin feeding.

The empty exoskeletons of hatchlings and settled juveniles were often found on the leaf surface, either in isolated positions or on lerp sites. The cause of death was attributed to the feeding activity of lacewing larvae, which use their mouthparts to remove the fluid contents from their prey. Predation by lacewing larvae on juvenile psyllids was observed in the laboratory, where green lacewing larvae (Family Chrysopidae) attacked hatchlings in this manner. Lacewing larvae were also observed under the microscope attacking relatively new lerps, of between five and fourteen days in age. The thickening and solidifying nature of the

lerp wall (see Section 6.3.2.1.1) appeared to be no barrier or deterrent to the lacewings' ability to feed on the psyllid juvenile inside. The lacewings were able to penetrate the soft, transparent layer of lerp honeydew with ease. These lacewing larvae were covered in psyllid wax tubes, rather than emptied psyllid cuticles, giving the lacewings a pronounced 'woolly' appearance. On one occasion, two lacewing larvae were introduced into an arena containing psyllid hatchlings, and before 30 seconds had elapsed, the larger of the two lacewings attacked and commenced feeding on the smaller (Bar and Gerling, 1985).

6.3.2 Growth and development of *M. fici* juvenile instars

6.3.2.1 Lerp components and structure

6.3.2.1.1 Notes on lerp states

A number of lerp states was observed in the main *in situ* field study: the list below names and defines these states:

- 'Soft' indicated that the lerp was pliable or 'rubbery';
- 'Hard' indicated that the lerp is stiff and not pliable (usually the case after several days of prolonged conditions of low atmospheric humidity);
- 'Labile' was a condition between 'soft' and fluid;
- 'Dissolved' indicated that the lerp honeydew was in a liquid or fluid state, but had not flowed across the leaf surface;
- 'Flowed' indicated that the liquefied lerp material had actually flowed across the leaf surface in a pool;
- 'Exploded' described the case where the lerp had appeared to have done just that, and was the result of soggy, tumescent lerp material succumbing to the force of gravity and falling from the leaf surface;
- 'Midvein lerp reformation' (*MVLR*) refers to the aggregation of juvenile psyllids remaining on the leaf surface after lerp 'explosion'; this aggregation always took place along the abaxial surface of the leaf midvein.

6.3.2.1.2 Initial (lerp-forming) stages

The *M. fici* lerp is an amorphous, very flexible structure, in contrast to, for example, the fine net-like or solid lerps constructed by *Cardiaspina* spp. (White, 1970a; White, 1970b; White, 1972). The lerp is formed from products of psylloid feeding, and is composed mostly of honeydew excreted by the psylloids as a waste product (Figure 6.7), which sets within a matrix of wax tubes (Figure 6.8), also produced by the psylloid. The wax tubes are produced as a secondary metabolite and are excreted via pores arranged in paired, approximately 30 μm diameter circular structures on the sixth abdominal segment (Figures 6.7 and 6.9). The pores extrude a large number (estimated by the author to be at least one thousand) of single wax filaments (Figures 6.9 and 6.10) which loosely associate upon exit from the pores to form the tubular structures. The wax tubes are produced by the juvenile psylloids from the first to fifth instars inclusive, the pores being lost at, or just before, the psylloid's last apolysis, which is the one from fifth instar to adult. The diameter of a single wax filament on leaving its pore of origin is approximately 0.7 μm (700 nm). The width of the completed tube is the same diameter as that of the pores on the psylloid, i.e., approximately 30 μm , and this diameter did not appear to change markedly over the life of the juvenile. The measurements of these structures given above were estimated using the scale bar placed on the micrograph by the SEM, in Figure 6.9. Extruded tubes were observed to dissociate into their component filaments when encased in honeydew (Figure 6.10). Once having chosen a suitable lerp site, the psylloid juveniles did not start to excrete honeydew or wax tubes immediately. The first droplets of honeydew (Figure 6.7) were not voided by the hatchlings until approximately 24 hours after settling, and wax tubes did not appear from their abdominal pores for a further 24 hours after the appearance of the honeydew (Figures 6.7). The wax tubes gradually built up to form a fluffy cover over the psylloids as the tubes lengthened (Figure 6.11 below). The excreted honeydew and the mass of wax tubes started to combine once sufficient of these ingredients had been produced, at between four and five days. Full coverage of the juvenile psylloids by lerp material was not complete until about seven days after settling had started at a particular spot. When the lerps had reached between 12 and 14 days in age, the honeydew and wax mixture began to become more opaque and less fluid, although still remaining quite elastic. The juveniles by this time resided under a dome-shaped structure, its 'ceiling' just clearing the psylloids. All apolyses and ecdyses took place under the lerp, with the exception of the fifth instar to adult ecdysis, which the psyllid underwent exposed on the abaxial leaf surface.

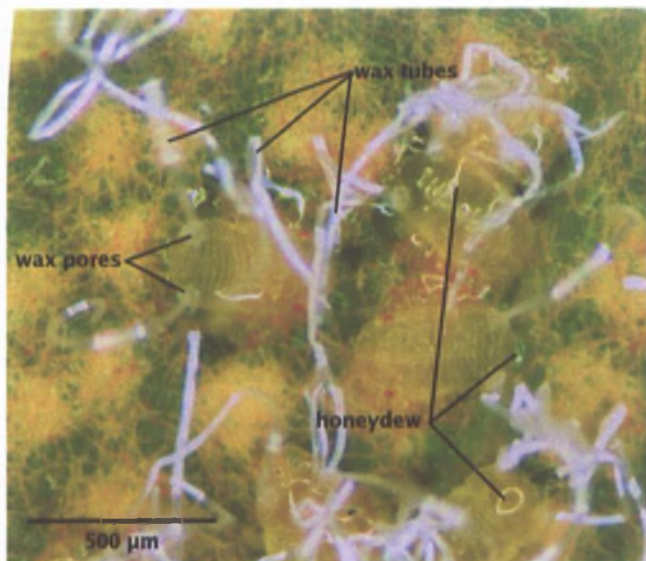


Figure 6.7: Settled *M. fici* hatchlings

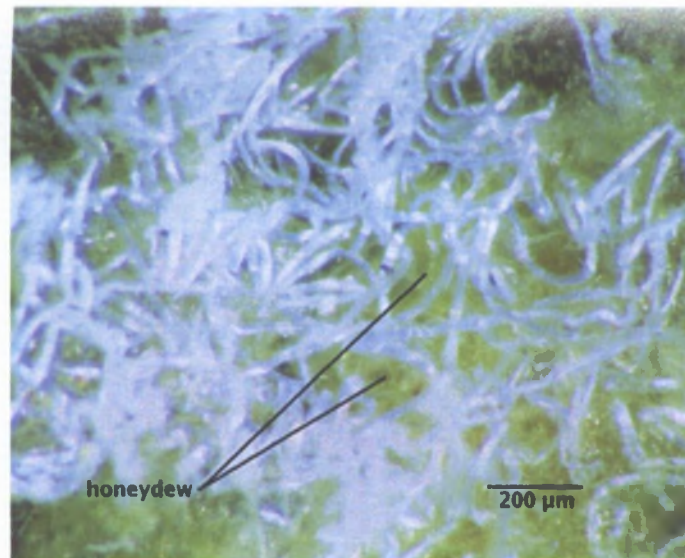


Figure 6.8: Developing matrix of wax tubes

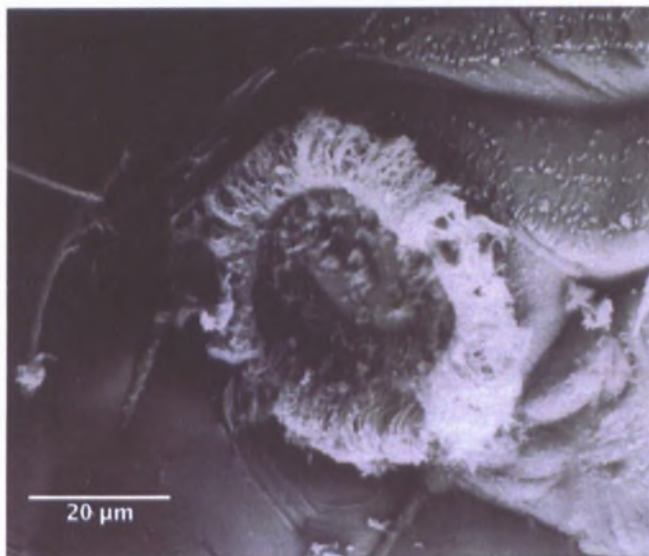


Figure 6.9: *M. fici* wax pore structure, $\varnothing \approx 30 \mu\text{m}$

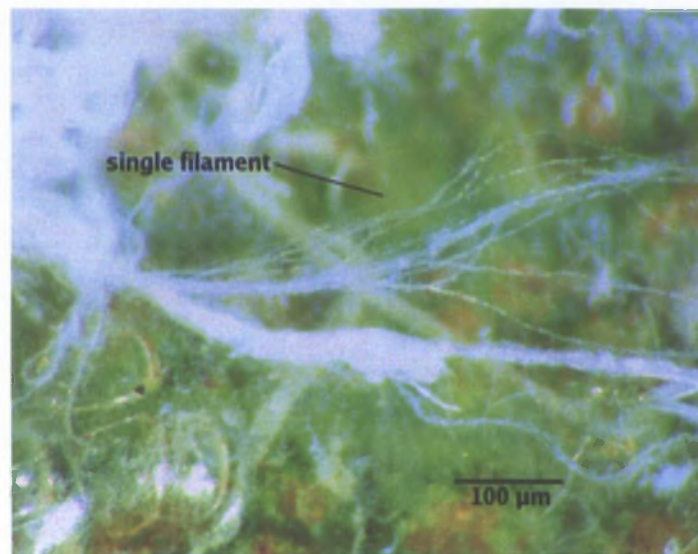


Figure 6.10: Psylloid wax tube disintegrating into component filaments

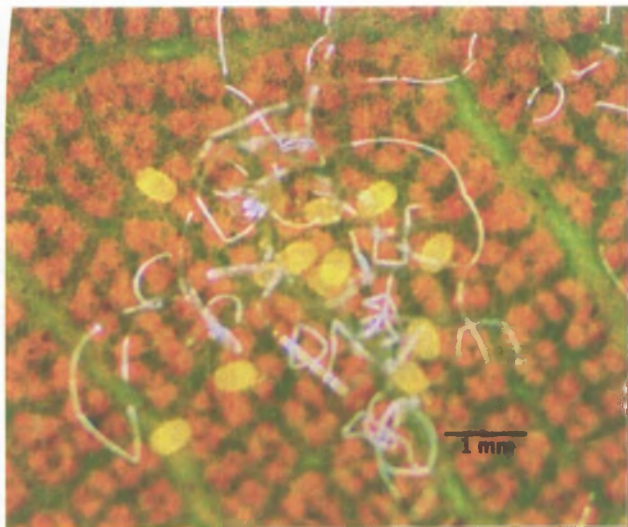


Figure 6.11: *M. fici* lerp at initial stage

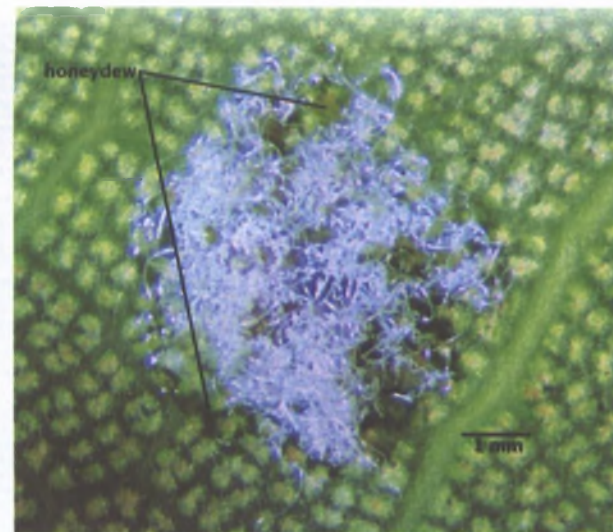


Figure 6.12: Lerp closure

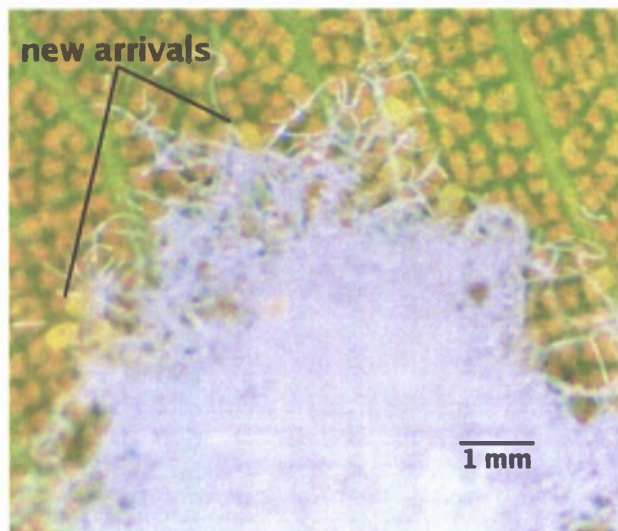


Figure 6.13: Recent recruits at lerp periphery

6.3.2.2 Life cycles stages within the *M. fici* lerp

Once a lerp had been started, or an aggregation of hatchlings had at least settled in one spot, more hatchlings appeared to prefer to join the establishing lerp, rather than set up a new one. A 'solid' core formed where the oldest psylloids had settled, the lerp gradually spreading outwards as younger recruits' honeydew and wax production began. Aggregation appeared to occur at least until another lerp was started, when new hatchlings would then be attracted to that lerp. The end result of the aggregative activity was lerps containing more than one instar of psylloid juvenile. Although the age profile of the lerp would tend, in the early stages, towards younger psylloids at the periphery, and older psylloids in the centre, all lerps dissected in the laboratory showed that the psylloids appeared to have a tendency to move about under the lerp after it had closed over the psylloids, so that the initial rather even population age gradient as described became disrupted.

Ecdysis from first to second instar occurred several days after psylloids were completely covered by a 1 mm layer of accumulated honeydew and wax tube mixture (Figures 6.11 and 6.12 above). The arrival of further hatchlings around an already closed lerp (Figure 6.13 above) further increased the lerp's diameter, the new portion closing over the new arrivals in about the same amount of time as the existing sealed structure around which they had gathered. The following five figures (Figures 6.14–6.18) show juvenile instar stages from second to fifth, a mature lerp, and a female winged adult. Figure 6.17 below shows an example of the mature lerp, from which those psylloids successfully reaching adulthood have emerged. The final moult exuviae can be seen at a short distance around the lerp: these moult skins were held firmly onto the leaf abaxial surface by the meshing of tarsal claws into the matrix of leaf hairs by the emergent pre-adults. The adhesion of the exuviae to the leaf hairs was strong enough to permit accurate counts of successfully emerging adult psylloids. At least six parasitoid exit holes (several labelled on figure) can be seen, as can structures (also labelled) which are associated with the fifth instar and/or pharate adult (see Section 6.4.2 and subsections). Also labelled on Figure 6.17 are two *Psyllaephagus* sp. adults which have become stuck in their exit holes and died before they could escape the lerp. A third stuck *Psyllaephagus* sp. adult had managed to climb out of its exit hole but had become stuck on the upper surface of the lerp. This individual may be seen above the middle of the three labelled exit holes on the right hand side of Figure 6.17. Figures 6.14–6.18 are commented on further in Section 6.4.2 and subsections.



Figure 6.14: 2nd and 3rd instars



Figure 6.15: 5th instar

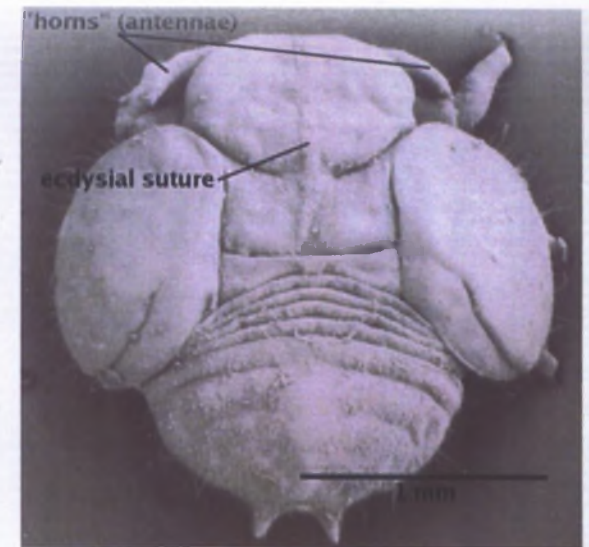


Figure 6.16: Pharate adult (*H5th*) (photo: RBGS)

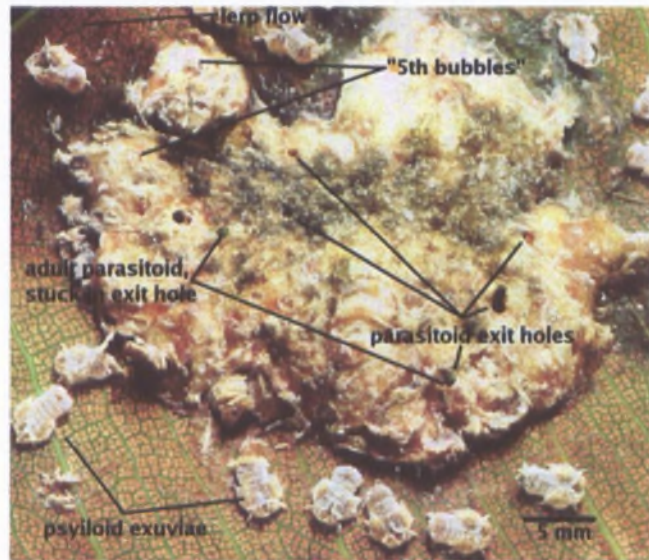


Figure 6.17: Mature *M. fici* lerp with final-moult exuviae



Figure 6.18: Adult female *M. fici* (photo: E Turak, RBGS)

6.3.2.3 Stadial duration of juvenile *M. fici* instars

The width and length data were analysed using a single linear regression model. The result of there was a relationship between head capsule width and length was accepted. Tests performed on the data set revealed it to be normally distributed with random variance. The variability in the data was relatively low, the regression coefficient (R^2) value for the test being 0.8632. Thus, a quite robust relationship between *M. fici* head capsule length and width is suggested in the data, and indicates an isometric growth relationship between the two variables.

The graph in Figure 6.19 below showing the relationship of head capsule width to length also gives the regression equation for this model:

$$\text{mean length} = 0.513 \text{ mm} + 0.439 * (\text{mean width at instar}) + \varepsilon \text{ (where } \varepsilon = \text{error term)}$$

The y-intercept value gives the mean head capsule length for the first instar.

Given the above relationship between width and length, the width data only were analysed with respect to date, using ANOVA. The results were statistically significant at the 5% error level, and gave mean values for seven groups (Table 6.3: see Section 6.4.2.3). Figure 6.20 below gives the seven groups with standard deviations and standard errors for each group. Figure 6.20 indicates the existence of five main size groups by the five groups on the left of the graph, which groups correspond to the five juvenile instars. Some lerps contained the whole range of instars, while others had a much narrower age range, depending on the way that recruitments had been made to a particular lerp. The issue of recruitment to and age structure of lerps will be discussed further in Sections 6.4.2.2 and 6.4.2.3. The two groups with the largest measurements equate to the pharate adult and revealed a number of distinct morphological differences, which all occurred in a specific sequence. These are reflected in Table 6.3 below and in the rightmost three columns in Figure 6.20, and are discussed in Section 6.4.2.4.

Table 6.3: Summary of *M. fici* development times from egg lay to end of adults

Period	Length of period in days
Mean population egg development time	56.9 ± 0.18
Total population egg-laying period	33
Mean population egg mass hatching period	3.8 ± 2.2
Mean population juvenile development time	39.6 ± 4.5
Est. mean time in 1st instar	12
Est. mean time in 2nd instar	7
Est. mean time in 3rd instar	6
Est. mean time in 4th instar	8
Est. mean time in 5th instar	7
Est. time in H5th (pharate adult)	2
Est. time in 1° PA (pharate adult)	1
Est. time in 2° PA (pharate adult)	1
Est. total, all three pharate adult stages	4
Total days adult population present	38
Population mean days post-egg including adults	49.3 ± 0.69
Total days duration of <i>M. fici</i> cohort	92

M. fici head capsules: width vs length

$$y = 0.587 + 0.438 \cdot x + e$$

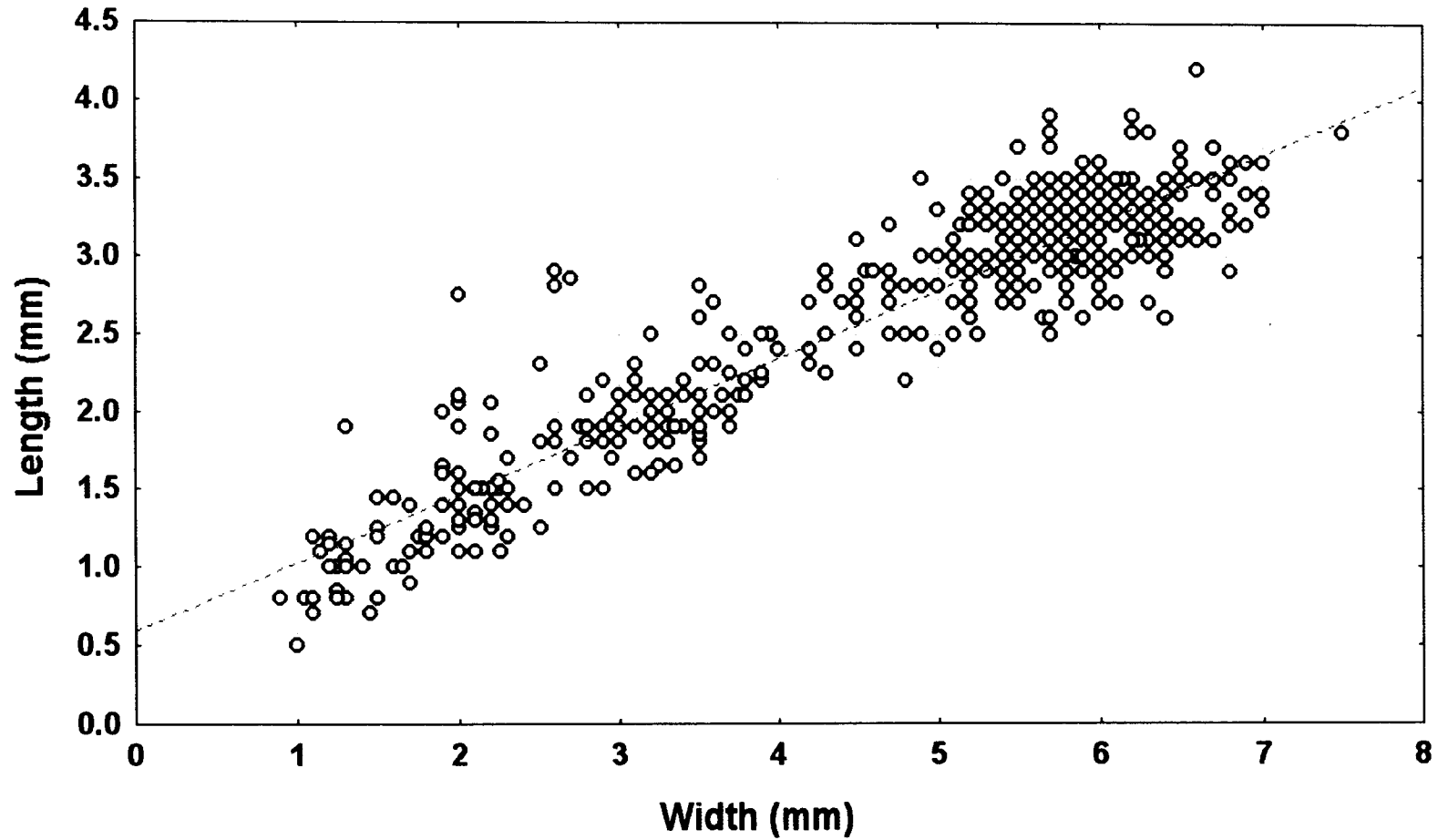


Figure 6.19: Relationship of *M. fici* head capsule widths to lengths

Instar sequence for *M. fici* (Mean/std dev./std err)

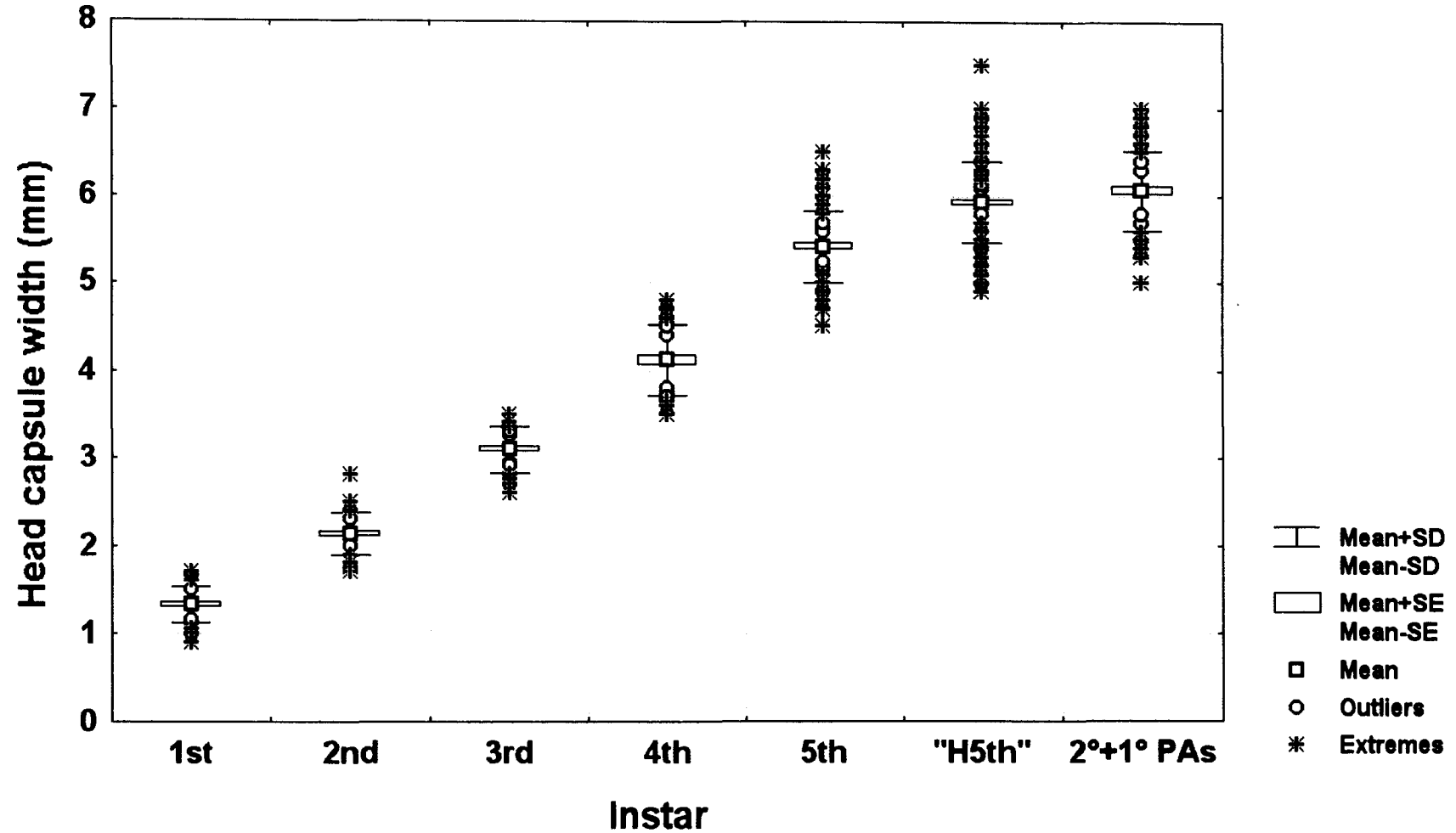


Figure 6.20: Seven mean *M. fici* head capsule width groups

6.3.3 Observations on the encyrtid parasitoid, *Psyllaephagus* sp.

During the AGS field survey, it was observed that female *Psyllaephagus* sp. stung (Figure 6.21) only into lerps of more than seven days in age. At no time were *Psyllaephagus* sp. females observed to sting exposed hatchlings or settled first instar psylloids; nor were they observed to sting into lerps that had not started to harden and cloud from their original viscous but still fluid and transparent state. Initial observations of parasitoids also revealed what appeared at first sight to be two species of *Psyllaephagus* stinging into psylloid lerps (Figure 6.22). These putatively separate species were of two distinct sizes, the smaller wasp being about half the size of the larger. The fact that the smaller wasp was seen with an ovipositor inserted into the lerp indicated that the parasitoid was a female. Specimens of the two sizes of wasp were sent to Dr Gary Gibson, Visiting Fellow at the Australian National Insect Collection (ANIC), CSIRO Canberra, who identified both types as being the same undescribed species of *Psyllaephagus* that is associated with *M. fici*. Female *Psyllaephagus* sp. individuals were also observed to consume material from lerps, while being watched under the microscope (Figure 6.23).

Observations under the microscope of psylloids, both in the field and in the head capsule study, revealed one or more blue or white threadlike traces (Figure 6.24) trailing from a number of juveniles of various instars. Closer study of this phenomenon revealed dark inclusions (Figure 6.24) inside the bodies of the affected insects, the inclusions being attached were attached to the proximal end of the blue or white threads. Individuals carrying the thread were dissected, and the dark inclusions then studied. The inclusions proved to be larvae of the obligate gregarious encyrtid endoparasitoid *Psyllaephagus* sp., one larva to an inclusion: the thread is a breathing tube, a structure common to the larvae of many encyrtid species (DF Hales, pers. comm.). The white colour of some of the breathing tubes observed (as in the examples in Figure 6.24) was possibly a result of age-related bleaching. Numbers of breathing tubes, and therefore parasitoids, varied between individual psylloids. A maximum of seven parasitoid breathing tubes was observed exiting from the body of one psylloid, although most parasitised psylloids contained one wasp larva, with approximately 30% of those psylloids observed to be parasitised containing two and three wasp larvae (see Section 6.4.3). Figure 6.24 shows at least two breathing tubes emerging from the body of a single juvenile psylloid. Figures 6.25 and 6.26 show the dorsal and ventral surfaces, respectively, of a *Psyllaephagus* sp. mummy; Figure 6.27 shows the pharate adult wasp present in the mummy shown in Figures 6.25 and 6.26, removed from the mummy case.

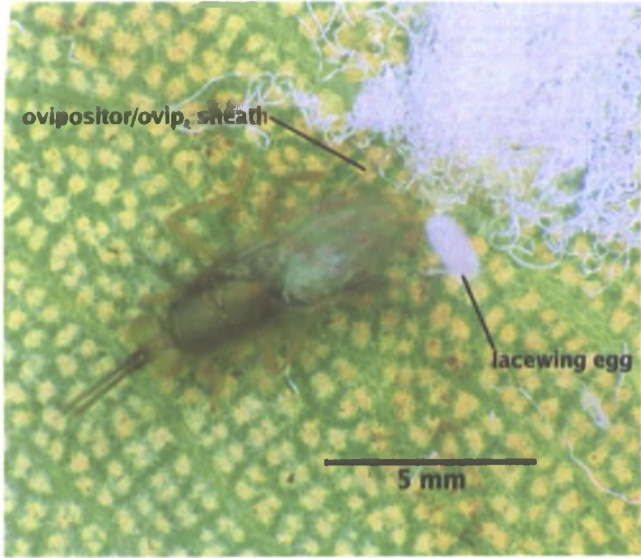


Figure 6.21: Large *Psyllaephagus* sp. female stinging

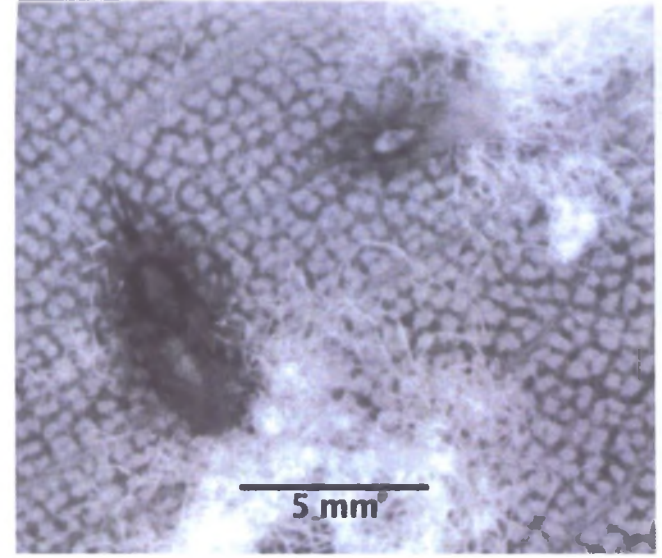


Figure 6.22: The two size forms of *Psyllaephagus* sp. females, both stinging



Figure 6.23: *Psyllaephagus* sp. female eating lerp components

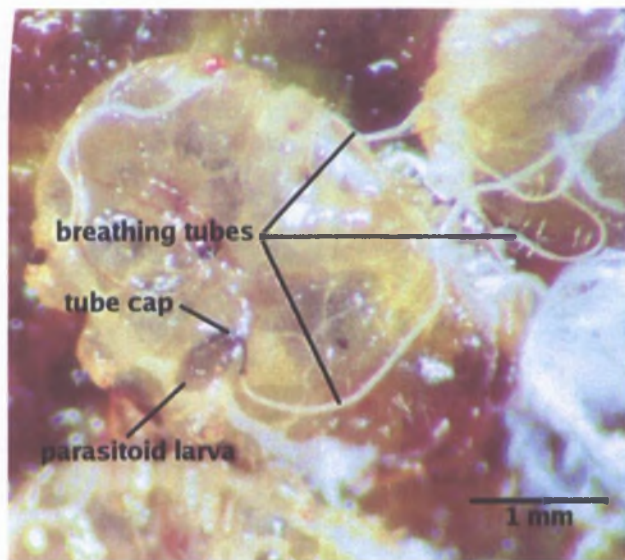
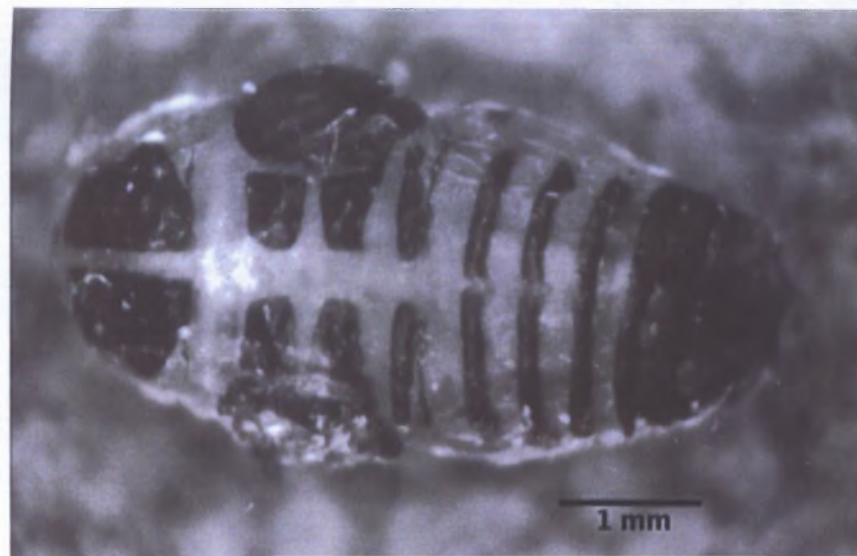


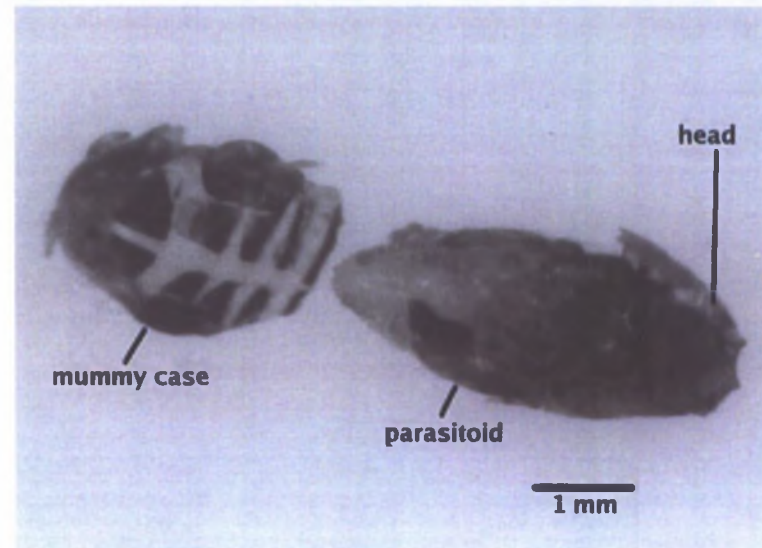
Figure 6.24: Psyllid larva with parasitoids



Figures 6.25: *Psyllaephagus* sp. mummy, dorsal surface



Figures 6.26: *Psyllaephagus* sp. mummy, ventral surface



Figures 6.27: *Psyllaephagus* sp. pharate adult ex mummy case

6.3.4 Reactions of lerps to relative humidity

Lerps exhibited two distinct, although related, reactions to conditions of elevated relative humidity (RH): in one reaction the honeydew of newly-established lerps would absorb moisture from the surrounding atmosphere and flow over the surface of the leaf (Figure 6.28 below); in the other reaction, older lerps similarly absorbed moisture, but instead of flowing over the leaf, became flocculent, and expanded to the extent that they split wide open, giving the appearance of having burst or exploded. In this latter instance, lerp covers would often fall from the leaf (Figures 6.29 and 6.30 below), carrying some of the psyllid juveniles with it, and exposing the remaining juveniles to the possibility of attack by predators and parasitoids. With sufficiently high humidity, sugary solution would dissolve out of the consolidated lerp and fall as a sort of “honeydew” rain (Figure 6.29). The psyllid, however, was observed to have a strategy to recover from this type of event: the rebuilding or reforming of a new lerp along the midvein of the leaf, by the inhabitants of one or more lerp populations whose cover had been lost (Figure 6.31 below), and was given the term “midvein lerp reformation”.

The lerp dimensions were found to be directly proportional to each other in both populations, and increased at about the same rate for both populations. Figure 6.32 (*AGS* population) and Figure 6.33 (*MFG* population) below show the coincidence of lerp dimensional changes with RH over time. The sudden decrease in mean maximum dimension values, which occurred on 28 December 1998 (day 14 of the *MFG* study and day 28 of the *AGS* study), immediately followed the maximum RH peaks that each population experienced. This sudden reduction in size was the direct result of the lerp becoming labile in the very humid conditions, and losing material (honeydew) to lerp flow. A plot of maximum and mean daily RH, mean daily temperature, and daily precipitation values, is shown beneath each of the two lerp dimension graphs, with all graphs having the same time scale (on the x-axes).

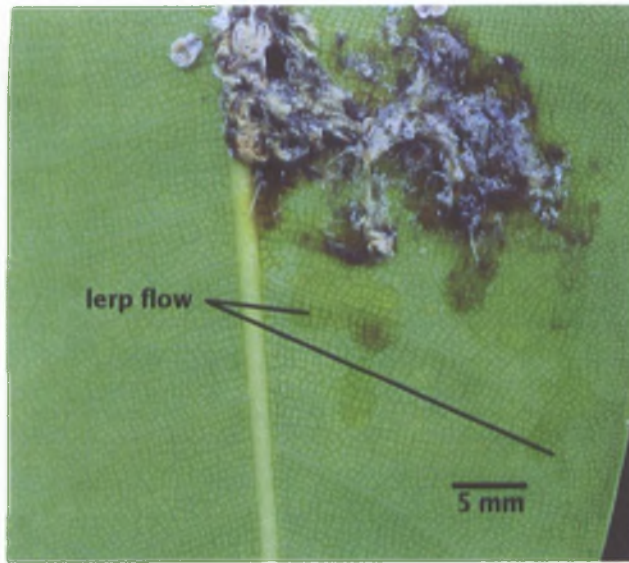


Figure 6.28: Lerp flow, induced by very humid conditions in glasshouse

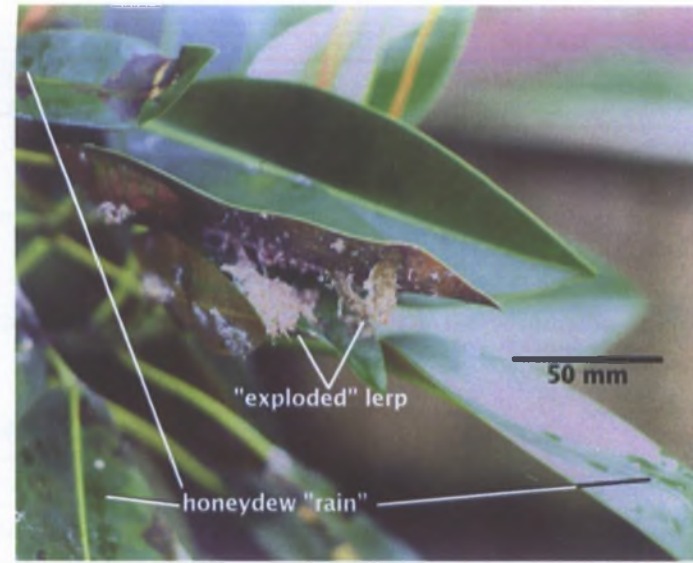


Figure 6.29: Lerp "explosion" and lerp flow



Figure 6.30: Lerp "explosion"

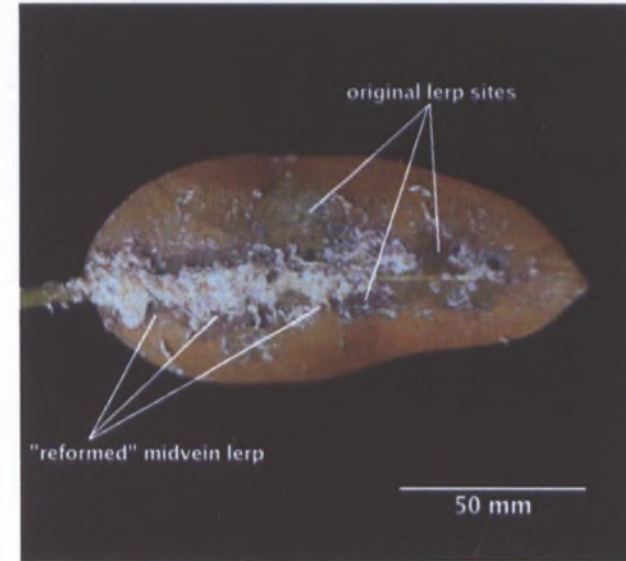


Figure 6.31: "Midvein lerp reformation" (MVLr)

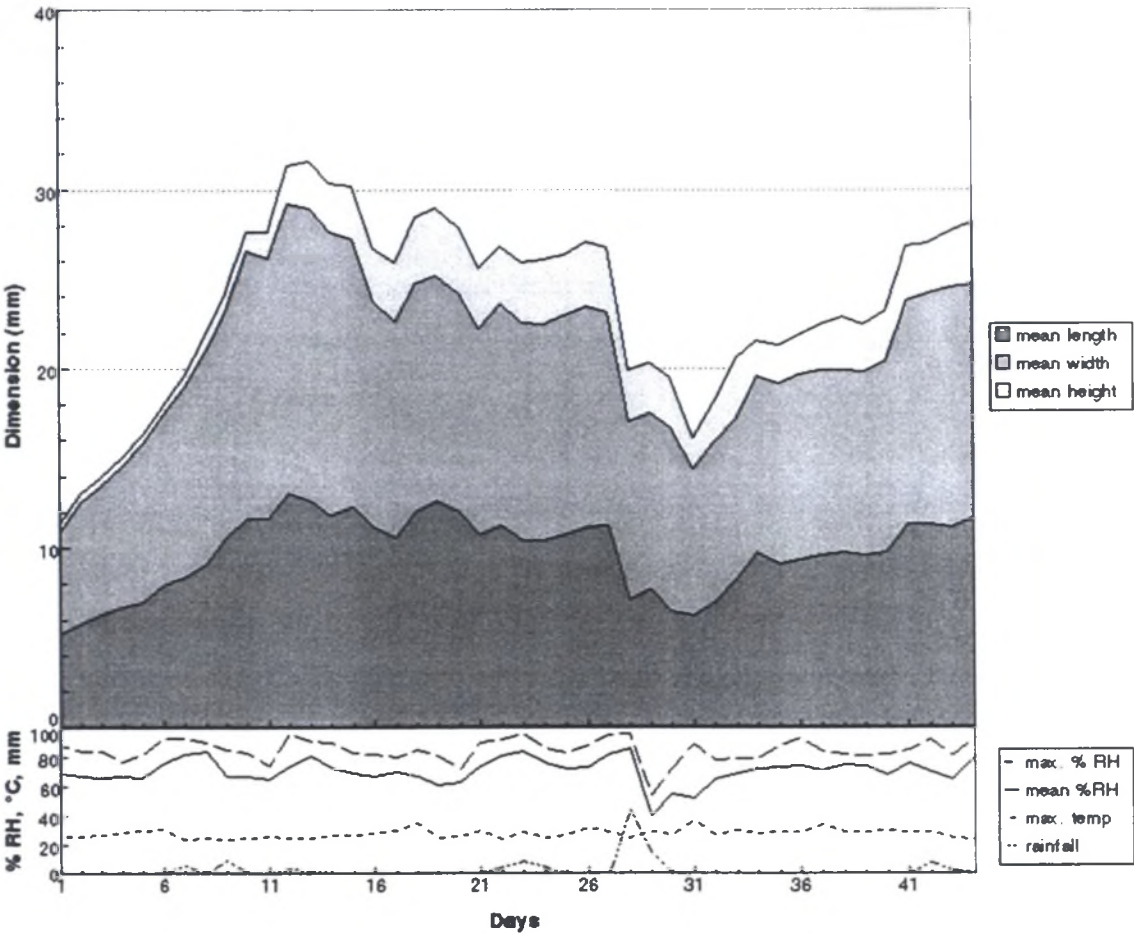


Figure 6.32: AGS lerp reactions to RH and temperature

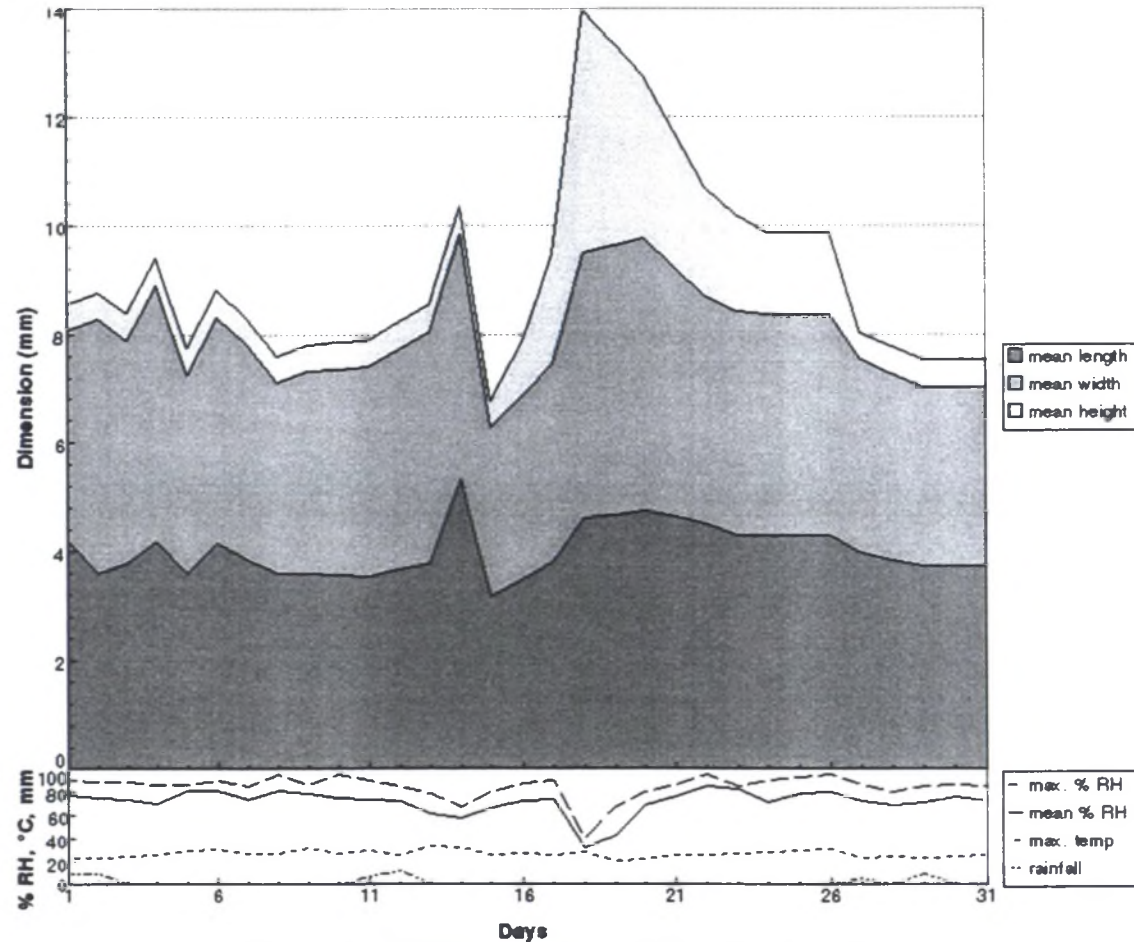


Figure 6.33: MFG lerp reactions to RH and temperature

6.3.4.1 The effect of leaf loss on *M. fici* and *Psyllaephagus* sp. survival

The rate of loss of the study population of leaves from *AGS* occurred at an average of one leaf per day, beginning some seven days after the main lerp ‘explosion’ event. 55% of the total study leaf population was lost by the end of the study period, with an estimated resultant death of 8500 (8498) psylloids and a smaller number (not able to be estimated) of *Psyllaephagus* sp. The estimate of 8500 psylloids lost represents a potential overall reduction of the psylloid cohort of close to 64%, depending on whether the psylloids on fallen leaves had reached the fifth instar or not. Fifth instars would be much more likely to survive to adulthood on fallen (desiccated or necrotising) leaves than earlier instars. The reasoning for this is that, since the midvein was the last part of the leaf to die, and most psylloids by this stage had congregated on the midvein in reformed lerps, it is possible that there may have been sufficient food to enable late-instar psylloids to reach the final moult stage. This was confirmed in a few instances when abscissant study leaves from *AGS* were brought back to the laboratory and placed in an insect-proof cage for observation.

6.4 Discussion

6.4.1 Egg laying and hatching dynamics of *M. fici*

6.4.1.1 Egg laying by *M. fici*

Insect egg development time varies highly between species, even between closely related species, but the long development time for *M. fici* observed in the field is somewhat unusual in comparison with most other insects. While some hemipterans (e.g., cicadas) can have a single generation time of thirteen years or even more (Ito, 1998; Marshall, 2001; Marshall and Cooley, 2000), most of that time is spent at the larval stage. *M. fici*, on the other hand, spends most of its time in the egg. Temperature profoundly affects all developmental stages in insects, including eggs. Eggs in winter periods will take longer to develop than those in summer, and may descend into a period of embryonic quiescence, where development is slowed down or suspended below a certain temperature threshold. Decreasing or shorter photoperiod is also likely to be a contributing factor to this phenomenon. Likewise, there is also an upper threshold or critical temperature above which embryonic quiescence may be initiated.

The long durations of both the egg-laying period of 33 days (Table 6.1) and the (mean) embryonic development period of approximately 57 days (Table 6.3) suggests a *K*-selected species (MacArthur and Wilson, 1967; Roff, 1992). The large total number of eggs laid, however, is consistent with an *r*-selected species, i.e., rapid turnover and/or large numbers in a cohort or population (MacArthur and Wilson, 1967; Roff, 1992).

The mean number of eggs per egg mass (26 ± 1) is likely to remain more consistent over the long term than the mean number of egg masses per leaf (19 ± 1); the mean number of eggs per leaf (142 ± 10), therefore, is likely to vary depending on the population size. Number of eggs in a cohort (and a generation as a whole) would be governed by such factors as predation of adults, temperature extremes outside the development thresholds, high relative humidity (see also Sections 6.4.2.1, 6.4.2.2 and 6.4.4), and number of leaves on trees suitable for egg-laying. This last item is also likely to be influenced by the numbers of psyllids in the preceding generation. If, for example, a plague population of psyllids in the preceding generation triggered a defoliation event, then egg numbers laid by the defoliating generation (for want of a better term) would be expected to be considerably lower than those laid by a succeeding generation with access to greater resources (i.e., *F. macrophylla* leaves), because of the loss of egg-laying sites to the defoliating generation.

Predation on the eggs was never directly observed, and determination of the cause of egg disappearance as predation is only inferential. The extent of egg losses that I was able to attribute to predation, more than a third of study eggs, was large compared with all other causes of mortality except for leaf fall. The failure to hatch of 14.7% of the total surviving eggs could have a number of causes, such as eggs having been unfertilised, or the embryo dying within the egg.

6.4.1.2 Hatchling ‘migration’ and fates

Hatchling ‘migration’ was an unexpected phenomenon, but hatchlings indisputably settled to produce lerps on leaves with no egg masses. A migrating psyllid could thus walk at least 600 mm before settling, based on the average length of a half-leaf lamina plus full petiole distance of 250 mm, doubled, plus an average internode distance between leaves of approximately 100 mm. Alternatively, the psyllid might have fallen from a higher leaf, as suggested above. As migrating psyllid hatchlings were not observed during the daytime during the field study, it would therefore have been most likely to have occurred at night. This apparent ‘migration’ also may have accounted for the disappearance of some of the first instars which I had recorded as having been eaten by predators. Nighttime observations with dim supplementary illumination would be necessary to clarify any possible migration. If migration were taking place, it could be hypothesised that it is as a response to overcrowding of hatchlings on leaves with an overabundance of egg masses (i.e., a density dependent response).

Lacewing predation of juveniles was not restricted to the freely wandering hatchlings: as reported in the results (Section 6.3.1.2), lacewing larvae were observed to penetrate through the material of the early-stage lerp wall and feed on the juveniles beneath. There are reports in the literature of lacewing larvae circumventing insect defensive structures (e.g., Muller, 2002). Once the lerp wall had reached a thickness greater than the length of the lacewing jaws, feeding on psyllid juveniles would be prevented. There would be limited time available, therefore, for lacewings to attack lerps, and this would limit the number of psyllids being eaten by lacewings while within the lerp: only the outer circle of psyllids would be eaten. The habit of lacewing larvae of placing empty prey exoskeletons on their backs would confound estimates of the numbers of psyllid predated by lacewings, since this phenomenon would remove dead individuals from the count. Numbers of prey bodies carried would necessarily depend on the size (instar) of the carrying lacewing larva. Those lacewings observed, however,

were actually covered in fragments of psylloid wax fibres, rather than psylloid cuticles. The fibres would most likely have been 'harvested' from very early lerps, where the honeydew had not yet amalgamated with the mass of wax tubes. This suggests that these particular individuals may have been feeding predominantly on psylloids within lerps, rather than on free hatchlings. Other chrysopids are known to use wax coverings of insects, e.g., woolly apple aphids, as camouflage (Milbrath *et al.*, 1993). This observation also suggests, however, that if the use of wax tubes as camouflage by lacewing larvae was more widely practised across the population of *M. fici*-feeding (green) lacewings, the number of empty psylloid cuticles lying on the leaf surface may have been a more accurate indicator of numbers fed on by lacewings, than previously assumed.

It is also feasible that the lacewing larvae may also have been feeding on the still-fluid lerp sugars, as omnivory, and consumption of saccharide solutions in particular, is known to occur in chrysopids (Limburg and Rosenheim, 2001). The observed cannibalism has implications for the effectiveness of lacewing larvae as predators (Rosenheim *et al.*, 1993) of *M. fici*. Given that this cannibalism would most likely be a density dependant phenomenon, and that the density of lacewing eggs and larvae was observed during the study to be very low (much less than one in every twenty leaves), the likelihood of two lacewings encountering each other would be low.

6.4.2 Growth and development of *M. fici* juvenile instars

6.4.2.1 Initial (lerp-forming) stages

The *F. macrophylla* leaf abaxial surface texture affords a certain degree of utility to psylloid hatchlings. The reticulate vein structure of the leaf is glabrous, or with sparsely distributed ascending hyaline hairs, and surrounds sunken interveinal (or intercostal) regions which are densely covered or partially filled with ferruginous, tomentose to felted hairs (Dixon, 2001b) (Figure 6.34). First instars were observed sitting directly on the intercostal regions, with their heads over, and their stylets in, the green veinal region (Figure 6.35). The hairs in the intercostal regions provided a stable substrate (a fluffy sort of matrix) for the psylloid juveniles to grasp firmly with their tarsal claws. The length of the tarsal claws in first instars are of a similar dimension to the width of the intercostal hair.

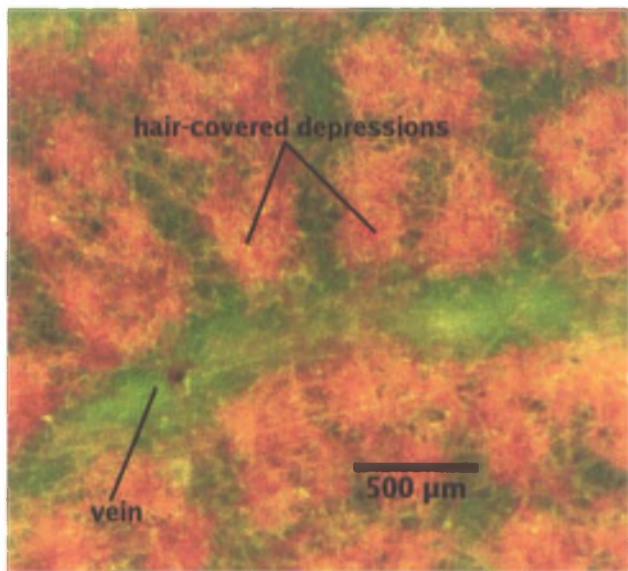


Figure 6.34: *F. macrophylla* leaf abaxial surface

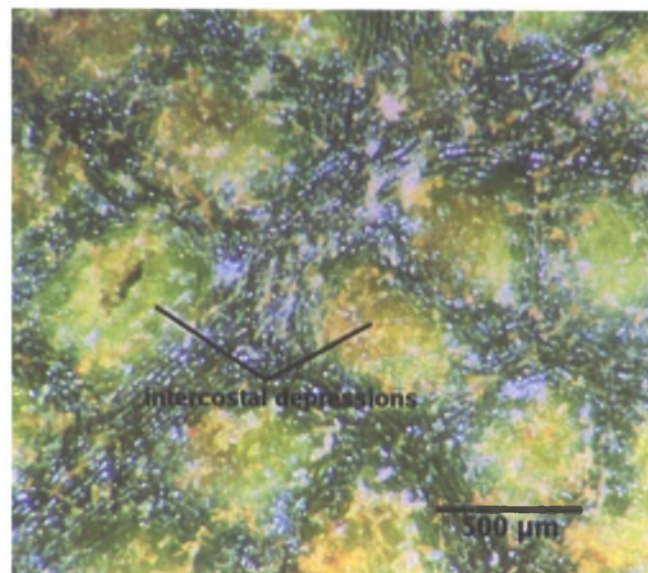


Figure 6.35: *F. macrophylla* leaf intercostal depressions

One possible reason for continued aggregation at a particular lerp site may be that the psylloids that aggregate at that site might have hatched from the same egg mass, or from the egg masses laid by the same female on that, or even another, nearby leaf. A possible advantage to ‘stragglers’ joining already established but unsealed lerp sites would be a ‘statistical’ protection by being part of a larger ‘herd’, especially as the established lerps’ older inhabitants would be producing honeydew and wax rapidly and in sufficient quantities to afford the newcomers some protection before the newcomers were able to begin producing their own lerp-building materials.

6.4.2.2 The age structure of the lerp population

The fifth instar psylloids were observed to go through several recognisable developmental stages. After the fourth to fifth instar ecdysis, the juvenile psylloid became flattened and discoid in shape, with the antennae more pronounced than formerly. The eyespots had moved closer to the front of the insect (earlier instar eyespots were set to the sides of the insect) and the adult compound eye structure was visible. I gave this stage the term “Horned 5th”, abbreviated to “*H5th*” in my field notes (Figure 6.16), and which referred to the horn-like (lateral) placement of the antennae from the head when seen in plan view. This change in shape from its more teretiform or sausage-shaped precursors was accompanied by the cessation of wax tube production and the appearance of a thin but general mealy coating on most external surfaces. This stage also coincided with the thinning and elevation or ‘bubbling’ of the lerp surface directly over the *H5th* (Figure 6.17). This ‘bubble’ was apparent on the surfaces of almost all active lerps (i.e., ones with living psylloids underneath) during the field observations. The attenuating ‘bubble’ would then split, revealing the *H5th* underneath. This bubbling was therefore termed a ‘5th bubble’. The ‘bubbling’ may have been caused by movement of the pharate adult in loosening its final mount cuticle, prior to emerging from under the lerp. This characteristic of the lerp was sufficiently common to be used as an indication that the appearance of the adults was imminent, as adults emerged from the lerps two to three days at the most after the ‘bursting’ of the ‘5th bubbles’.

The next distinct morphological change, which I termed the Secondary Preadult, abbreviated to 2° *PA*, was the stretching of the discoid shape back to something like the earlier more sausage-like form, with the junction of the thorax and abdomen starting to constrict markedly. Also at this juncture a set of about twelve or thirteen darkened, forward-pointing spines plus two spurs,

were noted on the lateral aspect of the rear tibiae. The tibial spines were just discernible in the early 2° *PA* stage as delicate, hyaline structures, and the hind wings had started to darken at this juncture. This indicated that the separation of the fifth instar cuticle, and the beginning of production of the adult cuticle, had already occurred, and that the psyllid was now present under the fifth instar cuticle as a pharate adult.

The final visible changes before eclosion were the completion of the elongation process and the darkening of the tibial spines and spurs as adult pigmentation developed. I termed this elongated, dark-winged stage the Primary Pre-Adult, or 1° *PA*. The 1° *PA*s emerged from under their lerps and walked several millimetres (but sometimes up to 75 mm) away from the lerp before eclosing from their fifth instar cuticles. The adult emerged by attaching its rear tarsal claws firmly into the abaxial or adaxial leaf surface, then climbing out of the exuviae at the ecdysial cleavage line on the dorsal surface of the head region (Figure 6.16). The newly emerged adult, a very light green in colour, then rested on the leaf surface where it had emerged, and expanded its wings. This was followed by hardening and darkening of the cuticle to a dark brown colour, with black head and upper thoracic plates, and the hardening of the wing veins. Some adult mortality was observed to occur by adults not being able to free themselves from the fifth instar cuticle, although this was a relatively rare occurrence, with perhaps 2% of all observed emergent adults suffering this fate. An even smaller percentage of adults failed to fully expand their wings.

The results of the analysis of the head capsule width data confirm a very slight increase for the 2° *PA* and 1° *PA* stages. On the emergence of the adult psyllid, the empty exuviae would more or less retain the teretiform shape (see Figure 6.17). The exuviae were observed to be persistent on the leaf for at least three weeks after the adults had emerged. The persistency of the exuviae on the leaf was sufficiently robust and long-lived to be used as a representative count of the number of emergent adults from a particular lerp (or leaf, in the instances where the adult's lerp of origin was untraceable).

6.4.2.3 Stadi al duration of juvenile *M. fici* instars

The analysis of the head capsule data suggested that there were five instars between egg hatch and adult emergence, which agrees with the literature (Hodkinson, 1974; Hollis and Broomfield, 1989).

The durations of the first, fourth and fifth instars were relatively easy to observe via the transparency of the early lerp (up to approximately the first to second instar moult), and by the exposed nature of the fourth and fifth instars (fourth to fifth and fifth to adult moults) brought about by either the loss of lerp covers or the bursting of '5th bubbles'. The estimating of the duration of the second and third instars was a different matter, however, because of the integrity and opacity of the lerp covers during these stages. The length of these two instar stadia were estimated by subtracting the mean first, fourth and fifth instar durations from the mean juvenile (post egg) development time (approximately 39 days with rounding) and dividing by two. The estimated durations for the second and third instars for the generation observed were both nine days in the field during January and February 1999. From this result it appears that each of the first to fifth instars has approximately the same duration. Figure 6.20 indicates that mean post-moult head capsule size from first instar through to fifth instar was probably linear: this also suggested that the method of determining the estimates for second and third instar durations was reasonably accurate.

Given the mean head capsule measurements of the various instars, psylloid juvenile instars could be determined in field quite easily by even untrained personnel, by using a set of dial or digital calipers in 0.02 mm divisions, after first exposing the juveniles if necessary. The appearance of a particular instar could be used to predict adult emergence with reasonable accuracy. This information could then be used to determine the timing of control measures such as the spraying of insecticide. The estimate of stadi al length in juvenile instars (Table 6.3) provides the basis for a reasonably detailed degree days model (Chapter 7).

6.4.3 Observations on the encyrtid parasitoid, *Psyllaephagus* sp.

The apparent delay in stinging juvenile psylloids suggests that *Psyllaephagus* sp. does not lay its eggs into psylloids that are not old enough (i.e., younger than seven days from egg-hatch) to support a feeding parasitoid larva. This would have the effect of allowing parasitoid eggs to

evade lacewing predation since the lerp would be reaching a stage where lacewing mouthparts would no longer be able to reach the psylloids inside.

6.4.3.1 Possible polyembryony in *Psyllaephagus* sp.

The number of breathing tubes emerging from the bodies of many parasitised larvae indicated that there was more than one parasite in each of these hosts. This in turn suggested that either *Psyllaephagus* sp. has evolved polyembryony (Chapman, 1998; Grbic *et al.*, 1998; Ivanova-Kazas, 1972; Noyes, 1989, Nenon, 1976; Nenon, 1978; Walter and Clarke, 1992), or that more than one non-polyembryonic egg laid was within the juvenile psylloids.

Polyembryony is the generation of multiple clonal copies of eggs within a host from one to several initial eggs laid by the mother, and is known to occur relatively often in encyrtids, usually within lepidopteran hosts. At least one report occurs of a polyembryonic wasp with a hemipteran host: in this case a chalcid parasitoid, *Pseudorhopus testaceus* Ratzeburg, a parasitoid of a coccoid, *Physokermes hemicryphus* Dalman (Voinovich and Sugonyaev, 1993). Chalcidae and Encyrtidae both reside with Superfamily Chalcidoidea, and are closely related groups of taxa. This relationship suggests that the polyembryonic trait may have been conserved as a result of having high adaptiveness, rather than evolving independently.

Polyembryony in encyrtids results in the differentiation of two castes: an “altruistic” soldier (or *precocious*) caste, and a reproductive form (Cruz, 1981, Cruz, 1986b; Cruz *et al.*, 1990; Grbic *et al.*, 1997; Hardy, 1996; Utsunomiya and Iwabuchi, 2002). The soldiers are altruistic in the sense that they forgo their reproductive potential to defend their reproductive siblings. Polyembryonic eggs are predominantly female in those hosts which were relatively young at the time of parasitoid egg-laying (Ode and Strand, 1995), and the proportion of these female eggs developing into precocious larvae is relatively high compared with polyembryonic eggs laid in older hosts (Ode and Strand, 1995). Older hosts, however, may have a more nearly equal sex ratio. The roles of the precocious “defenders” (Cruz, 1986) are: to protect the reproductives from other species of parasitoids by killing the “interlopers”; and to control the ratio of male and female reproductives by killing males (Grbic and Strand, 1991; Grbic *et al.*, 1992; Grbic *et al.*, 1997, Hardy *et al.*, 1993; Ode and Strand, 1995; Walter and Clarke, 1992). Precocious larvae die within the host before reaching adulthood, often from natural causes rather than from injuries sustained in their defensive activities (Cruz, 1986; Grbic *et al.*, 1992).

Active defence of larval reproductives against predation is a significant adaptive advantage for polyembryony, since it promotes the maximisation of adult reproductives. Deliberate alteration of sex ratio is also employed by polyembryonic encyrtids (Grbic and Strand, 1991; Grbic *et al.*, 1992; Hardy *et al.*, 1993; Ode and Strand, 1995; Walter and Clarke, 1992), as another means of maximising numbers of adult reproductives. Environmental conditions such as ambient temperature and host hormone titres have also been observed to promote changes in caste numbers and sex ratios in polyembryonic encyrtids, e.g., *Ageniaspis fuscicollis* Dalman (Hymenoptera, Encyrtidae) (Nenon, 1976; Nenon, 1978).

Rates of polyembryonic multiplication can vary from two or three to more than one hundred within a single species: in the latter case is *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), a parasitoid of the leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Edwards, *et al.* 1998). Other encyrtids will produce a more restricted number (e.g., 10–12 from *Holcothorax testaceipes* Ratzeburg in *Phyllonorycter ringoniella* Matsumura and *P. blancardella* Fabricius (Lepidoptera: Gracillariidae) (Wang and Laing, 1989)). One example of large numbers of eggs that has been reported in the literature has been that of 1893 individual eggs generated from two eggs laid in a larva of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Manickavasagam and Kanagarajan, 2003). The most extreme case officially presented in the literature so far was 2,796 eggs polyembryonically produced by *Copidosoma floridanum* Ashmead (Hymenoptera: Encyrtidae) within a larva of *Trichoplusia* sp. (Lepidoptera: Noctuidae) (Ode & Strand 1995).

There was no conclusive evidence arising from the observations made during this project to confirm whether or not polyembryony is a reproductive strategy on the part of *Psyllaephagus* sp. The existence of multiple breathing tubes issuing from a single host, however, is suggestive of the possibility that polyembryony may be functioning in this species. It should be noted that numbers of polyembryonic encyrtid parasitoids within a single host can be as few as 2–12, not just thousands, and this relatively small number certainly matches the observations made of parasitoids in *Psyllaephagus* sp. The following discussion on the size-dimorphism in *Psyllaephagus* sp. females suggests that polymorphism could be one possible mechanism by which the two sizes of females could have arisen.

6.4.3.2 Possible reasons for the observed size-dimorphism of *Psyllaephagus* sp. females, including polyembryony

Polyembryony, if it is functional within *Psyllaephagus* sp. may be a key to the smaller size form of the *Psyllaephagus* sp., since body size in polyembryonic, as well as other, parasitoids has a negative correlation with clutch size (i.e., numbers of larvae) (Ellers and Jervis, 2003; Jervis *et al.*, 2003; Ode and Strand, 1995). The observation that the smaller form were only females *Psyllaephagus* sp. appeared to support maximisation of reproductive potential.

An alternative mechanism for the observed size-polymorphism in *Psyllaephagus* sp. females is *eusocial*, as opposed to polyembryonic, caste formation (Wilson, 1971). Eusocial caste-size differentiation operates in the sense of size as a function of differing roles, e.g., a soldier being larger than a worker, or a queen being larger than a soldier, rather than purely a difference in body size as a result of clutch size (see above). Eusocial caste systems occur in a number of insect orders (e.g., Embioptera, Isoptera, Hymenoptera). In Order Hymenoptera eusocial caste-based structures appear to be restricted mainly to Families Formicidae (Taylor, 1991) and Apidae (Michener and Houston, 1991), with eusociality occurring to a much lesser extent in Families Halictidae (and absent in Australian halictids) and Anthoporidae (Michener and Houston, 1991). There is no evidence whatsoever of a specifically eusocial caste system in *Psyllaephagus* sp. having evolved, although it could be argued that polyembryonic reproductives are analogous to queens. This analogy is tenuous, however, since the typical ongoing (long-lived) eusocial colony controlled by a solitary queen, spanning multiple (non-queen) lifetimes, does not occur in the single-lifetime cohort of juvenile polyembryonic parasitoids living in turn within a single host that has itself a determinate lifespan that ends with the pupation of the parasitoids. Based on what has been observed of *Psyllaephagus* sp. so far, size differentiation in this species cannot be ascribed to eusocial, caste differentiation or function: polyembryony, however, may be a much more likely candidate for producing the size difference. Weighing against the hypothesis of polyembryony in *Psyllaephagus* sp., is the fact that there do not appear to be any rival parasitoids to compete against – or none that have come to light so far. The selection pressure to promote polyembryony is missing in this particular respect.

Another possible alternative is differentiation by allometric growth within a population (Wilson, 1971). This type of size dimorphism (or other degree of size polymorphism) is not

that known as *sexual size dimorphism* (Coelho, 1997; Hemptinne *et al.*, 2001; Persons and Uetz, 1999; Reeve and Fairbairn, 1999). Sexual size dimorphism is the phenomenon of size differences *between* sexes (e.g., in spiders, where the female can be larger than the male by a factor of five, ten or more), not size differences *within* a sex. A possible mechanism by which the smaller female might have arisen may be as a result of being laid in an older host than those in which the larger females were laid, with the older host having greater resistance to the rapid growth of an endoparasite than that of a younger host; or a host of lower quality; or a host of smaller size itself, or perhaps even all of these (Mackauer, 1996; Nicol & Mackauer, 1999). The timing of egg laying by polyembryonic encyrtids will influence the sex ratio (Cruz, 1986a; Cruz, 1986b; Ode and Strand, 1995; see also Ueno, 1998) in at least one species, *C. floridanum* (Ode and Strand, 1995). Polyembryonic host age also affects the number of eggs laid: older hosts receive fewer eggs, and those parasitoids successfully reaching adulthood will attain a larger size as a result of the clutch size being smaller.

6.4.3.3 Mortality estimates for *Psyllaephagus* sp.

Apart from the host itself overcoming and destroying the parasitoid, the fall of leaves with parasitised psylloids made the precise evaluation of parasitoid mortality difficult: parasitoid death would not have occurred if such parasitoids had already pupated, or were in the process of pupating, when the hosts' leaves fell from the tree. In this situation, the parasitoids would no longer have been at a stage where they required further resources from the psylloid host, apart from a shelter in which to pupate. The survival of leaf and psylloid (on or off the tree) would not be essential. Parasitoid pupae might, however, be damaged by trampling, or by ground level predators. Since the leaves were lost at a fairly constant rate over the last half of the study period, leaves falling earlier in the range would carry lerps containing a larger number of immature psylloid instars than in those lerps on leaves falling later. It would be likely, therefore, that more parasitoids would die on leaves falling earlier than on those leaves falling later, where the parasitoids on the latter type of leaf had had more time to pupate, or to become close enough to pupation for leaf fall not to matter. This was confirmed by observing abscissant study leaves that had been brought back to the laboratory and placed in a small insect-proof cage. A number of adult parasitoids were observed to be stuck emerging from the lerps, in an analogous manner to adult psylloids failing to emerge successfully from their fifth instar exuviae.

The range of ages within lerps, as discussed above in Section 6.4.2.2, would also have a confounding effect on the accurate estimation of parasitoid mortality. The confounding arises as a result of fallen leaves which were supporting lerps in which at least some of the psylloids (and therefore parasitoids) were still in earlier stages of psylloid development than the fifth instar. Even though the majority of psylloids in the lerps might have already reached the fifth instar and therefore were able to support the successful pupation of the parasitoids, there might be some psylloids not sufficiently advanced (i.e., younger than fifth instar) to support their parasitoids through to successful pupation. Determining the ratio of sufficiently advanced to too young hosts was too difficult. The danger of a parasitoid's host being too young would become less over time for *Psyllaephagus* sp. individuals whose leaves and therefore hosts fell later: the younger psylloids in the age gradient would have been able to reach the fifth instar and therefore be able to pupate before or even after arriving on the ground. As the age range within lerps covered at least one instar increment, the actual numbers of psylloids lost in the worst case might have been of the order of about ten per lerp. With a leaf with up to four or five lerps, or with one large midvein lerp, this number could reach fifty psylloids per leaf, and thus affect the number of parasitoids lost, proportionate to the number of psylloids that had been successfully parasitised.

6.4.4 Reactions of lerps to relative humidity

As mentioned in Chapter 1 (*Introduction*) of this thesis, *M. fici*'s lerps are quite different from those of many other species of psyllids and psylloids, for the following two reasons, which are reiterated here for emphasis.

Many psylloids are either free living (*Diaphorina citri* Kuwayama, *Heteropsylla cubana* Crawford, *Psylla pyricola* Förster, *Trioza erythrae* del Guercio, etc.), gall forming (*Apsylla cistellata* Buckton, *Neopelma baccharidis* Burkhardt, *Phytolyma lata* Walker, *Trioza hirsuta* Crawford, *Trioza jambolanae* Crawford, etc.) or spin a lerp over themselves using a fine tracery (e.g., *Cardiaspina albitextura* Taylor, *Cardiaspina fiscella* Taylor, *Glycaspis brimblecombei* Moore, etc.) or a shell-like structure (e.g., *Cardiaspina densitexta* Taylor, *Lasiopsylla rotundipennis* Froggatt) of poorly soluble or insoluble starches (Gilby *et al.*, 1976; White, 1972). In distinct contrast, *M. fici* produces a lerp which is highly labile and ultimately soluble, but yet almost completely obscures the juveniles from view for most of their post-egg pre-adult life. While most psylloids are solitary insects, whether they are free living or not, it is

the exception to find psylloids (or psyllids) which *live communally* as do *M. fici* and *Macrohemitoma* sp. (Takagi & Miyatake, 1993).

The saccharide components of *M. fici*'s lerp are highly soluble in the early stages of lerp formation, and can at least partially dissolve at later stages (Figures 6.28 and 6.29 above). Rather than flow over the leaf surface, the more mature lerps become flocculent and heavy as they absorb atmospheric moisture. The lerps lack any strong attachment to the leaf other than the adhesive power of the lerp saccharides, and respond to gravitational force and fall to the ground (Figures 6.29 and 6.30 above).

The graphs of dimensions of study lerps on *MFG* and *AGS* (Figures 6.32 & 6.33 respectively) demonstrate the dimensional fluctuation with relative humidity. The peaks of the lerp dimensions coincide with peaks in the RH graphs, and *vice versa*. The highly labile nature of the lerp in conditions of high humidity is well demonstrated. The graphs give a good representation of the degree to which the lerp and therefore the psylloid were affected by the weather. The very means for the psylloid's protection thus suddenly became a liability and a source of danger (see Section 6.4.2.1 above).

The lerp flow reaction to humidity, however, may have a more detrimental effect on the psylloids' chances of survival than the 'lerp explosion' event. The following reasons for this are suggested:

- very young psylloids can become coated with fluid but still quite viscous honeydew, then consequently become stuck to the leaf, then drown and/or become preserved in the sugary solution;
- very young psylloids can be carried for relatively large distances over the leaf, to sites which are not necessarily optimal for feeding and growth, and may be stuck to the leaf by their dorsal surfaces.

A much higher proportion of the lerp population is at risk than those whose lerp covers fall off the leaf. The juveniles, because not directly attached or adhering to the lerp covering when residing under it, mostly seemed to be able to remain attached to the leaf abaxial surfaces by their tarsal claws and by the insertion of their stylets into the leaf tissue. Judging from the time

that the lerp explosion event occurred, the psylloids under the lerp were several weeks older than those affected by the lerp flow event, and would therefore have had a firmer tarsal grip on, and stylet insertion into, the leaf surface than if they had been younger.

The flow of honeydew on leaves with large numbers of early-stage lerps in many instances covers the entire abaxial leaf surface, and thus may inhibit gas exchange.

6.4.4.1 The effect of leaf loss on *M. fici* and *Psyllaephagus* sp. survival

The lerp-flow phenomenon appeared, from observation, to hasten the decline, senescence and abscission of affected leaves, compared with those leaves that had not been thus affected. The cause of the leaf decline may be attributable to the prevention of sufficient CO₂/O₂ gas exchange across the honeydew film for normal leaf tissue metabolic processes. The honeydew barrier may also have prevented the diffusion from the leaf of built-up ethylene, a plant hormone that triggers ripening, senescence and abscission (Abeles, 1973; Beyer *et al.*, 1979; Lieberman, 1979). There may have been other abscission triggers operating, either singly or in concert with the honeydew barrier, such as the plant's phytoalexin response to the buildup within the leaf tissue of secondary metabolites present in psylloid salivary secretions. A phytoalexin response to the injection and subsequent translocation of psylloid-originated (or triggered) compounds may contribute to the rapid senescence of psylloid-affected leaves (Hori and Miles, 1993; Miles, 1999; Miles and Örtli, 1993; Taylor and Miles, 1994). Study leaves observed to fall from the tree during the observational period had supported or were still carrying large numbers of psylloids (of the order of around 300). The dehiscent leaves were also those that appeared to have been the most comprehensively coated by flowed lerp honeydew. Whatever the causal factors may have been, the loss of so many leaves (51% of the study population, a moderate *F. macrophylla* defoliation event) could be interpreted as the result of an adaptive mechanism in the tree by which it enabled it to divest itself of a debilitating insect load (Dayton *et al.*, 1992; Edwards *et al.*, 1993; Edwards and Wanjura, 1991; Edwards and Wanjura 1989). Such a mechanism would also have another beneficial side effect for the tree by reducing the size of the immediately succeeding psylloid generation. This would be effected by both the reduction in numbers of potentially reproducing females, and by the reduction of the number of leaves present and suitable for egg-laying for that part of the population of female psylloids which actually survived to mate and lay fertile eggs.

6.5 Summary of *M. fici* Phenology

The main discoveries arising from the *in situ* studies, with respect to the phenology of *M. fici*, are summarised as follows:

- the relatively long time spent at the egg stage, even during conditions of seasonally elevated temperatures;
- hatchling migration, which occurred over relatively large distances compared with body length, and at a developmental stage that appeared to be the most vulnerable to predation;
- the age (instar) range within the lerp;
- confirmation of Turak's identification of the honeydew composition of the major constituent of the lerp components;
- durations of the five juvenile psylloid instars
- quantifiable mortality in early instar stages resulting from lerp flow in high humidity;
- persistence of juvenile psylloids on the leaf, after lerp fall;
- midvein lerp rebuilding;
- the variety of reactions with which lerps responded to the activity of the psylloid itself (e.g., '*5th bubbles*'), and to fluctuating conditions of relative humidity;
- the extent of egg predation prior to hatching (*MFG* study data);
- two size forms of *Psyllaephagus* sp. females;
- the delay by *Psyllaephagus* sp. in stinging psylloid juveniles until late first or (more probably) early second instars were present;
- *Psyllaephagus* sp. is a gregarious parasitoid;
- the relatively large numbers of parasitoid larvae present within a single juvenile psylloid;
- *Psyllaephagus* sp. may be polyembryonic (like many other encyrtid endoparasitoids);
- putative polyembryony may be responsible for the smaller sized females;
- the lerp is a food source for *Psyllaephagus* sp.

The following chapter continues with the evaluation of the *in situ* field studies from the viewpoint of the population dynamics of *M. fici*. This exploration will be made using several types of survival analysis, including conventional life tables, and some more 'modern' numerical and graphical methods.

Chapter 7. Population Dynamics of *Mycopsylla fici*

7.1 Introduction

Whereas the emphasis of Chapter 6 was upon the phenology of *M. fici*, Chapter 7 is concerned with its population dynamics on and in relation to its host. This chapter includes the following main topics:

- Survival analysis, including life tables;
- The construction of ‘degree days’ models for *M. fici*, within the contexts of the Royal Botanic Gardens and Sydney Domain population and of the wider geographical, and therefore climatic, range of the psyllid;
- A comparison of psyllid activity with respect to leaves.

The following three sections (Sections 7.1.1 - 7.1.3) introduce these topics in their respective order.

7.1.1 Survival analysis interpretations of psyllid population dynamics

The aim of this part of the *in situ* field surveys was to study the population dynamics of *M. fici* post-egg through to adulthood, and to construct degree days models for the psyllid. Some attention was also given to the gross physical effects of the psyllid on host leaves.

The reasons for undertaking such studies were:

- to develop an age-specific life table to enable a better appreciation of mortality factors and dynamics affecting the psyllid;
- to be able to accurately predict psyllid population events on the scales of the single life cycle, and over the longer term (defoliation events);
- to be able to predict accurately the growth rates of the various stages of the post-hatch *M. fici* instars, in order to develop a control strategy.

Observational difficulties caused by the psyllid’s quasi-cryptic lifestyle (and which resulted, for example, in the inability to collect sufficiently accurate data for calculating useful population dynamics constants such as the ‘capacity for increase’ (r_c) (Laughlin, 1965) of

either the psylloid or its *Psyllaephagus* sp. parasitoid), have already been discussed in previous chapters, and need not be reiterated here.

The two egg-laying studies and the *in situ* field study data yielded much information on the general population dynamics of *M. fici*. The data sets thus generated were susceptible to a range of numerical analytical techniques, including several forms of survival analysis.

Life tables, developed in the late 19th and early 20th centuries by the insurance industry to predict risk, and therefore profit and loss, were originally the mainstay of elucidating survival and mortality trends in the biological sciences (Bellows *et al.*, 1992; Southwood, 1987), including medicine until the 1950s. The proliferation of increasingly more powerful and cheaper digital computers since the 1950s and the parallel development of advanced numerical (computational) methods have allowed the development of more complex models of analysis, such as Kaplan and Meier's 'Product Limit' method (Kaplan and Meier, 1958), analysis of covariance (ANCOVA) (Glasser, 1967; Jones and Crowley, 1989), multivariate analysis (Crouchley and Pickles, 1993; Kim and Xue, 2002; Guo and Lin, 1994; Prentice & Hsu, 1997) and regression models such as Cox's 'Proportional Hazard' model (Cox, 1959; Cox, 1972; Cox and Oakes, 1984; Lee *et al.*, 1992). Most of these analyses are nonparametric in nature, given the binomial nature of the central data: the subject either survives (it is 'censored' (Wald, 1949)), or it does not (it is 'completes'). Other techniques used in the interpretation and modelling of survival data include numerical simulation methods such as bootstrapping (Efron, 1982), and similar techniques combined with generalised linear models (GLIMs and GLMMs) (Tiller *et al.*, 1999). Life tables are still employed in survival/mortality analysis in general (Chi, 1994), but their use is now supplemented with or replaced by the more recent numerical and related graphical methods (Buonaccorsi *et al.*, 2003; Lancelot *et al.*, 2002; Pan and Chappell, 2002; Petersen and Adolph, 2001; Xu and Adak, 2002). 'Traditional' life tables, plus a number of the more recent numerical methods and their graphical interpretations, have been employed here in the analysis of psylloid survival and mortality from field-collected data. These analyses include the Kaplan-Meier Product Limit method, and the Cox Proportional Hazard tests.

7.1.2 Degree days models for *M. fici*

Day degrees (D°), alternatively known as degree days ($^\circ D$) (Allen, 1976; Baskerville and Emin, 1970; Morris and Fulton, 1970), are a particularly useful and widely used developmental index for many organisms ranging across the gamut of biological entities. This range includes viruses, bacteria, fungi, plants, and vertebrate and invertebrate animals (including terrestrial arthropods), whose development is either largely temperature dependent, or is influenced by other temperature dependent factors, such as sources of nutrients and nutrition, water availability, etc. The scientific literature is superabundant with references to this index. Degree days are employed to forecast insect and mite egg hatch and appearance of adults, the onset of particular diseases, crop harvesting times, response by plants to chilling, and in livestock management. This index is used in conjunction with lower, and less usually upper, development threshold temperatures. The term 'degree days' ($^\circ D$), is used in preference to 'day degrees' in this chapter, as the former term is more widely represented within the scientific literature.

Degree days is a thermal constant, K , or 'heat requirement' for a species, and is defined as:

$$K (^\circ D) = D (T - t),$$

where D = length of development period in days;

T = mean daily (24 hour) temperature for the period;

t = developmental threshold temperature.

Developmental threshold temperatures are determined as upper and lower limits, and may actually be higher than, say, a feeding threshold temperature (Morris and Fulton, 1970), and may also vary according to life cycle stage.

Depending on the degree of resolution required in the model, and the occurrence of 'substages' within a species' particular instar, the $^\circ D$ for an instar can be split up into components. Degree days for a particular instar (or its substage) are denoted by the use of subscripts, e.g., $^\circ D_E$ (or K_E) for degree days for the egg stage (Morris and Fulton, 1970).

Above the upper and below the lower thresholds, development will slow down considerably, or even cease. If time on the 'wrong' side of the particular developmental threshold is prolonged

beyond a certain limit, descent into quiescence will occur. The ‘developmental velocity’ (per cent development per day, or V) of a particular stage is defined as $100/D$ (Morris and Fulton, 1970), and may be linear or nonlinear, depending on life cycle stage (Morris and Fulton, 1970). Degree days for an insect are usually determined in the laboratory using a standard range of temperatures, usually in 5 °C steps, in order to establish the lower, and possibly upper, development threshold temperatures of that insect. Once the thresholds are established, the degree days value is then calculated for each stage of the life cycle, and for the life cycle as a whole (Morris and Fulton, 1970). Field based determinations of degree days are also calculated using mean daily temperatures (e.g., data from eight three-hour intervals per 24 hour period), or even simply from the daily maxima and minima ($(T_{24 \text{ hr max.}} + T_{24 \text{ hr min.}})/2$), where data with more frequent measuring intervals are not available. The resulting degree days model can then be used to predict various events of an insect’s life cycle. If the model reveals shortcomings in its ability to predict within certain limits (e.g., 5%), the model and the data which produced it are reevaluated, with further data being collected where necessary.

7.1.3 Comparisons of psylloid activity on five study trees

Variation in psylloid density may occur from tree to tree, and from time period (e.g., month, season, year) to time period. Data acquired during the egg-laying, life history and chlorophyll fluorescence studies was brought together in a set of tables to compare a number of aspects of the trees and the psylloid, with respect to dates, tree growing conditions, tree defoliation rates, and the visual condition of the trees (see Section 7.2.1.3, and Sections 7.3.3 and 7.4.3 and their respective subsections).

7.2 Materials and Methods

7.2.1 General materials and methods

To summarise information already given at the beginning of Chapter 6 (Section 6.2.1.1), the *in situ* field studies from egg hatch to adult eclosion were started on the *F. macrophylla* specimen project code *MFG*, on 11 December 1998. Death of early-instar psylloid juveniles resulted in this psylloid cohort becoming defunct in just over three weeks from the commencement of the study, and observations on this population were finally terminated on 11 January 1999. Observations of a psylloid cohort that had started hatching on 27 December 1998 were begun on 29 December. The chosen tree, *AGS* (Figure 6.2), was situated adjacent to the southeastern corner of the Art Gallery of NSW, in the Sydney Domain. Data obtained from the terminated *MFG* life history survey, however, were analysed with respect to hatching and settling events.

Daily observations in all four field studies commenced between 6:00 and 7:00 am, and on some occasions as early as 5:00 am. Observations were made on the chosen set of sample leaves until either no more eggs had hatched after three days (in the case of the second *MFG* egg-laying and hatching experiment), or until seven days after yellow sticky traps placed in the *AGS* canopy failed to yield *Psyllaephagus* sp. individuals (15 March 1999). The sticky traps were placed in the *AGS* canopy at the beginning of adult psylloid emergence from the lerps, for the specific purpose of determining the dates of extinction of the both the psylloid and the parasitoid cohorts. Traps were replaced on a daily basis until 16 March 1999.

Post hoc determination of the age range of the psylloid juveniles on *AGS* that were observed on the first day of the field survey was made by the use of observations and photographs of psylloids on the leaf having just emerged from the egg. The size of known newly-hatched psylloids was compared with the size of roaming and newly-settled hatchlings, and it was concluded that the most likely estimate for the start of hatching was two days before the start of observations, i.e., on about 27 December 1998. The first day's observations, therefore, represented a combined record of numbers hatched per day for the first three days of the post-egg cohort's life cycle (see Section 7.3.1). Many more new hatchlings and lerps entered the survey after the survey commencement date, and the duration of recruitment for a particular lerp was able to be estimated, and applied back to the first three days' hatchings. Observations made subsequently to those made on the first *AGS* survey day followed the formation and

growth of new lerps, as well as the growth of those lerps recorded on day 1 of the *AGS* survey (see also Chapter 6, Section 6.3.2 and subsections).

Using the methods already described in Chapter 6, data were collected daily during the *MFG* and *AGS* surveys for the following variables:

- number of newly hatched psylloids per day;
- number of dead psylloids observable, including probable cause of death;
- instar of psylloids; lerp measurements ($l \times w$, and $l \times w \times h$ when h began to exceed 0.5mm) made to the nearest mm;
- status of each lerp (soft, hard, labile, dissolved, flowed, exploded, reformed);
- number of parasitoids at lerp and behaviour;
- physical state of leaf;
- number of new psylloid exuviae per day;
- number of visibly parasitised psylloids;
- number of parasitoid exit holes;
- adult parasitoid presence;
- observations of parasitoid behaviour;
- status of substages immediately prior to adult eclosion;
- mating and egg laying;
- predation of adults by birds and spiders.

7.2.1.1 Survival analysis interpretations of psylloid population dynamics

7.2.1.1.1 *M. fici* survival analysis baseline: *MFG* 1998-99 egg survey data

Since neither the location nor the number of the eggs from which the 1998-99 *AGS* study psylloids hatched was known, estimates of egg numbers were generated using the results from the 1998-99 *MFG* egg laying and hatching survey, for which rates of hatching, survival and mortality were known.

7.2.1.1.2 Age-specific life-tables for *M. fici*: *AGS* field study data set manipulation

The *MFG* and *AGS* data sets were re-sorted with respect to *Leaf* and *Lerp* main variables, with further modifications being necessary to convert the date ranges of various events into simple time intervals of numbers of days, not date ranges, to allow the analysis program (survival analysis module of Statistica 5.1m (Statsoft, 1998)) to parse the data correctly. These modifications were made to estimate survival rates of both leaves and psylloids, and also to determine if there were any time-series effects in operation. Other information from the data set on various environmental influences such as sunlight, relative humidity, temperature, etc., was included in the analyses.

Other simple statistics (means and standard deviations) were calculated from a variety of data sources acquired during the course of the project's field work, in order to emphasise the importance of leaf age (position back up the branchlet from the shoot (= 0)) with respect to psylloid activity on any particular leaf, amongst other parameters. The treatment of much of this material concerns not only the survival of the psylloid, but comparisons between trees and geographical locations.

The survival analyses presented and discussed in this body of work were:

- life tables for psylloids on *MFG* and *AGS*;
- Kaplan-Meier product-limit analyses of lerp and leaf survival;
- Cox Proportional Hazard regression models for lerp and leaf survival.

The type of life table presented in this thesis is that of an age-specific life table (Southwood, 1987) (see Section 7.3.1.2), and uses the pivotal age of the age class x (e.g., eggs, juvenile

instars 1,...,5, etc.); the number surviving at the start of age x , l_x ; the number dying during age interval x , d_x ; the percentage mortality rate per age interval x , $100q_x$; and the percentage survival rate per age interval x , $100-100q_x$ (Southwood, 1987).

Data collected on leaf loss during the AGS field study were converted into censored/non-censored variables with respect to psyllid lerps ('*LERPICEN*') and with respect to leaves ('*ABSLFCEN*'). The censoring variable was the actual date on which a particular leaf and/or lerp was marked out of the survey as defunct (lerps) or fallen off the tree (leaves and lerps) (see Section 7.3.1.3).

7.2.1.2 Degree days models for *M. fici*

In an attempt to estimate the lower development temperature threshold for *M. fici*, consideration was given to the conditions under which the insect had evolved. The original range of *M. fici* has been obscured by the fairly indiscriminate dispersal of its host tree along the eastern seaboard of Australia by human (horticultural) activities. The current hypothesis, in the absence of other (contradictory) evidence, is that *M. fici* is subtropically adapted, since *F. macrophylla* is a part of a subtropical/Melanesian group of related *Ficus* species (Dixon, 2002; Dixon 2001a; Dixon 2001b; Dixon *et al.*, 2001). It could therefore be expected that the minimum temperatures that the psyllid is subjected to would be relatively high compared with most temperate insects, which typically have lower developmental threshold temperatures in the vicinity of 10 °C or less, and often not much more than 4 °C. Similarly, *Aonidiella aurantii* (California red scale, also known as citrus red scale), has a lower development threshold of 10 °C (AD Clift, pers. comm.). Since *A. aurantii* is also a subtropical insect, the 10 °C lower threshold estimate for *M. fici* was chosen as an appropriate first approximation.

7.2.1.2.1 Degree days estimates for *M. fici*: two degree days models using different temperature data sets

Psyllid development data used in the development of the degree days models, presented in Chapter 6, Section 6.3.2.3 and subsections, were derived from the following sources:

- *MFG* egg laying studies (1997-98 and 1998-99)
- *MFG* and *AGS* *in situ* field studies
- Head capsule study

- Records from the Bureau of Meteorology, Sydney (data for Sydney and seven other locations in southern and eastern mainland Australia, including Adelaide)

The lower temperature threshold was set to 10 °C according to the reasoning above, i.e., conditions pertaining for an insect putatively having evolved in a subtropical type of climate (Sutherst and Maywald, 1991). Daily mean temperatures were used in the development of the models (Watson and Beattie, 1995; Watson and Beattie, 1996) and comprised two sets: 'short term' mean temperatures, calculated from daily eight x three-hourly readings from 3:00 am, recorded by the Bureau of Meteorology at Observatory Hill in Sydney for the days on which the surveys took place; and 'long term' mean temperatures calculated from the mean daily temperatures, for period the 31 August 1995 to 30 March 2001 inclusive.

7.2.1.2.2 Degree days estimates for *M. fici*: using the two temperature models to compare eight separate geographical locations

Both short term and long term temperature sets used in the initial degree days models were applied to the eight separate geographical locations: all mean temperatures were based on 24 years' contiguous data. The data from the eight different geographical locations including Sydney were fed into the 'short term' and 'long term' degree days models, for the same range of dates as the 1998-99 *M. fici* *in situ* field studies.

7.2.1.3 Comparisons of psyllid activity on five study trees

To estimate seasonal and other time-related variation the following tables from the various data sets were prepared:

- Summary comparison tables of egg and lerp presence with respect to leaf position and tree; plus some other tabulated results relating to single tree information grouped by leaf position;
- Summary table of defoliation information for 5 trees, with respect to year.

Data from various studies used in the preparation of these tables, including the chlorophyll fluorescence study (see Chapter 8), the various *MFG* and *AGS* egg and life history surveys were pooled to produce the results presented in Section 7.3.3.

Table 7.1: *MFG* egg laying survey results, 1997 and 1998-99

Egg Survey	Variable	Numbers/means	Percentages
<i>MFG Nov. 1997</i>	Total number of leaves in survey	65	—
	Number of leaves lost from survey	65	100.00%
	Total eggs laid/counted	94640	—
	Number of days survey run where eggs were laid	39 days	—
	Raw average eggs laid/leaf	1456.00	—
	Mean eggs/leaf at egg lay \pm std error	611 \pm 24	—
<i>MFG 1998-99</i>	Total eggs laid/counted	13335	—
	Total leaves in survey	95	—
	Number of leaves lost to lawnmower ¹	2	2.11% ³
	Total leaves remaining after mowing incident	93	97.89% ³
	Number of egg masses lost to lawnmower (12+7)	19	3.79% ³
	Number of eggs lost to lawnmower (298+198)	496	3.72% ³
	Total eggs after two leaves lost ²	12839	96.28 ³
	Mean eggs laid/leaf (lost leaf data removed)	26.83 \pm 8.38	—
	Total egg laying period	33 days	—
	Mean eggs laid/leaf before leaves lost \pm std error	141.9 \pm 94.85	—
	Mean eggs/egg mass	25.8 \pm 14.45	—
	Mean days to egg hatch	56.9 \pm 0.18 days	—
	Total number hatchlings counted	8379	62.84%
	Total number settled hatchlings counted	7773	92.77%
	Number of eggs surviving to egg-hatch	9593	72.63%
	Number of counted eggs predated	3246	24.18%
	Number of counted eggs lost to lawnmower	496	3.71%
	Number of counted eggs not hatched	1973	14.80%
	Number of eggs hatching successfully	7620	62.84%
	Gross total number of eggs lost	5715	42.86%
	Total number of eggs lost minus eggs on lost leaves	5219	39.15%

¹ Branches trailing to ground: two survey leaves (*EE* and *EG*) run over by lawnmower² All values from here down are based on the data adjusted for the loss of leaves³ Percentage of original total (13335)

Table 7.2: MFG and AGS life history studies: causes of mortality

Survey	Variable	Numbers/means	Percentages
MFG 1998-99 Life History	Total number hatchlings counted	1343	—
	Total number settled hatchlings counted	1343	—
	Number of hatchlings observed removed by predation	382	28.44%
	Number of hatchlings attributed lost to predation (underestimate)	606	45.12%
	Mean number of hatchlings settled per lerp \pm std error	13.1 \pm 0.99	—
	Total number of leaves dropped from tree during survey	4	6.15%
AGS 1998-99 Life History	Total number hatchlings counted	13335	—
	Total number settled hatchlings counted	11499	86.23%
	Mean number of hatchlings settled per lerp \pm std error	51.5 \pm 2.8	—
	Mean population juvenile development time ¹	39.6 \pm 4.5	—
	Total days duration of <i>M. fici</i> cohort ¹	92	—
	Number of hatchlings removed by predation	1829	13.72%
	Number of counted emerged parasitoid adult (from exit holes)	636	4.77%
	Estimated total number of parasitoids (incl. those on fallen leaves)	1436	10.73%
	Number of hatchlings stuck emerging from eggs	7	0.05%
	Number of juveniles stuck or drowned in honeydew	23	0.17%
	Number of emerging adults dead, stuck in final moult cuticles	32	0.24%
	Number of adults dead, stuck in honeydew	14	0.11%
	Number of adults successfully emergent	1951	14.63%
	Number lost to cohort, attributable to leaf drop	8498	63.73%
	Total number of leaves dropped during survey	40	55.56%
	Mean overall number of <i>M. fici</i> juveniles lost per day \pm std error	217.9 \pm 38.7	1.64% \pm 0.29%

¹ Data from Table 6.4

Table 7.3: Survival analysis for *MFG* and *AGS* summer cohorts

Tree	Variable	Eggs laid	Eggs dead	Hatchlings counted	Hatchlings dead	1sts – 5ths	1sts – 5ths dead	5ths	5ths dead	Adults
<i>MFG</i>	<i>l_x</i>	1805¹		1343		961 (1159)		0 (300)		(196)
	<i>d_x</i>		462¹		382		961 (661)		(104)	
	% apparent survival (100 – 100 q_x)	—		74.40%¹		71.56% (86.28%)		0% (25.90%)		(65.51%)
	% real survival	—		74.40%¹		53.24% (64.19%)		0% (16.62%)		(10.88%)
	log ₁₀ population	3.26		3.13		2.98 (3.06)		— (2.47)		(2.29)
	Age specific mortality <i>k</i>		0.12		0.15		— (0.59)		(0.18)	
<i>AGS</i>	<i>l_x</i>	17914		13328		11499		2978		1951
	<i>d_x</i>		4586		1829		8521³		1431²	
	% apparent survival (100 – 100 q_x)	—		74.40%¹		86.28%		25.90%		65.51%
	% real survival	—		74.40%¹		64.19%		16.62%		10.88%
	log ₁₀ population	4.25		4.12		4.06		3.47		3.29
	Age specific mortality <i>k</i>		0.12		0.07		0.53		0.24	

¹ Egg numbers laid are estimates for both *MFG* and *AGS* samples, in this instance. The estimates are based on the results of 1998-99 *MFG* egg-laying/hatching survey

² Parasitism, stuck in exuviae, stuck in lerp, etc.

³ Includes estimate of psylloids lost by leaf-fall

Key to coloured numbers in Table 7.5	
Bold	— Calculations from existing data
Bold Italic	— Estimations based on <i>MFG</i> egg-laying survey survival rate (74.40%)
Bold Red	— Cohort defunct at around 2 nd –3 rd instars
<i>Grey Italic</i>	— Estimated values if the cohort had continued at <i>MFG</i> egg survival rate (74.40%)

7.3 Results

7.3.1 Survival analysis interpretations of psylloid population dynamics

7.3.1.1 *M. fici* survival analysis baseline: use of the *MFG* 1998-99 egg study results

The *MFG* egg study data set was adjusted to allow for the loss of 496 leaves in 19 egg masses on two leaves from the eastern side of the tree, as a result of lawnmowing activities. Nineteen complete data rows were therefore removed from the set before analysis of the data was attempted. The results of the preliminary counts and percentages arising from the egg-laying and hatching data set are presented in Table 7.1 above, and include both the original and the modified results, respectively footnoted. (Note: Table 7.1 is an identical copy of Table 6.1, and Table 7.2 is a copy of Table 6.2 with two additional lines from Table 6.3. This information is repeated here for convenience since it will be referred to relatively frequently). The adjusted percentage value of eggs hatching (74.40%) was used for *MFG* and *AGS* life table calculation (Tables 7.1 and 7.3 above; see also Section 7.3.1.2) to calculate estimates for the original number of eggs laid for the *MFG* and *AGS* life history surveys. These estimates were 1805 and 17914 eggs laid for the *MFG* and *AGS* study populations respectively (Table 7.3). This assumes a constant rate of survival to egg hatch for all three study populations (*AGS* and *MFG* spring 1998–summer 1999 study cohorts as the summer–autumn 1999 *MFG* study cohort).

The mean egg development time per egg mass for the 1998-99 *MFG* egg survey was calculated at 56.9 days (± 0.18 days standard error) from egg lay to egg hatch (Table 7.1). The mean time from egg-hatch to adult eclosion for the *AGS* study cohort, on the other hand, 39.6 days (± 4.5 days standard error)., The mean time spent in the (*MFG*) egg stage, therefore, was almost 58% of the total time span from (*MFG*) egg-lay to (*AGS*) adult eclosion (96.5 days, on average).

7.3.1.2 Age-specific life-tables for *M. fici*

Table 7.3 above presents the life-table (Southwood, 1987) for the psylloid, based on the data from both *MFG* and *AGS* surveys. The upper half of the table presents results (mostly estimates) for *MFG*; the lower half, those for *AGS*.

All estimates in Table 7.3 are shown in italics or italics within parentheses, with text colour and weight used to assist the reader in readily distinguishing between the different types of

estimate used for the *MFG* section. The grey italicized values are the potential numbers at life stage x that would have been generated had the cohort not become defunct, and are made using the percentage survival rates of the *AGS* study cohort, again assuming constant rates across both populations. The bold italicized figures for the *MFG* part of Table 7.3 are estimates based on the *MFG* egg-hatch result (74.40%). The bold red values indicate the extinction of the *MFG* study cohort.

The estimate of eggs laid for the *AGS* study was 17914, with an estimate of 4586 eggs either having been eaten by predators or not having hatched (Table 7.3). The remaining results in the *AGS* section of the age-specific life table were generated from known *AGS* results, or estimates based on actual *AGS* observations (Table 7.3). The life-table was used to estimate the number of adults that the observed number of hatchlings on *MFG* would have ultimately produced, had that cohort successfully reached adulthood. No corrections for abiotic mortality factors were necessary in this instance. The estimated number of adults using this procedure was 196 (Table 7.3, upper (*MFG*) set).

The numbers that were truly known (i.e., positively observed and counted) in these sets are flagged in normal bold text in Table 7.3, and were: egg numbers laid, egg numbers hatched, numbers of eggs eaten, and number of hatchlings settled (*MFG*); and numbers of hatchlings settled, numbers of hatchlings eaten, and the numbers of moult skins (*MFG* and/or *AGS*).

7.3.1.2.1 Specific causes of mortality for *M. fici*

The psylloids had already been hatching for an estimated two days at the start of observations on this cohort (see Section 7.2.1). The mean number counted hatched per leaf on day 1 of the survey was 61 juveniles, or 15 per lerp (both with rounding to the nearest integer): for the initial two-day period, this equates to 20 psylloids per leaf per day, or 5 per lerp per day (rounding to the nearest integer). The mean number of psylloids per leaf (with rounding) on day 2 was 29, with a mean number of 7 hatchlings per newly-formed lerp. The estimated means of psylloids per lerp and psylloids per leaf for the two uncounted days before the *AGS* survey was commenced are similar to the actual values recorded on *AGS* day 2 (30 December 1998).

A total of 24.18% of all eggs lost in the second *MFG* egg study (adjusted data set) was attributed to predation, and the percentage of total eggs (i.e., potential recruits) lost to all causes of mortality by end of the *MFG* egg-hatch period of that survey was 39.15%. This effectively means that a psylloid (on *MFG*, at least) had less than two chances in three of surviving on the tree from egg lay to egg hatch.

Neuropteran (lacewing) larvae, most likely chrysopids (DF Hales, pers. comm.) were observed actively feeding on new hatchlings, and on first instars resident under lerps of up to twelve days in age. The lacewing larvae were observed, under the field and laboratory microscopes, to penetrate the soft lerp covers easily with their piercing and sucking mouthparts, and feed on the early-instar psylloids underneath. The estimate of mortality in the *AGS* cohort as a result of lacewing predation was calculated from field data at 13.72%, which equates to a one in seven chance of being eaten.

Leaf loss from *AGS* (55% of survey leaves) was estimated to have accounted for the death of 63.73% of psylloid juveniles (Table 7.2). This estimate is based on the numbers of psylloids settled on the leaves lost, minus the grand total of: numbers of observed predations on those leaves; numbers of juveniles stuck in honeydew; numbers of final moult exuviae; and numbers of parasitoid exit holes counted up until the time that the particular leaves were lost from the tree.

Psyllaephagus sp. females were observed to sting psylloids under only those lerps of seven days or older (see also Chapter 6, Sections 6.3.3 and 6.3.4): no lerp upper psylloid age limit for stinging was determined. The observed cessation of adult parasitoids' attempting to sting into lerps was coincident with that generation's own disappearance at approximately day 85 (19 March 1999). The number of adult parasitoids was estimated by counting the number of exit holes cut in the lerps by the newly-emerging adults, and which estimate was 636. This is most likely to be an underestimate, especially if (a) *Psyllaephagus* sp. is polyembryonic (see Chapter 6, Sections 6.3.3.1.2 and 6.4.3.1.2), and (b) more than one parasitoid adult used the same exit hole to escape from the lerp (whether polyembryonic and either emerging from the same psylloid mummy, or emerging from a different mummy via the same exit hole).

Parasitoid adults were observed to emerge from psyllid mummies recovered from fallen leaves, but sufficiently reliable estimates of parasitoid survival on fallen leaves were not able to be obtained using this method. The unknown number of *Psyllaephagus* sp. successfully reaching adulthood (disregarding potential numbers of adults uncounted as a result of possible multiple uses of the same exit hole, uncounted exit holes, etc.) was estimated, however, by assuming a constant rate of parasitisation across the survey population of lerps, and multiplying the number of leaves lost by the mean rate of parasitisation for leaves that were still attached to the tree (19.875, rounded up to 20). The resulting estimate was 800 parasitoids. The final estimated maximum number of adult parasitoids was therefore 1436 (800 unknown adults + 636 parasitoid exit holes counted), or 10.73% of the total population of settled psyllid hatchlings counted during the survey (Table 7.2). This latter estimate does not take into account numbers of *Psyllaephagus* sp. larvae which died inside their hosts on leaves either on or off the tree from any of a number of potential causes such as disease, host resistance, parasitoid overload of the host, immaturity of hosts on leaves lost, etc. From the results, therefore, a psyllid (on AGS) had a minimum chance of one in ten (and probably higher) of succumbing to a parasitoid before it reached adulthood.

7.3.1.3 Survival analysis of lerp and leaf data

Tables 7.4 and 7.6 give the ANOVA tables produced as part the Cox Proportional Hazard regression analyses of the *LERPICEN* censoring variable and *ABSLFCEN* censoring variable data, respectively. A key to the variable names is given in Tables 7.5 and 7.7 for *Lerp* and *Leaf* models, respectively. Variables found to be significant in each of the analyses are shown in red in Table 7.4 (*Lerp*) and Table 7.6 (*Leaf*). The original survival analysis combined the data for both the eastern and western sides of the tree. In the cases of both *MFG* and *AGS*, the data collection automatically fell into this east-west partitioning, since these were the parts of the canopies that were accessible from the ground. In the collecting of the data, care was taken to preserve this partitioning, which was also maintained throughout the various permutations of the data set. Since the major proportion of leaves falling from the tree was on the western side, the data set was analysed a second time with *TREESIDE* as a covariate, to take this situation into account. The results of the analyses with *TREESIDE* included are those presented in Tables 7.4 and 7.6. The independent variables found to have had a significant effect on leaf and lerp survival (or mortality), apart from the sides of the tree were, for *Lerps*, temperature at the time of total lerp flow (*TLFTEMP*, -ve-signed coefficient), relative humidity at the time of total

lerp flow (*TLFRH*, -ve sign), and relative humidity at the time of the psylloid mid-vein lerp reformation (*MVLRRH*, +ve sign) (Table 7.4). For *Leaves*, the variables of significance were relative humidity at time of psylloid mid-vein lerp reformation (*MVLRRH*, -ve sign), and the temperature at time of leaf abscission (*ABSLFTEMP*, +ve sign) (Table 7.6).

Figure 7.1 presents the graph the cumulative proportion of lerps surviving over the period of the *AGS* life history field study, produced by the Kaplan-Meier Product Limit analysis. This graph shows clear differences in lerp survival rates, between the eastern and western sides of the tree. The western lerps are seen to be failing sooner than those on the eastern side of the tree. The graph in Figure 7.2 is of the cumulative proportion of leaves surviving during the *AGS* study, as generated by the Kaplan-Meier Product Limit survival analysis for leaves. This graph shows the even larger differences between the eastern and western sides of the tree, with respect to leaf survival, than lerp survival. The likely reasons for this difference will be discussed in Section 7.4.1.3.1 below.

Table 7.4: Cox Proportional Hazard regression analysis of lerps and various field-observed variables: key in Table 7.5 below

Lerps

With TREESIDE Covariate

Dependent variable: Survival times in days

Censoring variable: LERP1CEN

$\chi^2 = 17.2433$; $df = 8$; $p = 0.02770$

Independent variable	Beta	Std Error	t-value	Exponent β	Wald statistic	prob.
LMTEMP	0.04618	0.177566	0.26005	1.047259	0.067627	0.794826
LMRH	0.00779	0.050700	0.15372	1.007824	0.023630	0.877832
TLFTEMP	-1.35036	0.555813	-2.42952	0.259148	5.902548	0.015124
TLFRH	-0.32925	0.130423	-2.52451	0.719460	6.373173	0.011591
MVLRTEMP	-4.55814	3.581690	-1.27262	0.010481	1.619571	0.203161
MVLRRH	0.08072	0.038446	2.09952	1.084065	4.407972	0.035779
ABSLPTM	0.07398	0.131425	0.56291	1.076786	0.316866	0.573501
ABSLPRH	0.08926	0.047005	1.89899	1.093367	3.606147	0.057575

Table 7.5: Key to variables used in *Lerp* model in Table 7.4

Variable code	Explanation
LERP1CEN	date of lerp censoring event
LMTEMP	Temperature at “lerp merge” event (lerp data)
LMRH	Relative humidity at “lerp merge” event (lerp data)
TLFTEMP	Temperature at occurrence of “Total lerp flow” event (lerp data)
TLFRH	Relative humidity at occurrence of “Total lerp flow” event (lerp data)
MVLRTEMP	Temperature at occurrence of “midvein lerp reformation” event (lerp data)
MVLRRH	Relative humidity at occurrence of “midvein lerp reformation” event (lerp data)
ABSLPTM	Temperature at occurrence of fall of study leaf from tree (lerp data)
ABSLPRH	Relative humidity at occurrence of leaf abscission event (lerp data)

Table 7.6: Cox Proportional Hazard regression analysis of F_V/F_M with respect to leaves and various field-observed variables : key in Table 7.7 below

Leaves

With TREESIDE covariate

Dependent variable: Survival times in days

Censoring variable: ABSLFCEN

$Chi^2 = 13.4146$; $df = 6$; $p = 0.03693$

Independent variable	Beta	Std Error	t-value	Exponent β	Wald statistic	prob.
TLFLFTEM	1.75579	0.976610	1.79785	5.788039	3.232246	0.072211
TLFLFRH	0.25786	0.191511	1.34647	1.294163	1.812983	0.178160
MVLRLFTE	-6.62987	4.058940	-1.63340	0.001320	2.667994	0.102395
MVLRLFRH	-0.10989	0.050526	-2.17485	0.895936	4.729971	0.029649
ABSLFTEMP	0.55907	0.270764	2.06478	1.749040	4.263309	0.038952
ABSLFRH	0.00184	0.071360	0.02575	1.001840	0.000663	0.979454

Table 7.7: Key to variables used in *Leaf* model in Table 7.6 above

Variable code	Explanation
ABSLFCEN	Date of leaf abscission censoring event
TLFLFTEM	Temperature at occurrence of “Total lerp flow” event (leaf data)
TLFLFRH	Relative humidity at occurrence of “Total lerp flow” event (leaf data)
MVLRLFTE	Temperature at occurrence of “midvein lerp reformation” event (leaf data)
MVLRLFRH	Relative humidity at occurrence of “midvein lerp reformation” event (leaf data)
ABSLFTEMP	Temperature at occurrence of fall of study leaf from tree (leaf data)
ABSLFRH	Relative humidity at occurrence of leaf abscission event (leaf data)

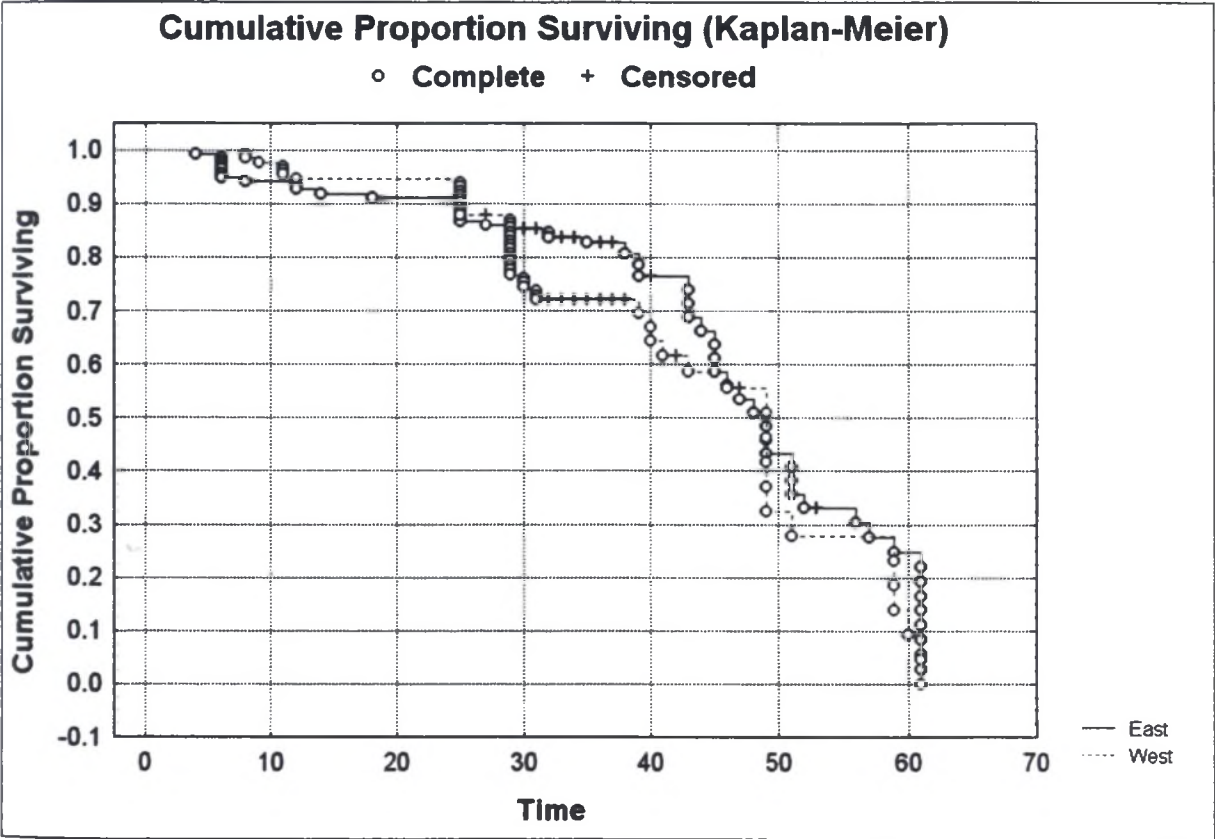


Figure 7.1: Differences in lerp survival with respect to side of tree (AGS data)

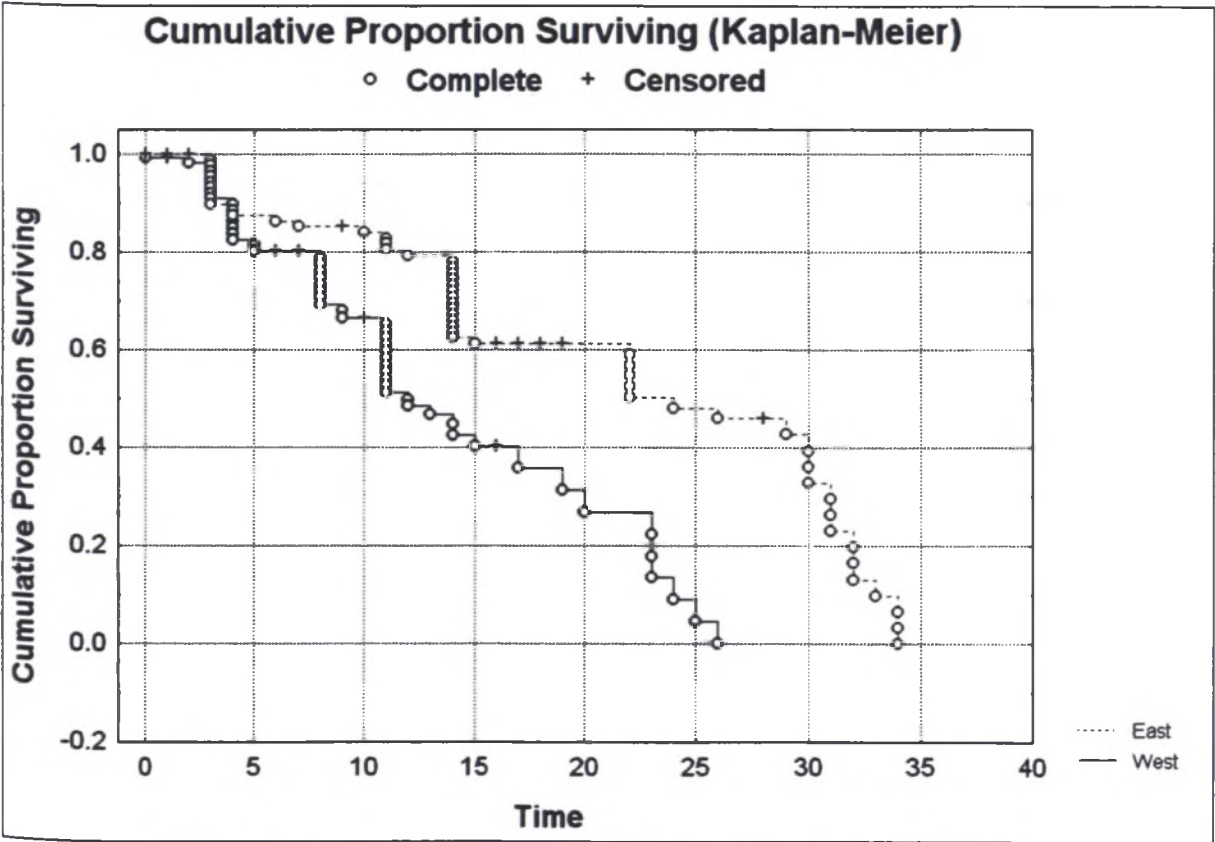


Figure 7.2: Differences in leaf survival with respect to side of tree (AGS data)

7.3.2 Degree days models

7.3.2.1 Degree days estimates for *M. fici*: two degree days models using different temperature data sets

Table 7.8 below presents two sets of degree days models for egg, post egg juvenile instars, total juvenile period, and total development. These models were constructed using the *MFG* and *AGS* psyllid field data, and the mean daily temperature for each actual date on which the survey was conducted. Temperatures were derived from Observatory Hill meteorological records. The ‘short term’ temperature model was calculated using the mean three-hourly temperatures for the 24 hour period from 3 am, for the actual days in late 1998 and early 1999 on which the surveys were conducted. The ‘long term’ temperature model was calculated using the five-yearly mean of mean daily temperatures occurring over the period 31 August 1995 to 30 March 2001 inclusive.

7.3.2.2 Application of the two temperature models to other geographical locations

The degree days estimates for a range of mostly geographically separate locations extending from temperate in the south (Melbourne) to subtropical in the north (Brisbane), with an additional hypothetical comparison with a Mediterranean-type climate (Adelaide), are presented below in a series of six tables from Tables 7.9 to Table 7.13 inclusive. Negative and positive prefixes in Tables 7.9, 7.11, 7.12 and 7.13 indicate one of two things, depending on the context. The first context (Tables 7.9, 7.12 and 7.13) is an indication that the quantity is less than that with which it is being compared (positive signs indicate larger values). The second context (Table 7.11) indicates latitude differences: negative indicates a difference in direction from the object of comparison towards lower latitudes (the north); positive, a difference in direction towards higher latitudes (the south).

The psyllid is well established in all locations modelled, with the exception of Adelaide, where *M. fici* has not been introduced.

Table 7.9 presents degree days calculated using the ‘short term’ model, and indicates a general shortening of development times (more available degree days per year) along a southerly to northerly transect, as a result of the mean daily climate becoming warmer. The climatic trend

as latitude decreases is also for less pronounced differentiation between ‘winter’ (low solar zenith) and ‘summer’ (high solar zenith) temperatures. There is also, hypothetically, a decreasing development time trend from east to west (Sydney to Adelaide). This latter development time is hypothetical in the sense that the psyllid does not exist in Adelaide. Latitudes and longitudes for each location are given in Table 7.10 below.

Table 7.11 presents the percentage differences in generation time as predicted by the ‘Long term mean temperature’ model between Sydney and each of the seven other locations, with reference to differences in latitude, mean daily temperature for 12 months, and the variability of the mean yearly temperature (as standard deviations). The ‘short term’ model was not used for this set of comparisons since the long term mean temperature should be a more reliable estimator of local climatic conditions than the ‘single season’ mean or ‘point’ temperatures.

Table 7.12 presents short term and long term model predictions for psyllid egg and (total) juvenile stage degree days, and complete-generation degree days, for the seven locations other than Sydney, and some direct comparisons of predictions for the two Sydney models with those for the other seven locations. The two sets of predictions were generated using the degree days values for eggs, juveniles and entire (summer) generation from the ‘short term’ and ‘long term’ models. The predicted number of *M. fici* generations per year for each of the eight locations was constructed using the two temperature degree days models and the results of the location generation predictions.

Predicted summer generation degree days and generations per year comparisons between Sydney and the seven other locations with respect to the ‘short term’ and ‘long term’ model predictions for Sydney, are given for separate egg and juvenile stages, as well as the complete generation, in Table 7.13 below.

7.3.2.3 Location summaries

7.3.2.3.1 Melbourne

Melbourne, with colder winter conditions and sometimes milder summers than the locations on the eastern coast to the north, had the lowest degree days available per year. The notoriously unpredictable nature of Melbourne’s weather is reflected in Table 7.11, where it is shown to

have the highest standard deviation for the yearly mean temperature of all of the eight locations.

7.3.2.3.2 Adelaide

The various models' predictions for a hypothetical psylloid population in Adelaide were close in value to those of Sydney's, although the 'short term' (1998-99) model summer generation development time is actually 6% longer than either the 'short term' or 'long term' Sydney models' predictions.

7.3.2.3.3 Wollongong

Wollongong and Melbourne share similar predicted summer generation lengths for both temperature models, but different numbers of generations per year compared with each other, Wollongong having, theoretically at least, fractionally more generations of psylloids per year than Melbourne. The Wollongong generation times indicate a warmer 'winter', but a cooler overall climate, than in Sydney: in fact Wollongong's yearly mean temperature was the lowest of all eight locations.

7.3.2.3.4 Sydney

The Sydney 'long term' temperature model differed from the Sydney 'short term' by 9.95% (10%) the average degree days per year above threshold, and 2.85% (3%) for the summer generation model (Table 7.13).

7.3.2.3.5 Parramatta

The Parramatta summer generation models predict a very slightly longer development time than those for Sydney. The western Sydney mean maximum temperatures (from Bureau of Meteorology records for both locations) for the same months were as hot as or hotter than the Sydney CBD temperatures.

7.3.2.3.6 Port Macquarie

The Port Macquarie predictions for summer development times and numbers of generations per year by the two models are the same as those for the Sydney CBD.

7.3.2.3.7 Grafton

For Grafton, approximately 3.3 ° of latitude north of Sydney, the model showed a 16% decrease in development time. While having shorter predicted summer generation times than Sydney, Grafton also had shorter predicted summer generation times than Brisbane, even though it is some 200 km south of Brisbane. and its mean daily temperature is lower by 0.3 °C.

7.3.2.3.8 Brisbane

The predicted number of generations per year for Brisbane (the largest for the eight locations at 3.10 per year), however, was 3.7% than higher than the number for Grafton. Overall higher temperatures year-round in Brisbane than in Grafton were observed in the yearly mean temperatures of 20.6 °C for Brisbane and 20.0 °C for Grafton.

**Table 7.8: ‘Short term’ temperature and ‘Long term’ temperature degree days models for life-cycle stages of *M. fici* for RBGS.
Climate data from Bureau of Meteorology, Sydney.**

Developmental Stage	Mean no. days	Degree days model		°D difference	% difference
		Short term mean temp.	Long term mean temp.		
Egg	56	726.3	704.9	-21.4	-2.99%
1st instar¹	12	162.2	141.7	-20.5	-12.7%
2nd instar¹	7	98.6	85.4	-13.2	-13.4%
3rd instar¹	6	80.4	73.2	-7.2	-9.0%
4th instar¹	8	99.2	97.6	-1.6	-1.6%
5th instar¹	7	87.9	85.4	-2.5	-2.8%
Total juvenile post egg	40	528.3	514.0	-14.3	-2.71%
Total cycle (egg + post egg)	96	1254.6	1218.9	-35.7	-2.85%

¹ Estimates: see Section 6.3.2.2.1

Table 7.9: Degree days for pre-adult stages and numbers of generations per year for *M. fici* for eight locations, predicted by the ‘Short term’ model

	Melbourne	Adelaide	Wollongong	Sydney	Parramatta	Port Macquarie	Grafton	Brisbane
Average yearly degree days available¹	1793.1	2583.3	2740.7	2778.2	2552.1	2757.0	3540.8	3849.3
°D difference from Sydney (eggs)	+15	-2	+3	—	0	+2	-10	-11
°D difference from Sydney (juvenile)	+10	-3	+2	—	0	+1	-8	-9
Predicted number of days of egg stage	71	54	59	56	56	58	46	45
Predicted number of days of juvenile stage	50	37	42	40	40	41	32	31
Total number of days in all pre-adult stages	121	91	101	96	96	99	78	76
Number of days per year above threshold	334	365	365	365	365	365	365	365
Predicted number of generations per year	2	4	3	3	3	3	4	4
Difference in generations per year wrt Sydney	-1	+1	0	—	0	0	+1	+1

¹10 °C lower development threshold temperature

Table 7.10: Latitudes and longitudes for the eight geographical locations

Location	Latitude	Longitude
Melbourne	37 ° 46' S	144° 58' E
Adelaide	34° 56' S	138° 35' E
Wollongong	34° 25' S	150° 54' E
Sydney	33° 52' S	151° 12' E
Parramatta	33° 50' S	151° 02' E
Port Macquarie	31° 26' S	152° 53' E
Grafton	29° 40' S	152° 57' E
Brisbane	27° 29' S	153° 02' E

Table 7.11: Percentage difference in generation time compared with Sydney, using the 'Long term' mean temperature degree days model

Location	Latitude difference ex Sydney	% difference in generation time	Mean daily temperature	Standard deviation
Melbourne	+3° 54' S	13.5% longer than Sydney	17.1 °C	4.3 °C
Adelaide	+1° 04' S	Same as Sydney	17.6 °C	3.3 °C
Wollongong	+0° 33' S	10.3% longer than Sydney	15.9 °C	3.8 °C
Sydney	—	—	18.5 °C	3.5 °C
Parramatta	-0° 02' S	2.04% longer than Sydney	17.7 °C	3.9 °C
Port Macquarie	-1° 26' S	Approx. same as Sydney	18.4 °C	3.5 °C
Grafton	-3° 12' S	15.7% shorter than Sydney	20.0 °C	4.0 °C
Brisbane	-5° 23' S	14.3% shorter than Sydney	20.3 °C	3.6 °C

Table 7.12: Comparison of *M. fici* stages using ‘short term’ and ‘long term’ mean temperature degree days models¹

Variable	Model	Locations								
		Melbourne	Adelaide	Wollongong	Sydney A ²	Sydney B ³	Parramatta	Pt Macquarie	Grafton	Brisbane
Closest egg degree days to Sydney ‘long term’ model	Short	673.5	678.2	677.0	—	677.4	677.7	674.4	683.3	680.9
	Long	710.8	700.8	711.5	726.3	—	699.6	708.3	699.5	699.1
Closest juvenile degree days to Sydney ‘long term’ model	Short	484.2	482.2	490.0	—	483.2	491.0	474.4	485.2	478.6
	Long	516.8	516.4	522.5	528.3	514.0	514.8	524.0	513.1	516.0
Total closest degree days to Sydney ‘short term’ model	Short	1157.7	1160.5	1167.0	—	1160.6	1168.7	1159.9	1168.5	1159.5
	Long	1227.6	1217.2	1234.0	1254.6	1218.9	1214.4	1232.3	1212.6	1215.1
Predicted no. days at egg stage	Short	71	54	59	—	56	56	58	46	45
	Long	65	55	62	56	58	56	57	48	48
Days’ difference from Sydney model: eggs	Short	+15	-2	+3	—	0	0	+2	-10	-11
	Long	+7	-3	+4	—	+2	-1.6	-1	-9.7	-9.7
Predicted no. days at juvenile stage(s)	Short	50	37	42	—	40	40	41	32	31
	Long	46	39	45	40	41	41	40	35	36
Days difference from Sydney ‘short term’ model: juveniles	Short	+10	-3	+2	—	0	0	+1	-8	-9
	Long	+5	-2	+4	—	+1	0	-1	-6	-5
Total estimated days’ duration of generation	Short	121	91	101	—	96	96	99	78	76
	Long	111	94	107	106	99	97	97	83	84
Average yearly degree days available for development	Short	1793.1	2583.3	2740.7	2778.2	—	2552.1	2757.0	3540.8	3849.3
	Long	2143.1	2618.8	2752.00	—	3085.3	2812.3	3081.1	3645.4	3774.4
Predicted no. generations per year	Short	2	4	3	3	3	3	3	4	4
	Long	1.75	2.2	2.2	2.46	2.53	2.3	2.5	3.0	3.1
Days per year available for development	Short	334	365	365	365	365	365	365	365	365
	Long	362	365	364	365	365	365	365	365	365

¹ Lower development threshold temperature = 10 °C

² Sydney A = ‘short term’ temperature model

³ Sydney B = ‘long term’ temperature model

Table 7.13: Predicted *summer generation* degree days and generations per year for *M. flci* for a range of geographical locations, compared with short and long term temperature models for Sydney. Lower development threshold temperature set at 10 °C

Variable	Locations ¹								
	Melbourne	Adelaide	Wollongong	Short term Sydney ³	Long term Sydney ⁴	Parramatta	Port Macquarie	Grafton	Brisbane
Average yearly degree days above development threshold	2143.1	2618.8	2752.0	3004.7	3085.3	2812.3	3081.1	3645.4	3774.4
Sydney degree days models for <i>summer generation</i>	—	—	—	1218.9	1254.6	—	—	—	—
Days per year above threshold ($t = 10\text{ °C}$)	362	365	364	365	365	365	365	365	365
No. of days at egg stage, short & long term Sydney models	—	—	—	56	55	—	—	—	—
No. of days at egg stage, using short term model °D	65	56	62	—	—	57	56	48	48
No. days at egg stage, using long term model °D	60	53	60	—	—	56	55	49	49
Difference, short & long term model egg stage duration	+5	+3	+2	—	—	+1	+1	-1	-1
No. of juvenile stage days, short & long term Sydney models	—	—	—	41	40	—	—	—	—
No. days at juvenile stage, using short term model °D	46	40	45	—	—	41	40	35	36
No. days at juvenile stage, using long term model °D	43	37	43	—	—	40	40	35	35
Difference, short & long term model juvenile stage durations	+3	+3	+2	—	—	+1	0	0	+1
Predicted duration summer generation, short term model	111	96	107	96	—	98	96	83	84
Predicted duration summer generation, long term model	103	90	103	—	96	96	95	84	84
Predicted difference between summer & short term models	+15	0	+11	—	—	+2	0	-13	-12
Predicted difference between summer & long term models	+7	-6	+7	—	—	0	-1	-12	-12
No. generations/year, short term model	1.71	2.08	2.19	2.40	—	2.24	2.46	2.91	3.01
No. generations /year, long term model	1.76	2.14	2.26	—	2.53	2.31	2.53	2.99	3.10
Percentage difference between long & short term models	2.92%	2.88%	3.19%	5.14%		3.13%	2.85%	2.75%	2.99%

¹ Arranged in approximate order of increasing latitude

² Time durations are in whole days where applicable

³ 'Short term' temperature model: °D calculated using daily temperatures from the mean of eight daily readings made at Observatory Hill, Sydney, for each of the *MFG* and *AGS* psyllid survey dates in 1998-99

⁴ 'Long term' temperature model: °D calculated using long term mean daily temperatures over seven years (1995-2001 inc.) for each location, for the same observation dates of the 'short term' temperature model.

7.3.3 Comparisons of psylloid activity on five study trees

7.3.3.1 Comparisons of psylloid activity with respect to leaves

Tables 7.14 and 7.15 below present summary information on the presence and absence of eggs (Tables 7.14) and lerps (Tables 7.15) with respect to the position of the supporting leaf on the branchlet, on the five of the six main study trees (*MFG*, *L44*, *HRB*, *BTC* and *AGN*) over several years. The tables give the mean proportion of presence or absence of eggs or lerps per leaf position per tree, as well as the overall average presence/absence proportions for tree and leaf. Leaf positions were those from 1–7, with Leaf 1 being defined here, and in Chapter 8, as the first fully unfurled (unfolded) leaf back from (older than) the shoot or not-fully-unfurled (youngest) leaf. The sixth or seventh leaf positions' values were calculated by combining the totals for all leaves older than 6 or 7 for a particular tree. Leaves older than positions 6 or 7 were much less represented in all of the chlorophyll fluorescence studies' sample data, as a result of the particular focus of those studies. The minimum proportions for both eggs and lerps were found to occur at the Leaf 1 (youngest) position.

7.3.3.2 Psylloid activity with respect to eggs on leaves

Egg masses were occasionally found on leaf no. 1 (the first fully unfurled leaf below the shoot or unfurling leaf). The proportions of eggs steadily increase on a leaf-position basis for the leaf average taken over the five trees, although the sequence for each tree is not monotonically increasing for all of the individual trees (Table 7.14), and the within-tree average for all leaf positions varies between the five trees, with a range of 95% (*BTC*) to 47% (*L44*) (Table 7.14). These results are discussed in Section 7.4.3.1. Eggs, being firmly attached to the leaf by a stalk, or pedicel, inserted into the leaf tissue, remain on the leaf even after the leaf has fallen from the tree. The author has seen dried, pressed specimens of *F. macrophylla* subsp. *columnaris* leaves held in the Collection at the National Herbarium of NSW at the Royal Botanic Gardens, Sydney, that were collected in 1898 on Lord Howe Island, and which still retain *M. fici* egg masses firmly fixed on them. Thus, it is not surprising to find eggs on leaves across the full range of leaf ages, whereas the range of leaf ages that *active* lerps may be found on is far more restricted.

7.3.3.3 Psylloid activity with respect to lerps on leaves

Active lerps were rarely found to be extant on leaves younger than leaf no. 2, on an average of 6 in 100 (Table 7.15). The per-leaf position average proportions of lerps (active, old lerps, and old lerp sites) per leaf generally increased with the age of the leaf (Table 7.15). Leaves 5 and 6 showed a marked overall decrease in lerp presence compared with most younger and one older (Leaves 7+) leaf position (see Section 7.4.3.2 below).

In most cases, the one exception being *L44*, the variability of lerp presence between trees was lower than that for eggs between trees. The variability for eggs with respect to leaf position was somewhat different, however, with no apparent pattern. The standard deviations of lerp presence for three leaf positions were higher than those for the same position with respect to eggs; and the standard deviations of lerp presence for three leaf positions were lower than those for the same position with respect to eggs, and in no particular order, either increasing or decreasing in age.

7.3.3.4 Other observations on psylloid activity with respect to both eggs and lerps

The most notable of the results presented in Tables 7.14 and 7.15 is that the average proportion of lerps per Leaf 1 (0.06) is lower than the average proportion of eggs per Leaf 1 position (0.51) by a factor of 8.5 (see Section 7.4.3.3 below).

A proportion of 100% for either eggs or lerps on any leaf position for any tree was relatively rare, occurring in only one instance on leaves younger than position 4 in the egg table (Table 7.14); two instances on leaves older than position 6 in the egg table; and one instance in leaves younger than position 3 in the lerp table (Table 7.15). The tree on which the single instance of 100% of leaves younger than position 4 which had eggs (Leaf 2) or lerps (Leaf 3) on them was *BTC*, which had heavy loads of both eggs and lerps on its leaves. The mean proportion of eggs present per leaf for *BTC* was 0.95 ± 0.030 , the highest of the within tree means for the egg table, and the lowest variation between leaf positions. The mean proportion of lerps present per leaf for *BTC* was 0.65 ± 0.38 , the second-highest mean for the group (*AGN* being the highest), and with the highest between-leaf variability of the group.

7.3.3.5 Comparisons of psylloid activity with respect to tree defoliation and environmental factors

Table 7.16 below presents comparisons of defoliation status of five of the project study trees with respect to various combinations of psylloid-related and environmental factors. Table 7.16 gives the mean number of egg masses counted for all leaf-positions from 1–5 inclusive on *MFG*, *L44*, *HRB*, *BTC* and *AGN*, mean proportion of eggs present on leaves at all leaf positions 1–5, mean number of lerps counted for all leaf positions from 1–5 inclusive, mean proportion of lerps present on leaves at all leaf positions 1–5. This leaf range is the one which carried the major proportion of *active* lerps, as opposed to old defunct lerps or old lerp sites from which the lerps had disappeared. The major proportion of defunct lerps and old lerp sites were on leaves at leaf-position 6 and older. The ‘average’ defoliation rate and the general soil conditions for each tree follow. From this table, presence of egg mass was no indicator of tree health, since *HRB* with low egg mass numbers had a high defoliation rate, while *MFG* with a low defoliation rate had a large mean number of eggs per sample population (leaves 1–5). Mean lerps per sample more or less followed the same pattern as mean eggs per sample, although at lower frequencies.

Table 7.14: Mean proportion of leaves with eggs for the five main study trees

Leaf age	Tree					Leaf avg.	Std dev.
	<i>MFG</i>	<i>L44</i>	<i>HRB</i>	<i>BTC</i>	<i>AGN</i>		
1	0.71	0.24	0.37	0.95	0.27	0.51	0.310
2	0.90	0.38	0.76	0.92	0.94	0.78	0.235
3	0.94	0.47	0.53	1.00	0.95	0.78	0.256
4	0.91	0.50	0.67	0.94	0.92	0.79	0.195
5	0.95	0.75	0.63	0.94	0.91	0.84	0.141
6(+)	1.00	—	0.75	—	0.94	0.90	0.131
7+	1.00	—	—	—	—	1.00	0.310
Tree avg.	0.92	0.47	0.62	0.95	0.82	0.80¹	0.235
Std dev.	0.099	0.187	0.148	0.030	0.271	0.151¹	

¹ Mean and std dev. for *Tree* average

7.15: Mean proportion of leaves with lerps (all ages) for the five main study trees

Leaf age	Tree					Leaf avg.	Std dev.
	<i>MFG</i>	<i>L44</i>	<i>HRB</i>	<i>BTC</i>	<i>AGN</i>		
1	0.18	0.04	0.01	0.00	0.06	0.06	0.072
2	0.48	0.46	0.18	1.00	0.89	0.60	0.337
3	0.66	0.26	0.87	0.78	0.79	0.67	0.242
4	0.59	0.50	0.78	0.76	0.92	0.71	0.166
5	0.65	0.25	0.67	0.71	0.51	0.56	0.188
6(+)	0.67	—	0.63	—	0.88	0.73	0.134
7+	0.46	—	—	—	—	0.46	0.072
Tree avg.	0.53	0.30	0.52	0.65	0.68	0.55¹	0.337
Std dev.	0.175	0.185	0.347	0.380	0.337	0.234¹	

¹ Mean and std dev. for *Tree* average

Table 7.16: Comparison of five Sydney study trees with respect to mean proportion of *M. fici* eggs and lerps on leaves 1–5, defoliation and hydraulic environment

Tree	Mean EMs ¹ /sample ²		Proportion with eggs		Mean lerps/sample ²		Proportion with lerps		Defoliation	Other conditions
	Mean	Std dev.	Mean	Std dev.	Mean	Std dev.	Mean	Std dev.		
<i>MFG</i>	33.2	0.213	0.882	0.098	17.5	5.43	0.51	0.199	Low	Tree roped off, irrigated
<i>L44</i>	11.0	0.447	0.47	0.187	4.7	3.33	0.30	0.185	Low-moderate	Tree roped off, irrigated and in wet area
<i>HRB</i>	16.8	0.530	0.59	0.149	10.7	7.66	0.50	0.383	High	Tree not roped off, not irrigated
<i>BTC</i>	18.4	0.736	0.95	0.030	5.7	4.68	0.65	0.380	Moderate-high	Tree not roped off, not irrigated
<i>AGN</i>	23.2	0.252	0.80	0.297	16.2	8.73	0.63	0.359	Moderate	Tree not roped off, irrigated

¹ EM = egg mass ² sample = combined total of eggs or lerps for leaf-positions 1–5

7.4 Discussion

7.4.1 Survival analysis interpretations of psylloid population dynamics

7.4.1.1 *M. fici* survival analysis baseline: *MFG* 1998-99 egg survey data

The second, complete egg-laying and hatching study revealed that the psylloid spent a relatively long mean time in the egg stage compared with the rest of the pre-adult life cycle – almost 60% of the total time from mean egg-lay date to mean adult eclosion date. The long development time within the egg suggests possibilities of either discontinuous or slow development, and also suggests that *M. fici* has *K*-selected species characteristics.

7.4.1.2 Age-specific life-tables for *M. fici*

If the assumption made that the *MFG* mean rate of psylloid survival from egg-lay to hatching was the same for the egg-lay to hatching survival for *MFG* and *AGS* life history study populations, the age-specific life table (Table 7.3 above) has some validity. In the absence of more controllable experimental conditions in which to acquire a more accurate set of data, the life table as presented above was the best that could be attempted. Every effort was made during the collection, collation and analysis of the data, to keep it as accurate and free from human influence as possible. The age-specific life table presented in Table 7.3 gives a basis for comparison with any future studies that might be made of *M. fici*'s life history.

7.4.1.2.1 Specific causes of mortality for *M. fici*

With the combining of the *MFG* egg and *AGS* post-egg cohort data to construct the psylloid's rates of survival and mortality at key stages of its life cycle, and assuming similar dynamics for the two populations, an *M. fici* individual was calculated to have an approximately 3.75% chance overall of surviving from egg to adulthood – or, 375 out of 10,000 eggs laid will become adult psylloids.

The major surprise with respect to psylloid mortality was the relatively large proportion of psylloids lost to what appeared from the evidence to be predation by, possibly, a chewing insect. The 'predation' of the egg population was, in fact, the second largest single psylloid mortality factor, the loss of leaves (on *AGS*) being the largest. Predation also appeared to account for a good proportion of the prematurely extinct *MFG* study cohort, but the results

appeared to be something of an anomaly when compared with both non-numerical observations of psylloid populations in general, and the *AGS* population in particular.

It is likely that lacewing larvae have a relatively restricted ‘window’ through which they can feed on psylloid juveniles: from egg hatch to about fourteen days after a lerp has fully closed over. At about this point, the lacewings would be prevented from feeding on juvenile psylloids by the thickness of the lerp wall exceeding the length of the lacewings’ jaws. In any case, only those psylloids within jaws’ reach at the periphery of the lerp would be vulnerable to lacewing attack: the majority of psylloids within the lerp would be out of harm’s way where the lacewings were concerned.

The situation for the psylloid with respect to attack does not disappear at this stage, however; attacks merely change form. Once free of lacewing predation, it appears that the psylloid almost immediately becomes vulnerable to its *Psyllaephagus* sp. parasitoid. *Psyllaephagus* sp. females were observed to commence stinging into lerps after the lerp had closed over at between 12 and 14 days of (lerp) age. This also suggests that the parasitoid mothers are selecting hosts which have a better chance of not being eaten by something else, and therefore provide a better quality host by stinging older and therefore more protected psylloids. Since parasitoid ovipositor lengths are generally many times their own body lengths long, the thickness of a lerp wall would present a fairly minimal impediment to the laying of its eggs within psylloid juveniles.

The relatively large number of leaves lost during the *AGS* study (55.6%) suggests that the psylloid may have been having such an impact on the tree that it was worth the loss of considerable photosynthetic area in order to rid itself of the problem (Blundell and Peart, 2000) – if that was in fact the train of cause and effect (Hodge *et al.*, 2000).

The results of the *MFG* and *AGS* surveys indicate that leaf loss is the major cause of psylloid mortality (55.6%), followed by egg predation (25.7%), followed by hatchling predation (14.6%), followed by parasitisation (10%), in decreasing order. Since these ascribed or calculated mortality rates (and excluding the egg mortality, of course) add up to about 80.2% mortality of the original settled *AGS* psylloid study population, and 14.6% of that original (settled) *AGS* study population successfully reached adulthood, underestimates have therefore

been made in some of these causes of psyllid juvenile death. The estimate most likely to be too low is that of mortality through leaf loss, although mortality through parasitisation is probably in error as well. There were a number of ways in which parasitoid estimates could have fallen short: on leaves fallen from the tree that otherwise might have been counted (via exit holes in lerps); by multiple parasitoid emergences through one lerp exit hole; by undercounting of exit holes; or an obscured parasitoid emergence through some other type of perforation in the lerp (e.g., a broken 'fifth bubble', a pharate adult psyllid exit hole, etc.).

Predation on adult psyllids was not determined, but the very high likelihood of its occurrence was inferred by the large presence of small birds such as fairy martins, flycatchers, noisy miners and Indian mynahs flying in and around the tree canopies at all times (in daylight) during the presence of adult psyllids.

Although the adults from the *AGS* cohort were observed to be present for a total of 24 days, mean individual adult life-span was not realistically determinable in the time available. The longevity of the adult stage is, however, likely to be promoted by the fact that adults were observed feeding directly from leaves using their well-developed stylets. The adult feeding sites were observed to be strictly on leaf midveins, but with no apparent preference for either abaxial and adaxial side.

The combined predation and parasitisation results suggest that natural enemies of the psyllid are responsible for a significant, and in instances where leaf loss is low, a major, proportion of psyllid mortality. The natural enemies should therefore be conserved by avoidance of pesticidal controls where possible. Until relatively recently at the Royal Botanic Gardens and elsewhere, it was the usual practice to rake up fallen *F. macrophylla* leaves to prevent them from sticking to people's shoes, clothing, etc. This practice has largely been abandoned at the Royal Botanic Gardens Sydney, to conserve nutrients for the trees, and to attempt to maintain the size of the parasitoid population at a higher level than if the leaves were removed to another site and composted. The potential parasitoid numbers represented by the 55 per cent of study leaves lost during the *AGS* study (if the majority of those parasitoids survived) give some support to the practice of leaving *F. macrophylla* leaves on the ground where they have fallen. The Botanic Gardens Trust has instituted explanatory signs next to a number of *F. macrophylla* specimens in the Royal Botanic Gardens, Sydney, to educate the public and to explain the

reasons for leaving the leaves intact under the tree, and to help ameliorate the perceived nuisance that the remaining leaves might have caused.

7.4.1.3 Survival analysis of lerp and leaf data

The results of the various survival analyses presented in Section 7.3.1.3 generally reflect the observations regarding lerp extinction and leaf fall made in the field at the time of the study that generated the data. The Cox Proportional Hazard regression models, however, give more of a numerical insight into specific forces at work on lerps and leaves. The factors (variables) found to be statistically significant in each of the two models (*Lerps* and *Leaves*) are now discussed in relation to their effects on the survival of lerps and leaves, respectively. The positive sign on a significant variable indicates that the variable is contributing to the promotion of the survival of the subject, while the negative (-ve) sign on a significant variable indicates that the variable is contributing to the decrease in the rate of survival (or increasing mortality) of the subject.

7.4.1.3.1 Lerp model

Relative humidity at the time of midvein lerp reformation, *MVLR RH*, was positive with a value of 0.081. The actual effect of increased humidity may help the reformation of the lerp along the midvein by preventing psyllid desiccation. Alternatively, increased humidity may contribute to the *MVLR* event because it destroys the original lerps and therefore necessitates the generation of the new midvein 'super-lerp'. The actual effect may include some degree of influence by both of the suggested mechanisms.

Temperature at time of total lerp flow, *TLFTEMP*, and relative humidity at time of lerp flow, *TLFRH*, were both negatively signed with values of -1.35 and -0.33, respectively. 'Total lerp flow' was the event where the entire honeydew component of a lerp absorbed sufficient atmospheric moisture that it became deliquescent and flowed downwards over the leaf surface, carrying some or all of the psyllids with it, drowning and/or crystallising a proportion of them in the process. A large proportion of the leaf surface could also be covered by the flowing honeydew, depending on how many lerps were on the leaf, and how far above the tip of the leaf a lerp was positioned. The negativity of the coefficients for *TLFTEMP* and *TLFRH* indicates that both temperature and relative humidity were contributing adversely to the survival, and therefore promoting the mortality, of the lerps thus affected – a result which

supports field observations. The deleterious effect of relative humidity on the lerp is easy to explain; it is a little more difficult, however, to attribute a mechanism whereby temperature at time of total lerp flow adversely affects a lerp's survival. It may be that higher temperatures contribute to a decrease in viscosity of the dissolved honeydew, thus promoting faster and therefore more complete flow from the lerp structure.

7.4.1.3.2 *Leaf model*

Temperature at time of leaf fall, *ABSLFTEMP*, had a positively signed coefficient of 0.56, suggesting that increased temperature promoted the fall of a leaf from the tree. Increased temperatures would assist the desiccation of the petiolar junction, resulting in contraction of the petiole tissue and withdrawal away from the junction, thus lessening the mechanical connection of the leaf with the branchlet. It should be noted in passing that despite the label name assigned to the variable, the actual abscission event at the petiolar junction would have occurred at some time before the leaf actually fell from the tree.

Relative humidity at the time of midvein lerp reformation, *MVLRLFRH*, had a negatively signed coefficient of -0.11 assigned by the analysis. This indicates that an increase in humidity at the time that the new 'super-lerp' is formed promotes an increased chance of leaf fall, an outcome that appears to directly oppose the mechanism suggested for the affect of temperature at time of leaf fall. It may be that since the *MVLR* event is promoted by increased humidity (see Section 7.4.1.3.2 above) the change in distribution of the psylloids changes the centre of gravity (or centre of mass) of the leaf, causing the torque applied by leaf movement due to wind to increase (a 'pendulum' effect), resulting in greater stress on the petiolar junction which has already undergone abscission. A similar argument applies to the fact that flowed lerp honeydew will also be at the leaf tip, and thus would increase torque on the leaf axis and weaken the petiolar junction, since total lerp flow event directly precedes the *MVLR* event. While the *TLFLFRH* variable is not specifically included in the *Leaf* model, this still does not prevent possible side effects of the lerp flow event influencing future events. The actual unsigned (*modulus*) value of the *MVLRLFRH* coefficient is the smaller of the two significant variables for the *Leaf* model, and is in fact the smallest *modulus* value of all significant variables for both models. The fact that *MVLRRH* (*Lerp* model) and *MVLRLFRH* (*Leaf* model) variables have opposite signs also reinforces the notion that what is good for the lerp (and therefore the psylloid) is bad for the leaf, and vice versa.

7.4.1.3.3 Leaf loss with respect to side of tree (*TREESIDE* covariate)

The possible causes of differences to leaf survival with respect to the eastern and western aspects of the tree are:

- higher air and leaf temperatures on the western side of tree;
- lower RH around the boundary layer of leaves on the western side of tree (tarmac and buildings on the western side, in contrast to shade at the hottest, driest and brightest part of the day on the eastern side, and shaded turf underneath the tree);
- higher incident and reflected light intensities for longer periods on the western side of tree in contrast to more shade and less intense reflected light on the eastern side's leaves.

All these factors would compound to produce higher stress levels on leaves on the western side of the trees than those on the eastern side. This in turn would be likely to lead to loss of leaf function *in extremis*, and consequent leaf abscission and loss from the tree.

Leaf-fall from the tree also means that the lerp was not only automatically removed from the study, but likely to be the cause of death of a proportion of the psylloids under lerps supported on that leaf. Thus, that which promotes the survival of the leaf will help promote the survival of the psylloids thereon.

7.4.2 Degree days models

7.4.2.1 Degree days estimates for *M. fici*: two degree days models using different temperature data sets

The degree days model (Allen, 1976) can be used to predict psylloid emergences at particular stages, given the mean daily maximum and minimum temperatures for the time of year. The model can also be used to predict the number of generations per year given local temperature records. An important component of the model for horticulturists and those others responsible for the maintenance (both 'short term' and 'long term') of *F. macrophylla* specimens growing in public recreation areas, etc., is the egg degree days, since this is the predictor for hatching time, the stage at which the psylloid appears to be physically the most vulnerable to attack by predators once it has left the egg (and not accounting for predation while in the egg, which also appears to form a significant proportion of juvenile psylloid mortality). The hatching stage is

also that at which the psyllid is most likely to be susceptible to control by, for example, insecticidal spraying.

At no point in the summer or entire-year temperature sequences used to calculate the Sydney degree days models did the mean daily temperature cross the arbitrary upper and lower development thresholds, indicating that psyllid development during either period was continuous. Given the close approximation of the models' numbers of psyllid generations per year to the observed numbers of psyllid generations per year, the models' lower development temperature threshold of 10 °C would appear to have been a reasonable first approximation.

Both 'short term' and 'long term' temperature models gave similar results, the differences being caused by long term climatic variability in the Long Term Temperature model, in contrast to what is effectively a 'spot' or 'point' reading of temperature in the 'short term' model. The degree days values for the various juvenile instars are in part dependent on the accuracy with which the instar durations were estimated (see Chapter 6, Section 6.3.2.3). Since these estimates are not wholly reliable, neither, therefore, are the instar degree days (see Table 7.8). A more accurate and complete (conventional) degree days model is dependent on detailed laboratory studies to determine the exact values for the upper and lower development thresholds, the mean durations of juvenile instars, and the mean duration of the (male and female) life-span. As an alternative somewhere between the *AGS* field study method and fully controllable laboratory experiments, it may be possible to undertake these detailed studies *in situ*, using an artificial plastic material such as 'Plasticine®' ('modelling clay') or picture-hanging putty (e.g., 'Blue-Tak®' or similar) to replace the psyllids' own lerp cover. Honeydew and wax could then be removed using, e.g., a dampened fine paintbrush, on at least a daily basis, or twice daily if time permitted. This would enable close scrutiny and measurement of the juveniles with respect to time, and allow accurate determination of moult events.

7.4.2.2 Application of the two temperature models to other geographical locations

The 'short term' model resulted in less available degree days (and therefore a longer time in any particular stage) than the 'long term' model. This is the result of the mean temperatures throughout the lives of the second *MFG* egg study and the *AGS* field study in late 1998 and early 1999 being cooler than the long term temperature average. Since the 'short term' 'snapshot', therefore, predicts psylloid events as occurring later than they would on average, the 'short term' model would tend towards being inaccurate over the longer term. If the 'short term' model were to be used to predict the hatching of psylloid eggs so that a control measure could be implemented, for example, the model would have a reasonable probability of failing and the psylloid would therefore have a good chance of escaping the control. The psylloid could well be under the lerp and protected from pesticides by the time that the sprays were scheduled to be applied. Hence, the 'long term' temperature model should be used in preference over the 'short term' model. The accuracy of the juvenile instar stadia also has a fundamental bearing on both of the degree days models, and until reliable mean lengths of each stadium are obtained, the models will also be biased by any stadial inaccuracies embedded within them. An even more accurate set of two or three separate seasonal models could be produced by using the mean daily temperatures for the approximate time period for a particular generation (e.g., spring, summer or autumn).

It is possible that number of generations of psylloids actually occurring per year is a significant factor in causing a psylloid outbreak, and is also a possible link between these outbreaks and the *F. macrophylla* defoliation cycle. A succession of years of warmer temperatures relative to long term averages, if sufficiently prolonged, would therefore result in a succession of three-psylloid-generation years. In turn, this could contribute to higher levels of stress within the tree than would arise during a succession of cooler, two-psylloid-generation years. The population densities would also continue to increase over this time as there would be more available degree days above the development threshold, resulting in the production of larger numbers of psylloids. The increasing psylloid numbers would place stresses on the tree by the amount of water and nutrients lost to the large psylloid population, and increasing concentrations of potentially debilitating secondary metabolites injected into the tree by the feeding processes of the psylloids. If the warmer conditions also happened to be accompanied by a marked decrease

in rainfall from the average, the putative stresses created within the tree by the psyllid would be compounded, with the possibility of the rate of defoliation events increasing.

7.4.2.3 Location summaries

7.4.2.3.1 Melbourne

Melbourne, the southernmost of the eight locations, had the lowest of the predicted degree days (all models) as the results of colder winters than the other locations.

7.4.2.3.2 Adelaide

The variability in Adelaide's mean yearly temperature was, at the time of the survey, lower than Sydney's (by about 6%). Adelaide lies on a line of latitude (Table 7.10) approximately 200 km south of Sydney. Since there is no *M. fici* population in Adelaide, this set of models is purely theoretical. The models do, however, predict to a certain extent how the psyllid population dynamics might unfold, if the psyllid were accidentally introduced there.

7.4.2.3.3 Wollongong

The Wollongong degree days are slightly lower by about 0.06% for the 'long term' model and by about 2% for the short term model than the corresponding Sydney models. These lower degree days values for Wollongong are consistent with its geographical location, being south of Sydney in closer proximity to cooler winds blowing from the Australian Alps.

7.4.2.3.4 Sydney

The Sydney prediction of 2.5 generations per year could be interpreted as two generations in cooler-than-average years and three generations in warmer-than-average years, and reflects what has been observed of numbers of psyllid generations per year in this region. In some years (as in 2002) the autumn generation has not appeared, while in other years (for example, in 1999 and 2004) there have been three psyllid generations observed.

7.4.2.3.5 Parramatta

The degree days estimate for Parramatta, in western Sydney, was lower than that of Sydney's itself, which is the result of winters being colder (and with a greater occurrence of frosts) than

those of Sydney central business district. The overall variability in Parramatta's climatic conditions is indicated by the fact that the standard deviation for Parramatta's mean yearly temperature is 11.5% higher than that of the Sydney CBD (i.e., Observatory Hill). Parramatta's climatic variability was in fact the third highest of all the eight locations. Parramatta's predicted degree days for all models except the 'summer generation' model (Table 7.13) were lower than that of Adelaide, thus further supporting the effect of colder Parramatta winters.

7.4.2.3.6 Port Macquarie

It could be expected, given its latitude, that Port Macquarie would have development times about midway between those of Grafton and Sydney, yet it is less than the Sydney 'short term' (Table 7.9) and 'long term' models' predictions. For Port Macquarie, approximately 1.5 ° of latitude north of Sydney, however, the model showed no appreciable difference in development time. The reason for this 'hiatus' at Port Macquarie is unclear, but may lie in the climate records for Port Macquarie, which, on closer inspection, gave the appearance of problems having occurred in the recording of measurements at certain periods. There was a wide and also random variation of time between recorded temperature measurements as supplied by the Bureau of Meteorology, amounting in some instances to gaps of several days, and sometimes even weeks, between readings. Climatic conditions at Port Macquarie caused by the flat topography of the area may also be contributory to the result.

7.4.2.3.7 Grafton

The somewhat anomalous result of model predictions for Grafton suggests a wider variation of climate around Grafton than that around Brisbane, with warmer 'summer' and colder 'winter' seasons, and is supported by the standard deviation for the yearly mean temperature at Grafton of ± 4.0 °C, compared with the standard deviation for the yearly mean temperature for Brisbane of ± 3.6 °C. Grafton is approximately 75 km inland from the ocean, with an inherently greater fluctuation in temperature at night. The models predict a full three generations per year for Grafton.

7.4.2.3.8 Brisbane

Brisbane is a subtropical coastal location with consequently less fluctuation in temperature because of the temperature-buffering capacity provided by the proximity of the very large body

of relatively warm water (i.e., the Coral Sea). This stabilisation of climate would thus provide the psyllid with a greater number of degree days above the development threshold than in Grafton. The models also predict a full three generations per year for Brisbane.

There were no data available for the actual number of generations for any of the locations other than Sydney, however, so no verification of these predictions was able to be made.

7.4.2.4 Degree days estimates for *M. fici*: possible effects of the *El Niño*/Southern Oscillation climatic phenomenon on the models

An organism's ability to adapt to major variations in climatic conditions, or irreversible shifts in long term climatic patterns, will determine its long-term survival. Since degree days is fundamentally a predictive device based on climate, major and long term, as well as short term (e.g., diurnal and seasonal), climatic events will have a bearing on an organism's growth rates over the long term as well as the short term.

The *El Niño*/Southern Oscillation (ENSO) climate cycle (Jaksic, 1998; Jaksic, 2001) is one possible mechanism by which the psyllid population boom-bust cycle might either be generated or reinforced. The *El Niño* climate oscillations are long term climatic cycles of alternate cooling and warming of surface ocean waters in the region of the equatorial eastern and central Pacific Ocean in the Southern Hemisphere. Lower atmospheric pressure produced by cooler ocean surface temperatures causes reduced evaporation and uplift, and in turn results in reduced rainfall, and is the part of the cycle termed *El Niño* (Jaksic, 1998). The second part of the cycle is the warming of surface (Pacific) ocean waters with increased surface evaporation, uplift of moisture-laden air, and hence increased rainfall, and is known as *La Niña* (Jaksic, 1998). When the *El Niño* part of the cycle is affecting western South America (the eastern side of the Pacific Ocean) with below-average rainfall, the *La Niña* part of the cycle is in effect on the western side of the Pacific Ocean, in Indonesia and eastern Australia. Cooler ocean currents lead to lower air pressures and less evaporation from the surface of the sea, and therefore lower rainfall, and vice versa. The ENSO cycles have an erratic, or possibly chaotic, periodicity, and are currently relatively unpredictable. *El Niño* events are correlated with dry conditions, although an *El Niño* event may not result in a full-blown drought.

The ongoing study of the phenomenon appears to indicate, however, that the occurrence of drought conditions during *El Niño* events may be more likely than not. Lower rainfall combined with the occurrence of higher than average atmospheric temperatures on land could combine to produce stresses in the plant, which would be compounded, possibly geometrically, by a climate-induced increase in psylloid numbers. A chain of events such as this might result in the increased frequency of defoliation events observed in *F. macrophylla*, not just in Sydney but in all locations that appear in Table 7.9. The psylloid outbreaks causing defoliation also have a somewhat erratic periodicity, suggesting that the outbreaking population may be under control of the climate and may even be directly correlatable with an Australian *El Niño* event. This suggestion is hypothetical, and it would require further long term observation of both climate and the insect tree system, as well as research into past climate records and known psylloid outbreak/*F. macrophylla* defoliation events, to confirm or reject this hypothesis. Based on the above, it could be predicted that psylloid plague outbreaks and *F. macrophylla* defoliation might occur just after the peak of a very dry and warm western Pacific *El Niño* event.

7.4.3 Comparisons of psylloid activity with respect to trees and dates

Eggs were present on most trees, for most of the time. Egg mass and lerp numbers, however, both varied considerably from tree to tree, suggesting variations in numbers of fecund/fertile females in the preceding generations. Quality and/or quantity of the juvenile psylloids' food source would feed back into this system at any point (Wratten *et al.*, 1988): either at egg lay as suitable oviposition sites, during juvenile development as actual food quality, or during adulthood since the adult psylloid feeds actively from either the adaxial or abaxial side of the leaf midvein.

7.4.3.1 Psylloid activity with respect to eggs

Females will lay eggs on first-position leaves (especially during psylloid plague conditions), even though the leaves may be relatively unpalatable to both the adult psylloids and the post-egg juvenile psylloids. One explanation for the Leaf 1 egg-laying behaviour is that by the time that the 60 or so days that the psylloids have spent in the egg stage have elapsed, the leaves have been given sufficient time to mature to the point where they will be a good quality source of food for the hatching psylloids.

7.4.3.2 Psylloid activity with respect to lerps

The fact that there are actually lerps recorded for leaf 1 positions (Table 7.15) suggests that either the leaves were sufficiently mature, or that the eggs were laid during the height of psylloid plague outbreak, when good laying sites were scarce. Psylloid females were observed to lay eggs on almost any leaf surface available whether *F. macrophylla* or not, under conditions of extremely high adult psylloid densities (see also Chapter 5, Section 5.4.1). Even partially unfurled leaves were selected in the frenzy of competition for (any) egg-laying sites. The chlorophyll fluorescence studies from which most of the data was extracted to produce Tables 7.14 and 7.15 were conducted just after the psylloid plague and just before the massed *F. macrophylla* defoliation event of late September-early October, 1996. The trees (before defoliation) would have had eggs on almost every leaf, including first-position leaves, explaining the presence of lerps. Another explanation for the appearance of lerps on the first-position leaves (and probably the more likely one) is the combination of the propensity for *M. fici* hatchlings to 'migrate' to leaves other than those on which their eggs were laid, and the intense competition for feeding sites created by extremely high numbers of psylloids during the plague. This provides a mechanism for the colonisation of a sub-optimal leaf that would normally be rejected. The low average proportions for the leaf positions may be explained by the defoliation event and the resultant crash of the psylloid population (eggs, larvae and adults). There has not been such an outbreak since 1996, and the numbers of eggs on older leaves had exceeded those numbers on the earlier first-position leaves. This explanation is supported by the fact that the average ratio of eggs to lerps on first-position leaves (see Tables 7.14 and 7.15 and Section 7.3.3.2 above) was calculated at 8.5 to 1, almost an order of magnitude difference in density.

7.4.3.3 Other observations on psylloid activity with respect to both eggs and lerps

A proportion of 100% for either eggs or lerps at any leaf position for any tree was relatively rare in the observations, occurring in only one instance on leaves younger than position 4 in the egg table (Table 7.14), two instances on leaves older than position 6 in the egg table, and one instance in leaves younger than position 3 in the lerp table (Table 7.15), out of a total of 58 possibilities: approximately 6.9%. The tree on which the single instance of 100% of leaves younger than position 4 which had eggs (Leaf 2) or lerps (Leaf 3) on them was *BTC*, which had heavy loads of both eggs and lerps on its leaves. The mean proportion of eggs present per leaf

for *BTC* was 0.95 ± 0.030 , the highest of the within tree means for the egg table, and the lowest variation between leaf positions. The mean proportion of lerps present per leaf for *BTC* was 0.65 ± 0.38 , the second-highest mean for the group (*AGN* being the highest), and with the highest between-leaf variability of the group. The subject matter of Tables 7.14 and 7.15 will be returned to in Table 8.17 in Chapter 8, with the addition of F_v/F_M ratio indices of tree health for each tree. This comparison is deferred until the subject of Fast Chlorophyll Fluorescence Kinetics and the F_v/F_M ratio, so far only alluded to in passing up to this point in the thesis, have been discussed with respect to *F. macrophylla* populations growing in Sydney. The tree health and growing conditions of a subsample of the Adelaide *F. macrophylla* population is also compared with the five main study trees in the next chapter.

7.5 Summary of Chapters 6 and 7

The following list summarises the main findings presented and discussed in these two chapters:

- Long egg development period is approximately 56 days (Chapter 6);
- post egg juvenile development period is approximately 40 days(Chapter 6);
- psylloid hatchlings will migrate relatively large distances to populate uncolonised leaves (Chapter 6.);
- lerps contain overlapping age groups (i.e., a range of instars) (Chapter 6);
- post-egg psylloid juvenile head capsule growth is linear (Chapter 6);
- five post egg juvenile instars as for the general psylloid (Chapter 6);
- stadial duration of each post-egg juvenile instar estimated (in days) (Chapter 6);
- lerps are highly sensitive to atmospheric moisture (Chapter 6);
- first and second juvenile instars are susceptible to lerp deliquescence under very humid conditions (Chapter 6);
- psylloids in developed lerps are robust with respect to loss of lerp (Chapter 6);
- psylloids migrate to the leaf midvein after loss of lerp and rapidly build a new one; midvein probably a better food source than laminal tissue (Chapter 6);
- adult psylloids are active feeders (on midvein), probably long lived (Chapter 6);
- obstacles produced by the *quasi*-cryptic lifestyle of the psylloid were overcome to a degree sufficient to enable reasonable conclusions about psylloid life history to be made (Chapter 6);

- parasitisation by *Psyllaephagus* sp. occurs only in lerps that have been established for at least ten days (Chapter 6);
- two size phenotypes of female *Psyllaephagus* sp. found (Chapter 6);
- *Psyllaephagus* sp. juveniles can be multiply parasitised successfully (at least in the short term) (Chapter 6);
- *Psyllaephagus* sp. may be one of the polyembryonic species of encyrtid (Chapter 6);
- age-specific life tables were developed for *M. fici* (Chapter 7);
- an individual psylloid has a chance of approximately 3.6% of surviving from egg lay to adulthood (Chapter 7);
- specific causes of psylloid mortality are: leaf loss, egg and juvenile predation, parasitisation of juveniles, lerp flow, leaf abscission (Chapter 7);
- predators are estimated to have caused 13.72% of overall population mortality (Chapter 7);
- parasitoids are estimated to have caused 4.77% overall population mortality (Chapter 7);
- parasitisation of psylloid juveniles does not occur until the lerp is well developed (Chapter 7);
- degree days models based on summer 1998-99 generation developed (Chapter 7);
- complete generation degree day model is potentially robust as it is based on field observations and long term climate data (Chapter 7);
- accuracy of juvenile instar sections of degree days models are dependent on the estimates of the juvenile stadal lengths being correct (Chapter 7);
- climatic comparisons and predictions of psylloid summer generation numbers for eight locations across a wide climatic range using the degree days models developed for *M. fici* (Chapter 7), including number of generations per year of psylloid for the eight locations.

The degree days models should provide a useful tool in predicting psylloid outbreaks. Further study of the psylloid with respect to long term climatic patterns (including the *El Niño* cycles) could be undertaken to study the hypothesis that psylloid plagues are generated by drier and hotter than average climatic conditions. This hypothesis has arisen as a direct result of the

development of the degree days models for the psyllid, and observations of other interactions of psyllid populations with other climatic elements (e.g., relative humidity, soil moisture and other edaphic conditions), during this project.

The findings also allow for a better understanding of the resilience of the tree with respect to the psyllid. The major cause of psyllid mortality was determined to be that of habitat loss, i.e., leaf fall. This suggests that promoting good tree condition, to withstand depredations caused by the psyllid, as well as the effects of other environmental factors such as drought and soil compaction, are the keys to the long term survival of the *F. macrophylla* specimens in urban situations. This idea is expanded upon in the next chapter, where physiological effects of *M. fici* on *F. macrophylla* are studied in detail using Fast Chlorophyll Fluorescence Kinetics analysis techniques.

Chapter 8: Using fast chlorophyll fluorescence kinetics in determining stress in mature *Ficus macrophylla* (Desf. ex Pers.) trees

8.1 Introduction

Previous chapters have mentioned a relationship between psylloid population dynamics and health of Moreton Bay figs trees without any evidence or further discussion being presented. This chapter addresses the issue of psylloids and fig tree health.

It is common for plants to suffer stress from the feeding activities of insects and other arthropods (see Section 8.1.1.4 for references relating to *FCFK* and plant stress). The degree of stress on a particular plant, however, will depend on the species of plant, the species of arthropod feeding upon it, and other environmental factors such as water deficit, and extremes of temperature outside the plant's normal range. Plants vary in the type and magnitude of their response to insect feeding. *F. macrophylla* is sensitive to fig psylloid attack to at least some degree, since fig defoliation events occur predominantly when the psylloids are present in plague proportions (more than two active psylloid lerps per leaf on most leaves between first unfurled leaf and leaf five or six).

A brief summary of the light reaction pathways of photosynthesis and the role of fast chlorophyll fluorescence kinetics (*FCFK*) within this complex system of redox reactions is given as a background to tests made on the fig trees. Results of *FCFK* tests conducted on five Moreton Bay fig trees over a twelve month period are presented and discussed.

8.1.1 Chlorophyll fluorescence

In order to determine whether *M. fici* has a measurable effect on *F. macrophylla*, I used a Plant Stress Meter (*PSM*; Öquist and Wass, 1988) to measure the status of the photosynthetic systems of leaves on five selected Moreton Bay fig trees in and around the Royal Botanic Gardens, Sydney, over a period of 12 months. This period included a psylloid plague and a simultaneous and widespread Moreton Bay fig defoliation event, followed by relatively low psylloid numbers for the rest of the observation period. The *PSM* uses the phenomenon of fast chlorophyll fluorescence (fluorescent light emitted from chlorophyll molecules during

photosynthesis) to estimate the efficiency of the photosynthetic apparatus in a rapid, non-invasive and non-destructive manner.

8.1.1.1 Fast Chlorophyll Fluorescence Kinetics: Theory and Application

8.1.1.1.1 Photosystems within the photosynthesis ‘light’ reaction pathways

Photosynthesis pathways fall into two fundamental groups (Arnon, 1992b): those that are photon-dependent (energy capturing or ‘light’ reactions), and those that are in turn dependent on the photon-dependent (synthetic or ‘dark’) reactions. In the light-side series of reactions, bombarding photons of the correct wavelength (*ca* 690 nm) initiate and drive a reduction-oxidation electron transport chain, resulting in the reductive splitting of water and the production of the reducing equivalents NADPH and ATP (Arnon, 1992a; Andersson, 1992). Light harvesting is performed by membrane-bound antenna pigments (Albertsson *et al.*, 1983; Albertsson *et al.*, 1992) which pass the energy along a transport pathway to Photosystem II (*PS II* or cyclic reduction system) and Photosystem I (*PS I*, or non-cyclic reduction system) reaction centres via reduced intermediates (Arnon, 1992a; Arnon, 1995; Losada, 1992; McCauley *et al.*, 1987). Light energy, from the point of view of the photosynthetic machinery, is a limiting resource: it is always available in excess for at least part of the 24-hour diurnal cycle, and the photosystems must operate in such a way as to avoid being damaged by this excess (Arnon, 1992a; Arnon, 1995). Chlorophyll fluorescence is one of a number of internal regulatory processes in the light-side photosynthesis reactions which cooperate in preventing this destructive buildup of energy. Excess energy manifests in the form of heat and oxidised (i.e., higher energy level) intermediates in *PS I* and *PS II*: safety mechanisms to reduce energy levels exist as tightly controlled feedback systems residing within both these photosystems.

In the light-side reaction chains following photon-capture by the antenna pigments (Arnon and Tang, 1988), *PS I* (non-cyclic photophosphorylation) is responsible for the reduction of NADP^+ to NADPH via the protein ferredoxin, as well as the photophosphorylation of ADP to ATP. *PS II* (cyclic photophosphorylation) is responsible for the reduction of NADP^+ via pheophytin, water, and ferredoxin. Byproducts of the *PS II* reactions include the evolution of molecular oxygen (O_2) (Björkman and Demmig, 1987), heat, and chlorophyll fluorescence (Arnon 1995). Although it was originally believed that *PS II* passed its captured energy on to *PS I* as a crucial step in the Z-scheme (Hill and Bendall, 1960; Walker, 2002), the two photosystems are now thought to be largely independent of one another from the points of view

of both inputs and outputs, the only links being the overall feedback control system which integrates and regulates their activities (Arnon, 1995). *PS I* and *PS II* were given alternative terms by Arnon (Arnon, 1992a; Arnon, 1995) as *non-cyclic photophosphorylation* and *cyclic photophosphorylation* respectively, with Arnon (Arnon, 1993) also suggesting the dropping of the term 'Z-scheme'. At the stage where both photosystems are fully energised, but before the light reaction series takes place, the light-reaction photosynthetic systems are said to have attained their light saturation point (Papageorgiou, 1975).

Energy over the saturation point level has a number of different fates. In normally operating photosystems the super-threshold energy is used in the production of reduction equivalents, and any excess above this that cannot be stored in this way is dissipated by radiant heat and by chlorophyll fluorescent photon emission (Arnon, 1992a). The ability of the photosystems to discharge surplus energy efficiently and effectively (Asada, 1999) depends on their own health, the status of which is the result of a complex of interactions with environment factors such as water and sunlight, within and between themselves, and between the photosystems as a whole and the rest of the organism. This is the case for all photosynthetic organisms, including plants and cyanobacteria. In damaged photosystems, e.g., in a plant under stress (see below), the electron transport chain becomes blocked, causing the phenomenon of photoinhibition (Andersson, 1992; Ball, *et al.*, 1995; Krause, 1988; Matile *et al.*, 1999). *PS II* is initially affected (Ludlow and Powles, 1988; Andersson 1992), with the fluorescence mechanism under photoinhibited conditions usually being suppressed to at least some degree (Arnon 1992a; Cleland, 1988): photoinhibition can be measured directly and easily using a fluorimeter (Öquist and Wass, 1988).

8.1.1.1.2 Chlorophyll fluorescence mechanisms in photosynthesis pathways

The buildup of surplus energy beyond the light saturation point in terms of both molecular integrity and equilibrium oxidation states, and consequent damage to the system, has been stated above as being controlled by several mechanisms. The mechanism of interest here is that of chlorophyll fluorescence; energy in the form of photons is emitted (fluoresced) by photosystem excitation quenchers. These quenchers are embedded within the *PS II* (Barber, 1992; Demmig and Winter, 1993) and *PS I* (Arnon *et al.*, 1981a) reaction centres. Fluorescent emission is known to occur in two bandwidths: the fast fluorescence band of ca 689 nm associated with the *PS II* reaction centre; and the slow fluorescence band of ca 703 nm

associated with the *PS I* reaction centre (Arnon, 1992a; Arnon, 1995). *PS II* contains pheophytin (P680), a form of chlorophyll *a* which lacks the porphyrin-centred Mg^{2+} ion; a binding protein; a manganese-containing protein; and a quinone designated by the letter *Q* (Arnon, 1992; Andersson, 1992; Demmig and Winter, 1988). Oxidised (activated) *Q* accepts an electron from an energised P680 molecule, and lowers its own consequently energised state by passing the electron on to the H_2O -splitting process (itself cyclical: see Arnon, 1992a), or by emitting the energy from that electron as fluorescent light in the 689 nm band, thus removing energy from the system (Arnon and Tang, 1988; Papageorgiou, 1975). An Fe-S containing protein similar to ferredoxin is the *PS I* equivalent of *PS II*'s *Q* (Arnon, 1988; Arnon, 1992a). Both photosystems are independent of one another, the only linkages between them being those of the feedback control systems (Arnon, 1995). It is the *PS II* fast fluorescence which is used by the *PSM* to determine the health of photosynthesis systems in assayed tissue or cells.

8.1.1.1.3 Stages in the chlorophyll fluorescence mechanism

Chlorophyll fluorescence, also known as the Kautsky effect after the first person to observe the phenomenon, comprises several distinct stages (Papageorgiou, 1975), and is represented in the literature as the Kautsky curve shown in Figure 8.1 below (Bohlár-Nordenkampf, *et al.*, 1989). The entire sequence is divided into two separate stages: a fast phase corresponding to line segment *O-S* in Figure 8.1, and a slow phase corresponding to line segment *S-T* in Figure 8.1 (see also Govindjee and Papageorgiou, 1971). The two phases are in turn divisible: four subphases in the fast phase and two sub-phases in the slow phase. Referring again to Figure 8.1, the four fast fluorescence subphases are: activation rise or induction phase (line segment *O-I*); first fast fluorescence decay (line segment *I-D*); main fast fluorescence rise (line segment *DP*); and the second fast fluorescence decay phase (line segment *PS*). The slow fluorescence subphases are: slow fluorescence rise (line segment *S-M*) and slow fluorescence decay (line segment *MT*). As a reflection of this, the Kautsky curve is sometimes referred to in the literature as the *OIPSMT* curve (Bohlár-Nordenkampf *et al.*, 1989). The slow rise phase involves both photosystems, whereas the fast rise phase is restricted to *PS II*.

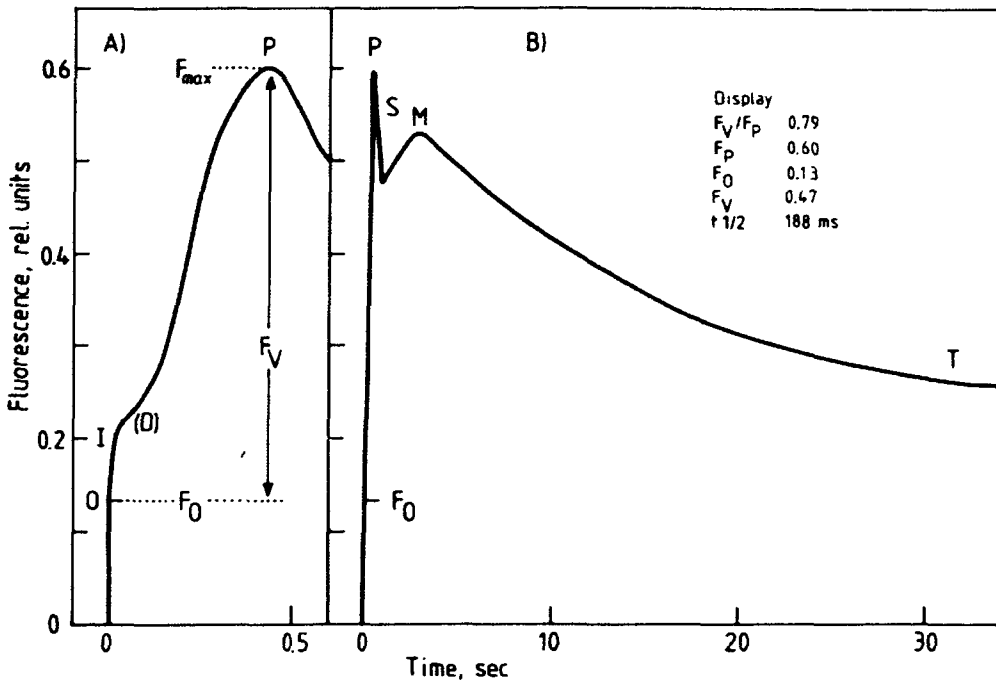


Figure 8.1: The Kautsky or 'OIPSMT' Curve: from Bohlär-Nordenkamp *et al.*, 1989.

The fluorescence value at the end of the induction phase (at point *O* in Figure 8.1) is the light saturation point, also known as the constant yield component, and is designated by the symbol F_0 . (Papageorgiou, 1975). The fast fluorescence rise (*D-P*) begins from this point. The peak of this phase at point *P* is defined as the maximum fluorescence value, or F_M (Papageorgiou, 1975). F_0 and F_M are used to calculate a measure of the efficiency of *PS II* and therefore of the photosynthetic apparatus in general. The difference between the value of maximum fluorescence F_M and the constant yield component F_0 , is known as the fast-phase variable fluorescence, or F_V . Dividing F_V by F_M gives the F_V/F_M ratio, which is a unitless quantity (Papageorgiou, 1975). The lower that the value of F_0 is in relation to F_M , the larger the F_V value will be, and hence the higher the F_V/F_M ratio. Conversely, the closer that the value of F_M is to the value of F_0 , the lower F_V/F_M will be. The usually accepted value of F_V/F_M for a healthy plant is around 0.800 (Lichtenthaler, 1988; Lichtenthaler and Miehé, 1997, Öquist and Wass, 1988). The remaining variable of importance deriving from chlorophyll fluorescence kinetics that was used in this study is that of the half raise time, or $t_{0.5}$, which is mathematically defined as $(t_{F_M} - t_{F_0})/2$ (i.e., the midpoint between t_{F_M} and t_{F_0}). This value is half the time taken for the fluorescence value to reach F_M from F_0 , and is measured in milliseconds. F_0 is approaching F_M in value, the F_V/F_M value is approaching a minimum, and vice versa: F_V/F_M and $t_{0.5}$ therefore have an inverse relationship.

8.1.1.1.4 Use of fast chlorophyll fluorescence kinetics in the detection of plant stress

Chlorophyll fluorescence generated by both *PS I* and *PS II* has been used since the 1970s (Leverenz and Öquist, 1987; Papageorgiou, 1975; Öquist *et al.*, 1992) to study parts of the light-side reactions within the photosynthetic apparatus of plants and cyanobacteria (Andersson, 1992). Fast chlorophyll fluorescence analysis has been employed in the study of plant growth and stress in biome/species combinations as different as wheat plants grown in experimental glasshouses (Lu and Zhang, 1988) and plants growing in forest communities (Ball *et al.*, 1995). The fast-phase fluorescence rise emitted from *PS II* is used in the analysis of drought-induced stress in plant tissue (Balota and Lichtenthaler, 1999), in validating remote sensing in forestry management (Stone, 2000; Stone *et al.*, 2001), and has been used as a research tool in the elucidation of electron donors in both *PS I* and *PS II* (Arnon, 1992a; Arnon, 1995).

As outlined above, photosynthetic structures in stressed plant tissue sustain damage, resulting in a decrease in the ability of the photosystems (especially that of *PS II*) to quench surplus energy via fluorescence. Quantitative analysis of plant tissue using machines exploiting fast chlorophyll fluorescence kinetics (*FCFK*) has become an established tool in the evaluation of stresses in plants (Azcón-Bieto, 1992; Azcón-Bieto *et al.*, 1983; Ball *et al.*, 1995; Bohlär-Nordenkamp *et al.*, 1994; van Kouten and Snel, 1990; Lichtenthaler, 1988; Lichtenthaler, 1996; Mohammed *et al.*, 1995; Schreiber and Bilger, 1987; Shimazaki *et al.*, 1988; Stone and Bacon, 1995a; Stone and Bacon, 1995b; Stone and Clarke, 1998) since the first measuring devices dedicated to this technique became realistically usable in field situations in the mid 1980s (Bohlär-Nordenkamp *et al.*, 1989; Öquist and Wass, 1988).

Types of stress affecting plants include: chilling/freezing; high temperatures and/or high light levels; water deficit; and insect and pathogen attack. Chilling-stress suppression of F_v/F_M values in plants is known from a number of *FCFK* field experiments on conifers in northern Europe (Adams and Demmig-Adams, 1992; Bauer *et al.*, 2000; Binder *et al.*, 1997; Cregg *et al.*, 2001), on olive trees in the Mediterranean (Bongi and Long, 1987) and maize (Hetherington *et al.*, 1983). Chilling damage can also be long-lived, and depending on the cause (Bongi and Long, 1987; Andersson 1992; Arnon 1992), it is sometimes irreversible for the affected photosystems. The ability of the plant to counteract and recover from chilling-

induced photoinhibition can be further retarded by high light conditions (Bongi and Long, 1987). High light levels following chilling damage prevent affected *PS II*s from recovering quickly enough to avoid still further damage to the overall photosynthetic machinery. Unusually elevated temperatures can also produce the same effect (Bongi and Long, 1987); in conjunction with high light levels and previous chilling damage, high temperatures can lead to irreversible damage. Water stress-induced photoinhibition and photoinhibition-recovery in wheat plants was examined by Lu and Zhang (1998) using chlorophyll fluorescence techniques. *FCFK* analysis of aphid feeding-induced stress in barley plants was used by Blanco (1994). *FCFK* analysis has also been used in laboratory research on maize plants (Hetherington *et al.*, 1983).

Using specialised spectrophotometric equipment (Bohlàr-Nordenkamp, 1989; Öquist and Wass, 1988), the state of the light-quenching components within *PS II* in leaf chloroplasts can be estimated. From this estimate, the condition of the leaf and, given sufficient data, the whole plant, can be inferred. The measuring equipment used to analyse fast chlorophyll fluorescence is essentially a fluorimeter with a central processing unit and random access memory. Data from the fluorimeter is processed by the CPU and stored in memory (Öquist and Wass, 1988). Calculated results for each analysis are usually displayed as the F_V/F_M ratio and $t_{0.5}$ values. Results are compared with the generally accepted reference value of ca 0.800 for leaves in good photosynthetic condition.

8.2 Materials and Methods

Specimens in Sydney and Adelaide were subjected to chlorophyll fluorescence analysis. The Adelaide population of figs was of particular interest as there is no population of *M. fici* there (see also Chapter 2, which discussed the geographical range of the psyllid), and in that sense acts as effective comparison subjects for the Sydney population.

Five *F. macrophylla* trees were chosen from the population in the Royal Botanic Gardens and Sydney Domain: these trees have appeared earlier in this thesis as the subjects of other studies (Chapters 4, 5, 6 and 7). The trees were growing in different microclimates: sheltered or directly exposed to salt spray and/or sea breezes. Each tree was given a distinguishing 3-letter code for identification during analysis. Two trees were situated in the Gardens (Code *MFG*: RBG accession number 15743, grid reference *10i3m*; Code *L44*: accession number 14066, grid reference *7f7l*). Accession number 15743 is situated close by the Morshead Fountain Gate (near Macquarie St), hence *MFG*; and accession number 14066 is situated on Lawn 44, near the Sea Wall, hence *L44*. Three trees were located in the Domain between the Andrew (Boy) Charlton Pool and the RTA Bus Turning Circle towards Mrs Macquarie's Chair, and close to the southeastern corner of the Art Gallery of NSW. These were: Code *BTC* (Bus Turning Circle): accession number 19181, grid reference *3g7t*; Code *HRB* (Herbarium): accession number ZL 19100, grid reference *5j10s*; and Code *AGN* (Art Gallery North): accession number ZV19100, grid reference *7m1*. *L44*, *BTC* and *HRB* were close to the harbour (less than 50 m), and *MFG* and *AGN* were more than 500 m from the harbour, in positions sheltered from windblown salt spray. *MFG* was the only one of the five trees to have been fenced off from the public to alleviate the effects of soil compaction caused by pedestrian traffic. The measurements were made on five separate occasions over twelve months from September 1996 to September 1998. Measuring of the whole group usually took two whole days.

Each study period (i.e., the *Date* variable) was also given a distinguishing code: *SO96* for September-October 1996; *ON96* for October-November 1996; *DEC96* for December 1996; *JUN97* for June 1997; and *AS97* for August-September 1998.

F_v/F_m and $t_{0.5}$ values from *F. macrophylla* leaves were measured using the Plant Stress Meter Mark II (*PSM*), a field-portable fluorophotometer designed and manufactured by BioMonitor S.C.I. (Öquist and Wass, 1988). The *PSM* had an onboard memory capacity for storing 1000

individual chlorophyll fluorescence analyses. Photosynthetic (actinic) light (the range between 300-660 nm) from a quartz-halogen globe irradiated leaf tissue at variable photon flux densities between 100 and 400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in discrete steps of 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The duration of the irradiating light charge was variable between one and five seconds in one second intervals. The PSM returned the results of the analysis as the F_V/F_M ratio and $t_{0.5}$ values on its liquid crystal display (LCD). Leaf tissue was dark-adapted for at least 15 minutes to allow the photosystems to run down to the oxidised or de-energised 'open' state. The dark-adapting device used was a simple plastic clip or 'clamp cuvette', which had an attachment end for accepting a fibre-optic light-delivery cable, and a sliding retractable blind enabling dark-adaptation of the specimen tissue. Measurements were taken by inserting the light cable, drawing back the blind and running the machine at the chosen settings for runtime and light density.

Twenty four leaves from both the eastern and western aspects of the five sample trees were subjected to analysis, making a total sample of forty eight leaves per tree per sampling period. Sample leaves were removed from the tree immediately before testing, as the limited reach of the light cable (approximately one metre) did not allow the leaves to be left *in situ*. The complete list of variables collected fell into three main groups: psylloid-related; tree-related; and environmental and others (Table 8.1 below). Data on the variables were collected by observation and counting, and were entered into the field log book. The range of values for each variable was set as appropriate: presence/absence (e.g., *Lerps*, *Egg mass*), integer (e.g., *Sunlight*: 0, 1, 2), tenths (*Cloud cover*), and percentage (e.g., % *Leaf missing*, % *leaf necrotised*).

Variables populated with discontinuous integer series such as Leaf age were coded and the data set was subjected to Multiple Analysis of Covariance and Multiple Regression tests using the IBM personal computer version of the software package 'Statistica 5.1m' (StatSoft Inc., 1998). The dependent variables were F_V/F_M and $t_{0.5}$. Graphs of salient analyses were also generated from within the Statistica package. All tests were made at the 5% error level ($\alpha = 0.05$).

Table 8.1: Variables collected during FCFK studies of five *F. macrophylla* specimens**Environmental and other variables**

Date (SO96, ON96, DEC97, JUN97, AS97)
 Cloud cover ($n/10$)
 Mechanical and non-psyloid arthropod damage (+/-)¹
 Proportion of leaf missing because of damage of any type (%)
 Sunlight (shaded; semi-shaded/dappled; full sun: 0, 1, 2)
 Sooty moulds (+/-)
 Predators (+/-)

Tree-related variables

F_V/F_M and $t_{0.5}$ values
 Branchlet number (1,..., n)
 Leaf number on branchlet (1, 2)
 Leaf number of particular survey (1,...,48)
 Leaf age relative to terminal bud (1,..., n ; terminal bud = 0)
 Leaf maturity (1,..., n)
 New leaf (+/-)
 Leaf surface (abaxial/adaxial: variable abandoned early in the survey)
 Leaf orientation (cardinal and intermediate points, i.e., north, northeast, etc.)

Psyloid-related variables

Egg masses (+/-)
 Number of egg masses
 Lerps (+/-)
 Number of lerps
 Number of new lerps
 Number of old lerps
 Leaf necrosis (+/-)

¹ +/- = presence/absence

The sample set of five Sydney trees was studied from September 1996 to June 1997 in the Royal Botanic Gardens and Domain. A sample set of five Adelaide trees was studied on 31 December 2000 and 1 January 2001, at the Adelaide Botanic Gardens and adjacent Botanic Park. It should be noted that the Adelaide cohort of trees of which the study trees are a part, are of a similar age to those in Sydney, i.e., about 150 years old. The trees in Adelaide were chosen for a range of conditions, reflecting those in Sydney as much as possible. The range was from irrigated and fertilised through to unirrigated and unfertilised. Like the Sydney trees, the Adelaide trees were assigned simple alphanumeric codes to distinguish them in relation to their locations. *BP1* was growing in the harshest conditions of the group, without irrigation or fertiliser. *BP2* received more irrigation and fertiliser than *BP1*, about the same as *BP3*, and less irrigation than *BP4*. *BP4*, on the other hand, while receiving more water, had more soil compaction as it was directly opposite the main northern entrance to the Gardens, and this is a very popular picnic area. *ABG1* was in quite a different situation compared with the previous four trees, in that it received the most irrigation, and also received frequent applications of water on the lower regions of the trunk as a side effect of the deliberate watering of various species of epiphyte growing thereon. The tree was also the recipient of the large quantities of well-composted organic mulch that were applied to the flower beds arranged around the base of the trunk of this tree. Soil water levels would have been higher than the other four trees since the surrounding area received regular watering for nearby smaller plants requiring high levels of soil and atmospheric moisture. Soil compaction may also have been reduced by the presence of paving blocks laid over the root zone of the tree acting as a buffer against compression. The gaps in the paving blocks allowed a steady supply of water to trickle through from regular hosing clean of the pavement, which was close to the Gardens' Restaurant.

8.3 Results

8.3.1 Results for Sydney population of *F. macrophylla*

8.3.1.1 Preliminary analysis

8.3.1.1.1 Relationship of F_V/F_M and $t_{0.5}$

The initial analysis was a simple linear regression between F_V/F_M and $t_{0.5}$, purely to test the relationship between the two variables. The graph of total F_V/F_M vs total $t_{0.5}$ data in Figure 8.2 below appears to indicate the theoretically-expected inverse relationship between the two variables: but see Section 8.4.1.1 in the Discussion regarding this. Figure 8.2 also presents the simple linear regression model for the relationship.

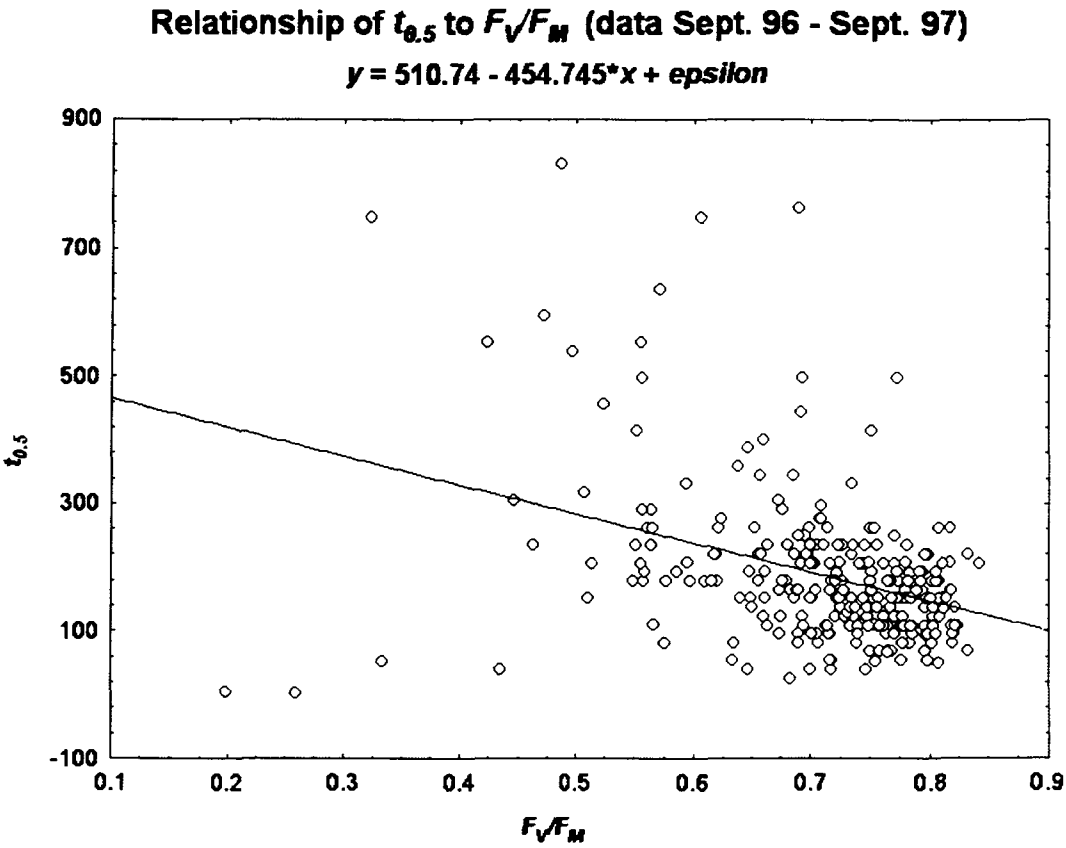


Figure 8.2: The inverse relationship between F_V/F_M and $t_{0.5}$

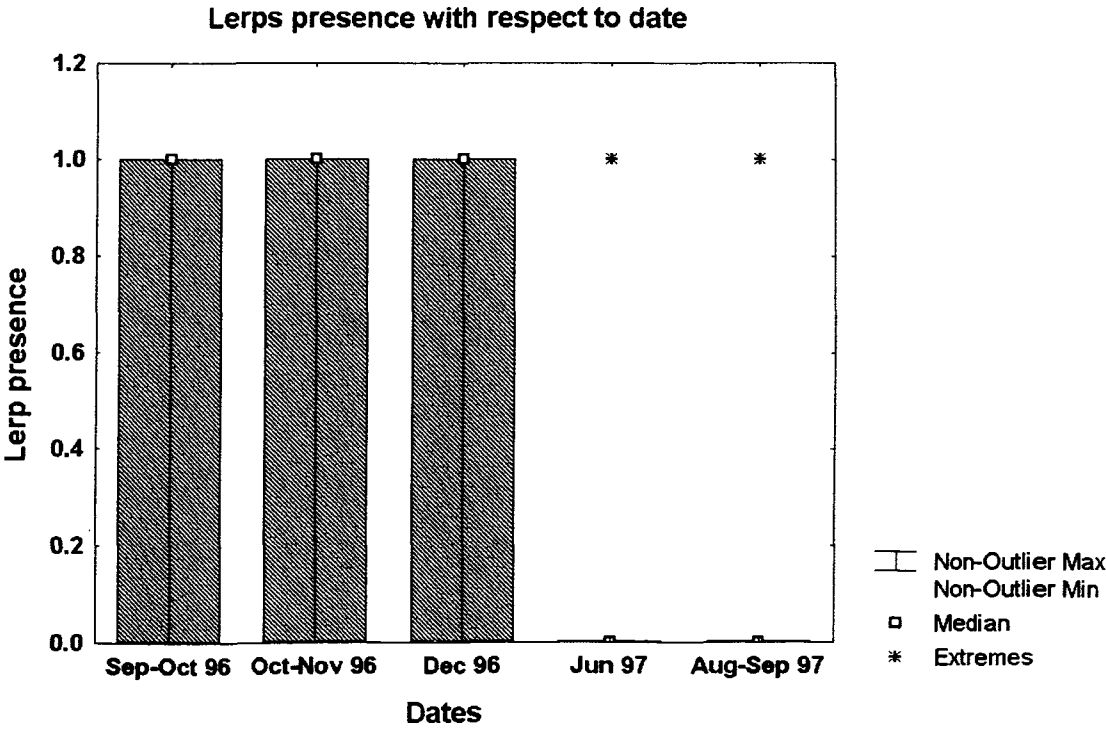


Figure 8.3: *M. fici* is only present in the egg stage during winter in Sydney

8.3.1.1.1.1 General overview of study data

The variation in F_V/F_M and $t_{0.5}$ values across the entire data set was very high, more so in the case of $t_{0.5}$; its standard deviation, however, was more than 63% of the value of the $t_{0.5}$ mean. Table 8.2 below presents some simple statistics arising from the ANOVA of F_V/F_M and $t_{0.5}$.

8.3.1.1.1.2 *Lerp* (+/-) vs *Date*

The *Lerp* (+/-) variable was used as the measure to represent the presence of actively feeding psylloids on the sample fig trees. A simple one-way ANOVA with *Lerp* (+/-) as the dependent variable and *Date* as the independent variable tested for the presence of active lerps throughout the year. The result was highly significant (Table 8.3 below), with the analysis identifying a partitioning of the data into two groups of study dates with respect to lerps: winter and spring-summer. The winter studies contained no active lerps (Figure 8.3 above).

8.3.1.1.1.3 *Lerp* (+/-) vs *Tree*

The results for the ANOVA of *Lerp* (+/-) with respect to *Tree* are presented in Table 8.4 below were not statistically significant at the 5% error level.

Table 8.2: Overview of the first pass over the data

Variable	Mean	Std dev.	<i>n</i>	Std. error
F_V/F_M	0.698	0.107	884	0.00057
$t_{0.5}$	191.950	121.904	884	4.10129

Table 8.3: ANOVA table for *Lerp* (+/-) with respect to *Date*

	df	MS	$F_{(4, 879)}$	prob.
<i>Effect</i>	4	7.893957	45.47171	0.000000
<i>Error</i>	879	0.195593		

Table 8.4: ANOVA table for *Lerp* (+/-) with respect to *Tree*

	df	MS	$F_{(4, 879)}$	prob.
<i>Effect</i>	4	0.527759	2.260711	0.060928
<i>Error</i>	879	0.233448		

Table 8.5: ANOVA table for F_V/F_M with respect to *Tree*

	df	MS	$F_{(4, 879)}$	prob.
<i>Effect</i>	4	0.218010	20.92845	0.000000
<i>Error</i>	879	0.010417		

Table 8.6: Mean F_V/F_M value for five *F. macrophylla* trees for five survey periods

	Mean F_V/F_M	Std dev.	<i>n</i>	Std error
<i>MFG</i>	0.659	0.11852	252	0.00747
<i>L44</i>	0.725	0.08708	170	0.00688
<i>AGN</i>	0.736	0.08879	237	0.00577
<i>BTC</i>	0.695	0.09373	97	0.00952
<i>HRB</i>	0.666	0.11432	193	0.00823
<i>All Trees</i>	0.695	0.10812	949	0.00351

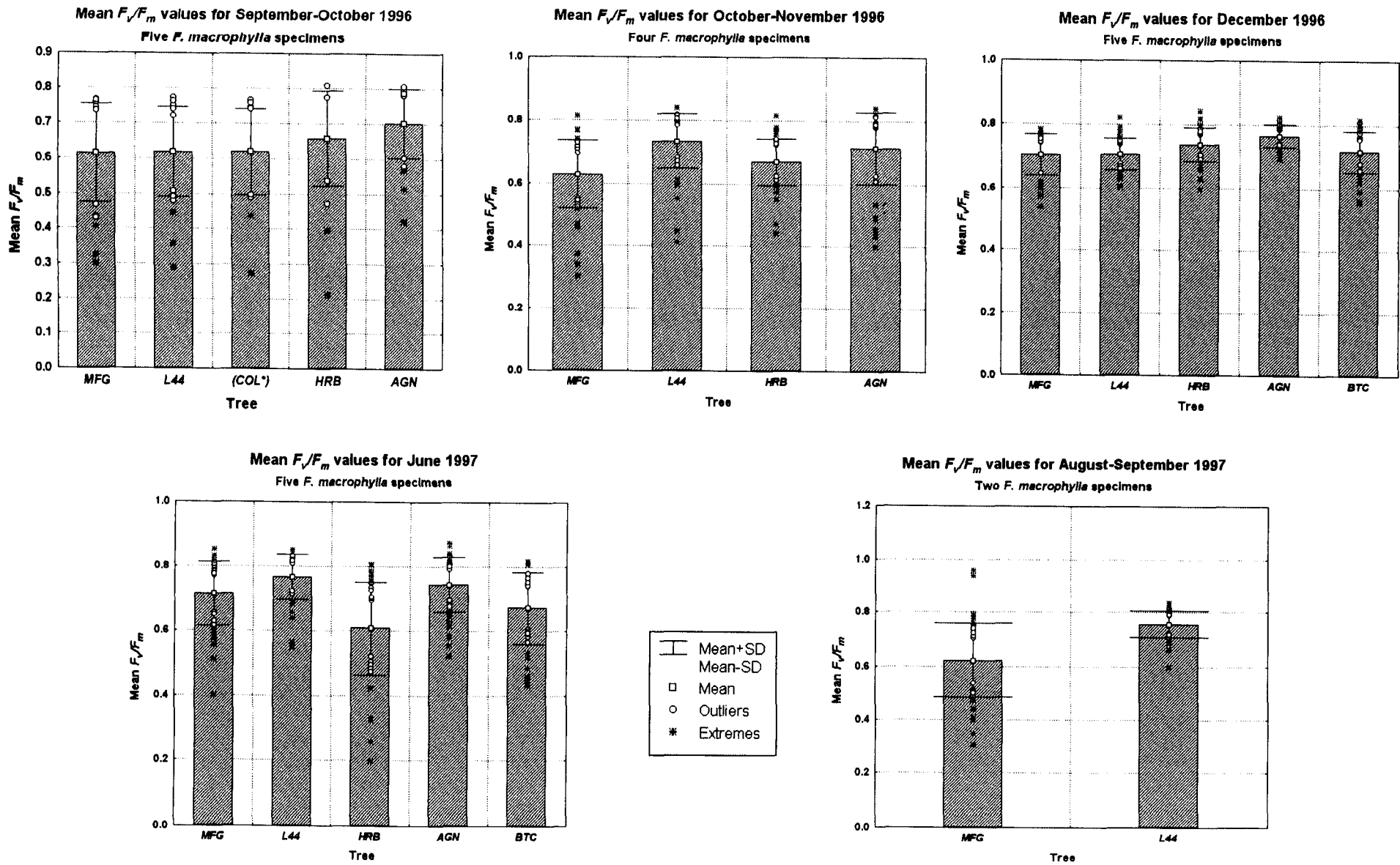


Figure 8.4: Mean F_v/F_m values for five *F. macrophylla* study trees, grouped by CF sampling period

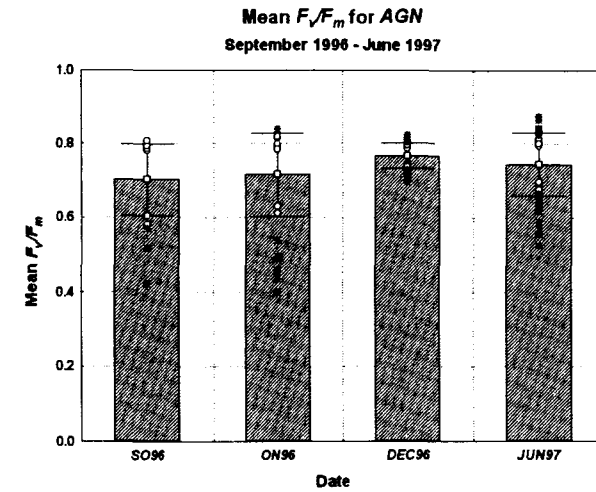
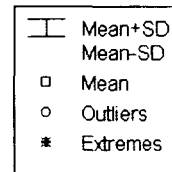
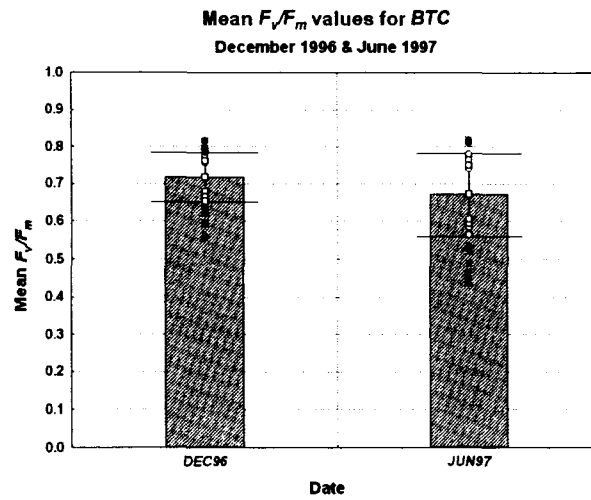
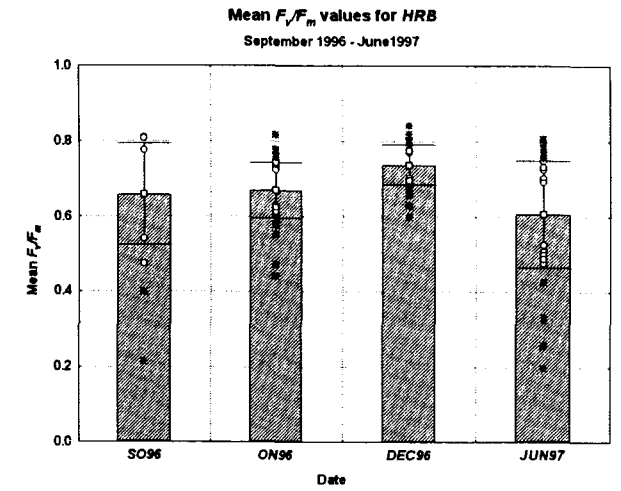
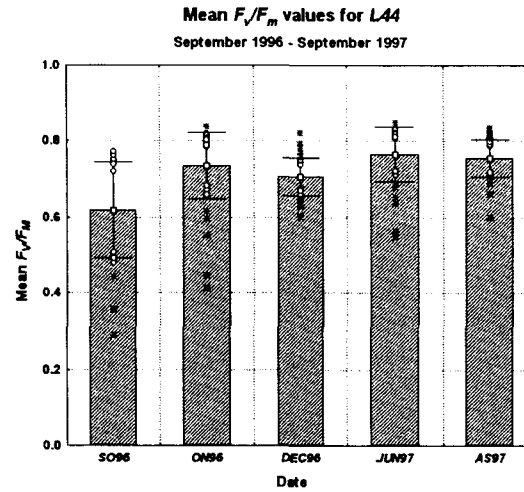
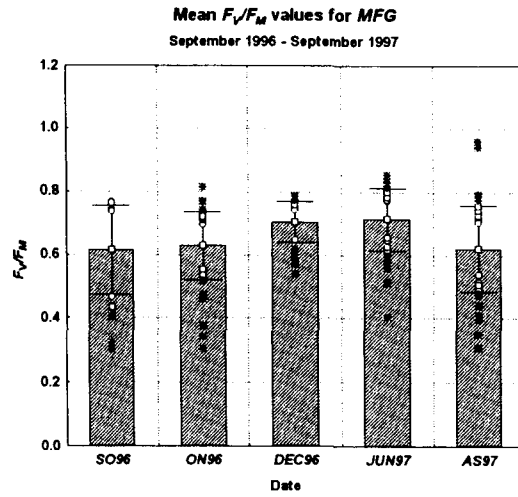


Figure 8.5: Mean F_v/F_m values for *F. macrophylla* sampling periods, grouped by tree

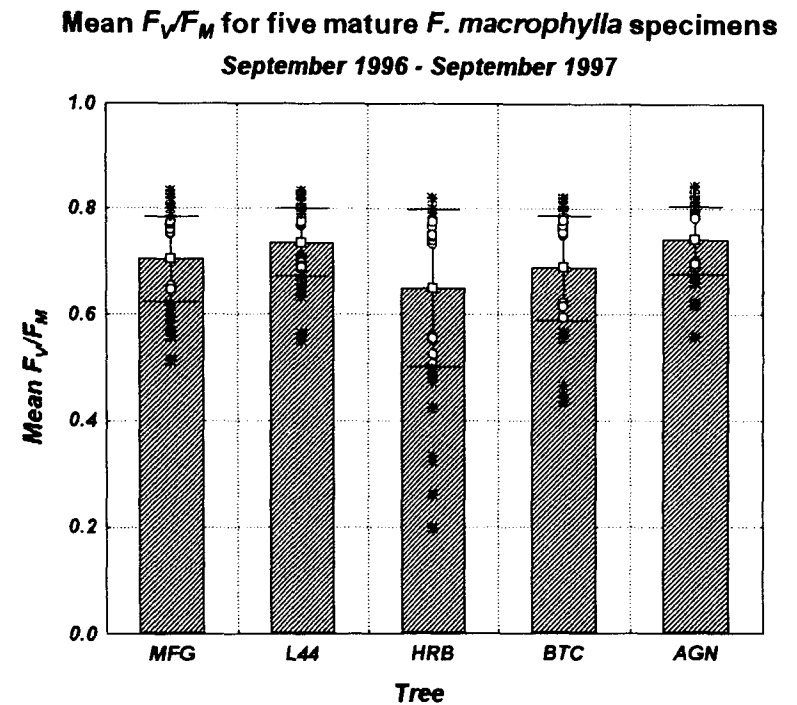
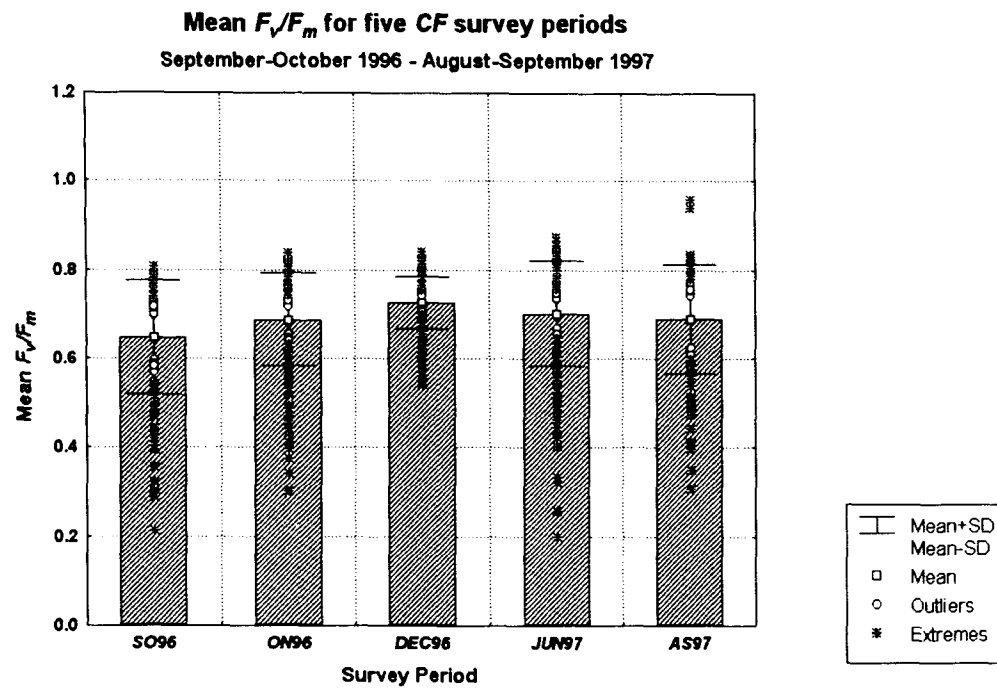


Figure 8.6: Comparison of mean F_v/F_m values for one year, with respect to CF sampling periods (left-hand graph) and trees (right-hand graph)

8.3.1.2 Exploratory ANOVAs - complete data set

8.3.1.2.1 F_V/F_M with respect to *Tree*

The data set was subsequently explored using simple bar graphs, and a one-way ANOVA with F_V/F_M and *Tree* as the dependent and independent variables respectively. The ANOVA table for this analysis is presented in Table 8.5 above, and the mean F_V/F_M values and other associated statistics for *MFG*, *L44*, *HRB*, *BTC* and *AGN* over the 12-month period are presented in Table 8.6 above. Figure 8.4 above presents five graphs, each graph containing the mean F_V/F_M values for the group of study trees groups for one sampling date, and Figure 8.6 (left-hand graph) shows a summary histogram for the mean F_V/F_M values for the five sample trees over the whole year. Note that *COL* in the first graph (September–October) in Figure 8.4 is adventitious, as *BTC* had not been added to the study at that point. *COL* is an *F. macrophylla* subsp. *columnaris* growing adjacent to *L44*, and was sampled for interest, but was replaced with *BTC* for the next survey as it (*COL*) was not an *F. macrophylla* subsp. *macrophylla*.

8.3.1.2.2 F_V/F_M with respect to *Date*

The same analysis was applied to F_V/F_M (dependent variable) with respect to *Date* (independent variable). The result was significant: the ANOVA table and the associated table of means appear in Tables 8.7 and 8.8, respectively. Figure 8.5 above presents five graphs as in Figure 8.4, each separate graph giving the mean F_V/F_M values for one individual tree for each of the sampling periods (*SO96*, *ON96*, *DEC96*, *JUN97* & *AS97*) conducted over the twelve months from September 1996 to September 1997. In some instances (*HRB*, *BTC* and *AGN*) trees were sampled less than five times during that period, because of a severe illness suffered by the author. The total mean F_V/F_M values for each sampling period are presented in the right-hand graph in Figure 6.

The same analysis was also performed on the $t_{0.5}$ data with respect to trees and dates, but further analysis was restricted to F_V/F_M because of extreme variation in the data, and the consequent lack of statistically interpretable information in the $t_{0.5}$ results. Section 8.4.1.1 in the Discussion presents the reasoning for this decision.

Table 8.7: ANOVA table for F_V/F_M by *Date*

	SS	df	MS	$F_{(4, 879)}$	prob.
<i>Effect</i>		4	0.12254	11.2924	0.00000
<i>Error</i>	9.53720	879	0.01085		

Table 8.8: Mean F_V/F_M values for five survey periods (*Date*)

<i>Date</i>	Mean F_V/F_M	Std dev.	<i>n</i>	Std error
<i>SO96</i>	0.649	0.12850	107	0.0124
<i>ON96</i>	0.688	0.10460	196	0.0075
<i>DEC96</i>	0.727	0.05889	242	0.0038
<i>JUN97</i>	0.702	0.11825	243	0.0076
<i>AS97</i>	0.689	0.12290	96	0.0125
<i>All Dates</i>	0.698	0.10657	884	0.0036

Table 8.9: Planned Comparison of F_V/F_M with respect to *Date* and *Tree*

	SS	df	MS	$F_{(1, 864)}$	prob.
<i>Effect</i>		1	116.6130	635.4830	0.00
<i>Error</i>	157.54350	864	0.1835		

Table 8.10: ANOVA results for F_V/F_M with respect to *Plague* status

	SS	df	MS	$F_{(1, 882)}$	prob.
<i>Effect</i>		1	0.25755	23.2479	0.000002
<i>Error</i>	9.77134	882	0.01108		

Table 8.11: Summary of means for *Non-plague/Plague* analysis

	Mean F_V/F_M	Std dev.	<i>n</i>	Std error
<i>Non-plague</i>	0.710	0.0998	581	0.00414
<i>Plague</i>	0.674	0.1149	303	0.00660
<i>All Groups</i>	0.698	0.1066	884	0.00358

8.3.1.3 First major alteration to data set

8.3.1.3.1 Removal of redundant variables

A number of variables from the three main groups (see Table 8.1) was deleted from the set for two reasons: redundancy (e.g., between *Leaf maturity* and *Leaf age* - *Leaf age* retained); and because the recording of details for some variables was incomplete for all trees on all dates (e.g., *Number of eggs*; *Number of lerps*). The truncated data set was analysed by a two-way ANOVA, with F_V/F_M as the dependent variable and *Tree* and *Date* as the independent variables. The analysis failed to complete because of missing values in both *Tree* and *Date*. It was possible, however, to perform a planned comparison on the data (Table 8.9): the *Date* variable was significant, *Tree* was not.

8.3.1.3.2 Balancing the design - removal of *BTC* and *AS97* data

As it stood, the data set was unable to be analysed in sufficient depth because of lack of balance in the data as collected: unbalancing data was removed to allow analysis incorporating both *Tree* and *Date* variables to proceed. The data set was truncated further: early inconsistencies in data recording, and gaps in data collection arising from a serious illness, necessitated the removal of the *BTC* data (two periods: *DEC96* and *JUN97*), as well as the removal of all of the data for September 1997 study date (*AS97* data were collected from *MFG* and *L44* only, because of ill-health).

8.3.1.4 Second major alteration to data set – *Non-plague/Plague* variable partitioning

After some reflection on the results presented in Section 8.3.1.2.2 (*Lerp* (+/-) vs *Date*), I decided to try partitioning the data set into subsets according to *Plague* vs *Non-plague*. While not mapping onto the psyllid plague period directly, the grouping of data in *Lerp* (+/-) vs *Date* histograms suggested a possible differentiation between the periods during psyllid plague and non-plague conditions, respectively. During the collection of data in September and October 1996, a psyllid plague and complete fig defoliation event occurred concurrently. In the light of this, the set was re-partitioned by sorting the data with respect to a new variable: *Psyllid plague*, which consisted of two groups: September to November 1996 (*Plague*: *SO96* + *ON96*) and December 1996/June 1997 (*Non-plague*: *DEC96* + *JUN97*).

Table 8.12: *Non-plague* and *Plague* ANCOVA models

<i>Non-plague model</i>							<i>Plague Model</i>						
	Effect df	Effect MS	Error df	Error MS	$F_{(n, 369)}$	prob.		Effect df	Effect MS	Error df	Error MS	$F_{(n, 285)}$	prob.
<i>Tree</i>	3	0.06045	369	0.00582	10.396	0.0000	<i>Tree</i>	3	0.03776	285	0.01034	3.653	0.0130
<i>Date</i>	1	0.07567	369	0.00582	13.013	0.0004	<i>Date</i>	1	0.09616	285	0.01034	9.303	0.0025
<i>Lerp</i>	1	0.00265	369	0.00582	0.456	0.5000	<i>Lerps</i>	1	0.00039	285	0.01034	0.038	0.8466
<i>Tree × Lerp</i>	3	0.00068	369	0.00582	0.117	0.9501	<i>Tree × Lerp</i>	3	0.03874	285	0.01034	3.747	0.0115
<i>Date × Lerp</i>	1	0.04691	369	0.00582	7.067	0.0048	<i>Date × Lerp</i>	1	0.00400	285	0.01034	0.387	0.5344
<i>Tree × Date × Lerp</i>	3	0.00501	369	0.00582	0.862	0.4611	<i>Tree × Date × Lerp</i>	3	0.05448	285	0.01034	5.271	0.0015
Regression component: two covariates							Regression component: two covariates						
	SS	df	MS	$F_{(2, 369)}$	prob.			SS	df	MS	$F_{(2, 285)}$	prob.	
<i>Effect</i>	0.2712	2	0.1356	23.325	0.00000		<i>Effect</i>	0.2123	2	0.1061	10.268	0.00005	
<i>Error</i>	2.1456	369	0.005				<i>Error</i>	2.9460	285	0.0103			
Regression coefficients							Regression coefficients						
Multiple $R = 0.3350112$; $R^2 = 0.1122325$							Multiple $R = 0.2592493$; $R^2 = 0.0672102$						
	β -weight	Std. Err.	b	$t_{(285)}$	prob.			β -weight	Std. Err.	b	$t_{(369)}$	prob.	
<i>Leaf age</i>	-0.008	0.0019	-0.2032	-4.13665	0.00004		<i>Egg mass</i>	-0.061	0.0266	-0.1310	-2.28259	0.02319	
<i>Sunlight</i>	-0.035	0.0067	-0.2564	-5.22062	0.00000		<i>Sunlight</i>	-0.030	0.0074	-0.2345	-4.08580	0.00006	

8.3.1.4.1 One-way ANOVA of *Plague* status

A one-way ANOVA with *Plague* status as the independent variable and F_V/F_M as the dependent variable produced a significant result. The ANOVA table is given in Table 8.10, and the mean F_V/F_M values are given in Table 8.11. The analysis determined that statistically *Non-Plague* and *Plague* subsets differed significantly from each other with respect to mean F_V/F_M values, and that the *Plague* mean was lower than the *Non-plague* mean. This line of exploration was continued on into separate analysis of the two subsets. The *Non-Plague* and *Plague* subsets were therefore analysed separately, using multi-way analysis of covariance (MANCOVA, hereafter abbreviated to ANCOVA) tests with F_V/F_M as the dependent variable. The main aims of this approach were to see if the components of each hypothetical model differed, and thus to possibly build predictive models using multiple regression techniques. *Date* (four levels), *Tree* (four levels) and *Lerp* (two levels) were used as independent variables. Other variables were included in the models as covariates. Table 8.12 above presents the variables and covariates retained in the *Non-plague* and *Plague* models by the analyses of the respective data sets.

To summarise Section 8.3.1.4, the variation in the *Non-plague* and *Plague* ANCOVA models can be satisfactorily explained from a statistical view point by:

***Non-plague* (Model 1A):**

Date and *Tree* single factor terms;

Tree × *Date* interaction term,;

Leaf age and *Sunlight* covariates.

***Plague* (Model 1B):**

Date and *Tree* single factor terms;

Date × *Tree*, *Tree* × *Lerp* and *Date* × *Tree* × *Lerp* interaction terms;

Egg mass and *Sunlight* covariates.

8.3.1.5 Regression models of F_V/F_M

The same set of variables that was used as covariates in the ANCOVA *Plague* and *Non-plague* models was then analysed by multiple linear regression (Table 8.13 below) in order to fit an equation that might be used to predict F_V/F_M for *F. macrophylla*, given various parameters.

8.3.1.5.1 *Non-plague* regression model

The only independent variable that was retained in the *Non-plague* regression model that was also present in the ANCOVA model was *Date: Tree* fell out of the regression model. The only variable that was present in the ANCOVA model, as a covariate, that was retained in the *Non-plague* regression model as a significant term was *Sunlight*, *Leaf age* falling out of the model. There were, however, a number of additional variables retained in the regression model that were not retained (as covariates) in the ANCOVA *Non-plague* model. These were: *Egg mass*, *Necrosis*, *Predator*, *Orientation*. All of the retained variables were given high significance by the analysis. The R , R^2 and adjusted R^2 test of overall validity of the model were rather low (Table 8.13), but were actually higher than the equivalent tests for the regression component of the ANCOVA model.

8.3.1.5.2 *Plague* regression model

The *Plague* regression model was different again from its ANCOVA counterpart. Not only did both covariates present in the *Plague* ANCOVA model (*Egg mass* and *Sunlight*) fall out of the regression model, but no further variables were added from the stock of those used as covariates in the ANCOVA analysis. *Lerps*, however, an independent variable that was rejected from the ANCOVA *Plague* model, was retained as highly significant in the multiple regression model. The R , R^2 and adjusted R^2 values for the *Plague* regression model were somewhat higher than those for the *Non-plague* regression model, indicating that the *Plague* regression model was a little more robust than its *Non-plague* counterpart (Table 8.13, right-hand side).

Table 8.13: *Non-plague* and *Plague* multiple linear regression models

<i>Non-plague Model</i>							<i>Plague model</i>						
<i>R</i> = 0.38155735; <i>R</i> ² = 0.14558601; Adjusted <i>R</i> ² = 0.1358394							<i>R</i> = 0.38963789; <i>R</i> ² = 0.15181768; Adjusted <i>R</i> ² = 0.13991337						
<i>F</i> _(6, 526) = 14.938; <i>p</i> < 0.00000; Std. Error of estimate: 0.09594							<i>F</i> _(4, 285) = 12.753; <i>p</i> < 0.00000; Std. Error of estimate = 0.10771						
	beta	SE of beta	<i>B</i>	SE of <i>B</i>	<i>t</i> ₍₅₂₆₎	prob.		beta	SE of beta	<i>B</i>	SE of <i>B</i>	<i>t</i> ₍₂₉₈₎	prob.
<i>Intercept</i>			2.9395	0.64944	4.5262	0.00001	<i>Intercept</i>			-4.8688	1.45526	-3.3456	0.00093
<i>Date</i>	-0.1487	0.04468	-0.0215	0.00645	-3.3274	0.00094	<i>Tree</i>	0.1825	0.05501	0.0192	0.00579	3.3179	0.00103
<i>Egg mass</i>	-0.1302	0.04459	-0.0277	0.00949	-2.9209	0.00364	<i>Date</i>	0.1458	0.05539	0.0361	0.01372	2.6318	0.00896
<i>Necrosis</i>	-0.1486	0.04163	-0.0405	0.01136	-3.5697	0.00039	<i>Lerps</i>	-0.1903	0.05517	-0.0442	0.01282	-3.4486	0.00065
<i>Predator</i>	-0.1501	0.04058	-0.0482	0.01304	-3.6976	0.00024							
<i>Orientation</i>	-0.1160	0.04115	-0.0051	0.00182	-2.8193	0.00499							
<i>Sunlight</i>	-0.2247	0.04102	-0.0258	0.00471	-5.4773	0.00000							
Analysis of Variance; dependent variable: <i>F</i> _V / <i>F</i> _M							Analysis of Variance; dependent variable: <i>F</i> _V / <i>F</i> _M						
	SS	df	MS	<i>F</i> _(5, 526)	prob.			SS	df	MS	<i>F</i> _(4, 285)	prob.	
<i>Regression</i>	0.82503	6	0.13751	14.9378	0.00000	<i>Regression</i>	0.59185	4	0.14796	12.7532	0.00000		
<i>Residual</i>	4.84194	526	0.00921			<i>Residual</i>	3.30656	285	0.01160				
<i>Total</i>	5.66698					<i>Total</i>	3.89841						

8.3.2 Chlorophyll fluorescence study of five trees from the Adelaide population of *F. macrophylla*

Table 8.14 below presents the results of a one way ANOVA with F_V/F_M as the variable of interest, and *Tree* the independent variable. The results were significant at the 5% error level, and the table of means for the analysis is given in Table 8.15 below. Table 8.16 below is a comparison of the mean F_V/F_M ratio values for the Adelaide sample trees in late December 2000-early January 2001, and the Sydney sample trees in December 1996, and overall means for all five Sydney chlorophyll fluorescence studies.

8.3.3 Comparisons of *F. macrophylla* specimens at different locations, with respect to F_V/F_M , psyllid presence and environmental conditions

Observations of individual study trees during psyllid outbreaks, and at other times, revealed that some trees defoliated more, and at different rates, than others. Three tables were constructed using a range of data acquired from the chlorophyll fluorescence and life history studies (see also Chapter 7, Section 7.3.3.4 and subsections). The equivalent tables in this chapter are Tables 8.17, 8.18 and 8.19. Table 8.17 is essentially Table 7.16 with the addition of the F_V/F_M ratios for the five study trees *MFG*, *L44*, *HRB*, *BTC* and *AGN* from the chlorophyll fluorescence analyses, the addition of some environmental indices, and the removal of the standard deviations for all variables, for clarity.

In order to provide an overview of the relationships of the environmental factors and defoliation for each tree, a simple ‘environmental conditions’ index was constructed in Table 8.17 by two stages, first by assigning arbitrary ranks to the following environmental factors originally appearing in Table 7.16, then adding the ranks together to create the index.

The ‘environmental conditions’ were:

defoliation status: *RD* (0, ..., 4; where 0 = **high** defoliation);

whether the tree had been roped off or not: *RR* (0, 1; where 0 = **not** roped off);

whether the tree received supplementary irrigation or not: *RI* (1, 2; where 1 = not irrigated).

The ‘environmental conditions’ index (or ‘simple environmental conditions index’), *RT*, was the sum total of ranks: $RD + RR + RI$. Strictly speaking, the index represents a comparative and combined index of *tree condition* and *growing situations*. The higher the value of *RT*, the better

off the tree supposedly is. RD is given for each tree in the rightmost column of Table 8.17. $L44$ is given a '+' to indicate the fact that the tree is growing in what was at the time of the project very wet soil conditions as a result of the effects of tidal action on an already high water table. The RT index for a particular tree could then be matched against its F_V/F_M value.

The RT values for trees follow almost exactly the same pattern as the F_V/F_M values for trees, with the notable exception of MFG , which is opposite to the trend in the sense that it has a *high* RT value but a *low* mean F_V/F_M value. Separate graphs of RT and F_V/F_M with respect to the trees, which show the above-mentioned trend very clearly, are presented in Figures 8.7 and 8.8 below. The behaviour of RT with respect to F_V/F_M for the five trees, including the MFG 'anomaly', is discussed in Section 8.4.3.

Tables 8.18 and 8.19 present more detailed comparisons of the growing conditions of the five main Sydney study trees, and the Adelaide chlorophyll fluorescence study trees. Both tables contain the overall F_V/F_M means for each tree. In the case of the Adelaide trees the results arise from only the one sample. The Sydney table (Table 8.18) does not contain any of the psylloid related statistics of Table 7.16 (Chapter 7) and Table 8.17. The Adelaide table does not contain psylloid information because, as already reported in this thesis, the psylloid does not occur there.

Table 8.14: ANOVA results for chlorophyll fluorescence analysis of five *F. macrophylla* specimens growing in Adelaide Botanic Gardens and Botanic Park.

	Effect SS	df	Effect MS	Error df	Error MS	$F_{(4, 251)}$	prob.
Tree	1.87780	4	0.296945	251	0.014381	20.64799	0.00000

Table 8.15: Mean F_V/F_M values for five specimens of *F. macrophylla* growing in Adelaide Botanic Gardens and Botanic Park

Tree	Mean F_V/F_M	Std dev.	<i>n</i>	Std error
BP1	0.641	0.2040	54	0.02776
BP2	0.618	0.1211	52	0.01679
BP3	0.723	0.0611	50	0.00864
BP4	0.720	0.0969	50	0.01370
ABG1	0.810	0.0161	50	0.00228
All trees	0.702	0.1372	256	0.00858

Table 8.16: Comparison of mean F_V/F_M values for ten *F. macrophylla* specimens, trees growing in Adelaide and Sydney

Tree	Mean F_V/F_M		
	Adelaide, Dec. 2000	Sydney, Dec. 1996	Sydney, all surveys
1	0.641	0.704	0.661
2	0.618	0.707	0.744
3	0.723	0.767	0.666
4	0.720	0.718	0.695
5	0.810	0.738	0.736
All trees	0.702	0.727	0.699

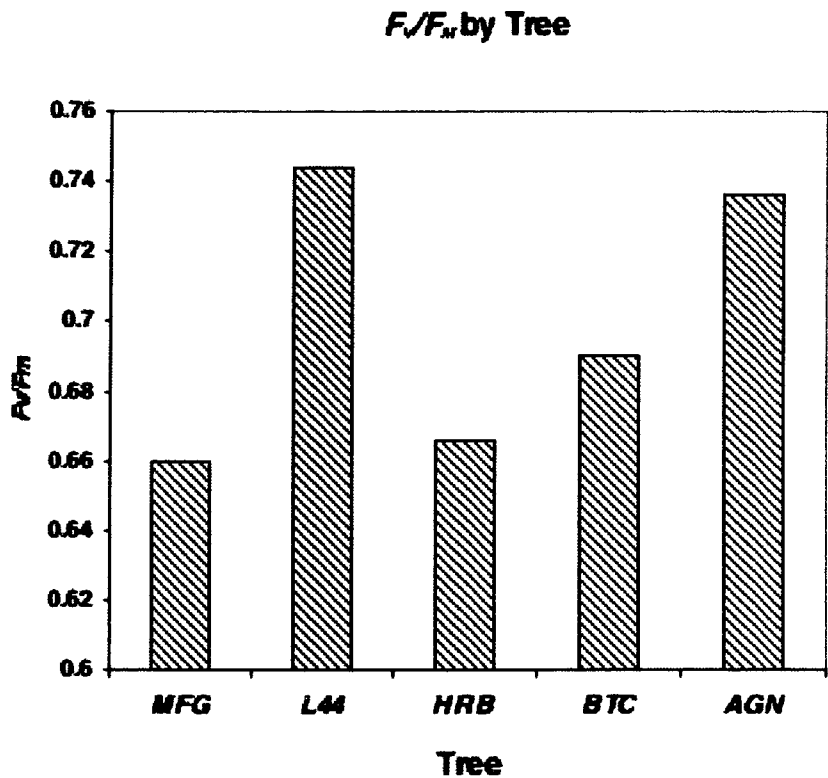


Figure 8.7: Mean F_v/F_m values for the five Sydney *CF* study trees over a one year period

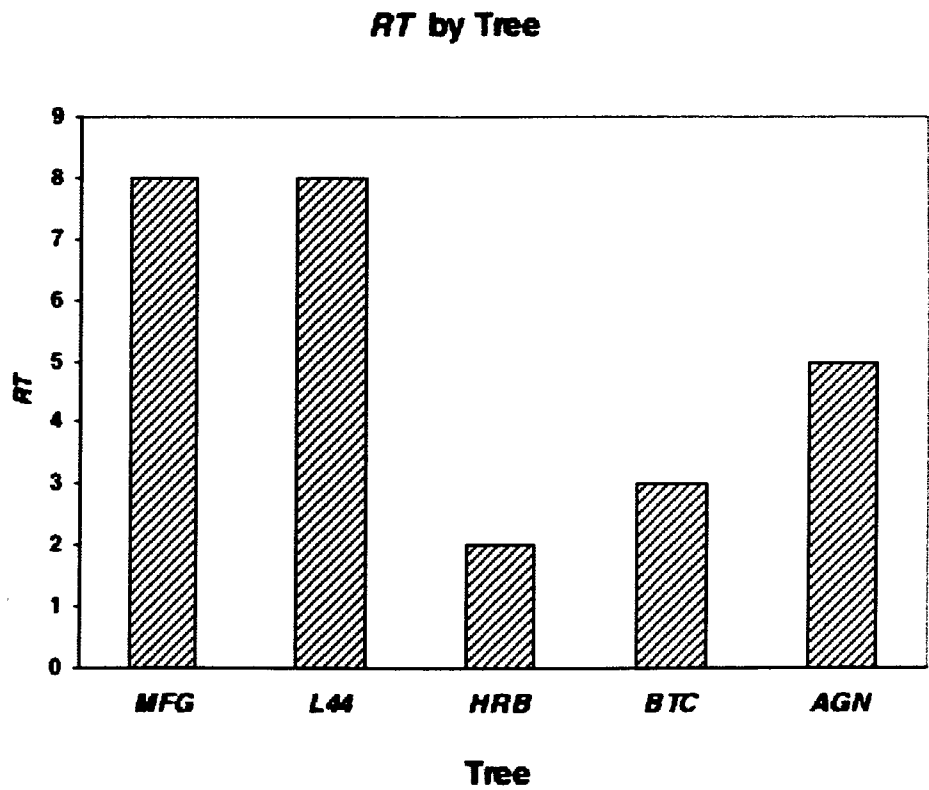


Figure 8.8: RT 'tree + environment condition' indices for the five Sydney *CF* study trees

Table 8.17: Comparison of five Sydney study trees with respect to mean proportion of *M. fici* eggs and lerps on leaves 1–5, defoliation and hydraulic environment

Tree	Mean F_V/F_M	Mean EMs^1 /sample ²	Mean % eggs	Mean lerps/sample ²	Mean % lerps	Defoliation	RD^3	Other conditions ^{4, 5}	RR^4	RI^5	RT^6
<i>MFG</i>	0.660	33.2	0.882	17.5	0.51	Low	4	Roped off, irrigated	2	2	8
<i>L44</i>	0.744	11.0	0.47	4.7	0.30	Low-moderate	3	Roped off, irrigated, in wet area	2	2	7+
<i>HRB</i>	0.666	16.8	0.59	10.7	0.50	High	0	Roped off, not irrigated	1	1	2
<i>BTC</i>	0.690	18.4	0.95	5.7	0.65	Moderate-high	1	Roped off, not irrigated	1	1	3
<i>AGN</i>	0.736	23.2	0.80	16.2	0.63	Moderate	2	Roped off, <i>irrigated</i>	1	2	5

¹ *EM* = egg mass ² sample = combined total of eggs or lerps for leaf-positions 1–5
³ *RD* = defoliation rank (0–4) ⁴ *RR* = roped rank (1 or 2) ⁵ *RI* = irrigation rank ⁶ *RT* = rank totals

Table 8.18: Comparison of five Sydney study trees with respect to defoliation and various environmental conditions

Tree	F_v/F_M		Defoliation	Soil moisture	Soil compaction	Average environmental conditions	Location
	Mean	Std dev.					
<i>MFG</i>	0.661	0.1175	Low	High	None	Relative shelter from high winds; shelter from sea spray; fenced off from pedestrian and vehicular traffic; irrigated; aerial root overcoats; regularly maintenance;	Near corner of Macquarie St & Cahill Expressway
<i>L44</i>	0.744	0.0880	Moderate	High	Low–moderate	Tree infected with <i>Ganoderma</i> sp.; Exposed to sea spray; not fenced off at time of CF studies	Near sea wall, 400 m south of Opera House
<i>HRB</i>	0.666	0.1142	High	Low	Low, moderate & high spots (patchy)	Lower RH and higher temperatures on western side of tree; area of radius 2 m around trunk protected by banks	On Mrs Macquaries Rd opposite National Herbarium
<i>BTC</i>	0.690	0.0936	High	Low	Moderate–high	Lower RH and higher temperatures on western side of tree; some exposure to foot traffic	Near Andrew Charlton Pool (400 m south of Mrs Macquaries Chair)
<i>AGN</i>	0.736	0.0886	Low	Moderate–high	Low–moderate	Shelter (50%) from prevailing (SE-SW) winds, no sea spray; No shelter from prevailing N-NE winds; salt spray	Near NW of Art Gallery coffee shop; between AG and Cahill Expressway
<i>All trees</i>	0.699	0.03875					

Table 8.19: Adelaide Botanic Gardens: *F. macrophylla* comparisons

Tree	<i>F_V/F_M</i>		Soil compaction	Soil moisture	Defoliation	Average environmental conditions	Location
	Mean	Std dev.					
<i>BP1</i>	0.641	0.2040	High	Low	High	Tough: dry, no irrigation, top of river bank; soil cracking	Southern bank of River Torrens, near Dequetteville Terrace Bridge
<i>BP2</i>	0.618	0.1211	Moderate-high	Moderate	High-moderate	On edge of turfed area, would receive irrigation on one 10 m south of <i>BP2</i> , irrigated area side of root zone	
<i>BP3</i>	0.723	0.0611	Moderate	High-Moderate	Moderate-Low	Receiving irrigation on one (park) side of root zone; some fertiliser applied to surrounding grassed area	On roadside by ABG northern entrance gates
<i>BP4</i>	0.720	0.0969	Moderate	Moderate-Low	Moderate-Low	Receiving irrigation on one (park) side of root zone; some fertiliser applied to surrounding grassed area	On road-side near Zoo
<i>ABG1</i>	0.810	0.0161	Low	High	Low	Optimal: canopy and root-zone irrigation; fertilised	Near ABG Restaurant
All trees	0.702	0.0762					

8.4 Discussion

8.4.1 Sydney main study

8.4.1.1 Preliminary analysis

8.4.1.1.1 Overview of F_V/F_M and $t_{0.5}$ results (all trees and all dates)

8.4.1.1.1.1 Relationship of F_V/F_M and $t_{0.5}$

The $t_{0.5}$ data (191.95 ms and 121.904 ms respectively) showed a negative relationship with respect to F_V/F_M (Figure 8.2) the regression line having a negative slope of almost 500 to 1, but this was overshadowed by the wide variation of approximately 64% (63.7%) of the actual mean $t_{0.5}$ value, including some large outliers (Table 8.2 and Figure 8.2, respectively). This departure from heterogeneity and normality in the distribution of the residuals suggests that there was something wrong with the way that the measuring instrument (*PSM*) determined the half-raise time, or that there was something unusual occurring in the photosystems of the leaf. After the sampling study was completed the author had the opportunity to check the calibration of the *PSM* against a Walz ‘*Teaching-PAM*’ instrument of known, very high reliability, from Sydney University Department of Biological Sciences. The “*Teaching-PAM*” was unfortunately not available to the candidate until after the *FCFK* data surveys had been completed. The *PSM*’s mean F_V/F_M results were within less than one per cent of mean of the *Teaching-PAM*’s results, for leaves from the same tree. The author is confident, therefore, that the F_V/F_M results reported here are valid.

8.4.1.1.1.2 Summary F_V/F_M statistics

The mean F_V/F_M value across the whole data set as determined by simple summary statistics was 0.695 units, with a standard deviation of 0.108 units, indicated a rather low state of tree health for all five study trees, relative to the optimum level of *ca* 0.800 units, but with a high degree of variation between the means for each (Table 8.6).

8.4.1.1.1.3 *Lerp* (+/-) variable with respect to *Date*

The results of the ANOVA of *Lerp* (+/-) with respect to *Date*, showing the absence of active, populated lerps in the winter period (Table 8.3 and Figure 8.3), is consistent with an expected slowing down in psyllid development caused by the ambient temperature falling below that of the psyllid’s lower development threshold. The only part of the psyllid’s life cycle to be present during the winter months is that of the egg stage.

8.4.1.1.1.4 *Lerp* (+/-) variable with respect to *Tree*

Table 8.4, from the *Lerp* (+/-) vs *Tree* analysis, suggests that psylloids appear to have no preference for one particular host tree over another; this in turn implies that site (sheltered vs exposed; more saline vs less saline, etc.) is not a significant factor for psylloids selecting (or staying on) a host. An alternative explanation may be the existence of differing psylloid meta-populations between trees, differing populations having an actual preference or even requirement for a particular tree. The implications of this latter explanation are a tight coevolutionary tendency on the part of the psylloids with respect to fixing on the physiology and biochemistry of a particular plant. It was beyond the scope of this project to test this possibility.

8.4.1.2 Exploratory ANOVAs - complete data set

8.4.1.2.1 ANOVA: tests of underlying assumptions

Tests for normality of distribution amongst the data showed a skewed tendency towards values below the mean, which is consistent with the mostly low F_V/F_M values, but a couple of relatively healthy trees were present. The departure from normality was not considered sufficiently serious to be concerned about. Homogeneity of variance as evaluated by residual vs fitted values was not as randomly distributed as is preferred for a parametric analysis, but the robustness of the least-squares methods used in ANOVA and linear regression is sufficiently high (Steele and Torrie, 1981) to consider that the departures of the assumptions underlying these analyses could be ignored with reasonable safety in the case of this data set.

8.4.1.2.2 ANOVA – F_V/F_M with respect to *Tree*

F_V/F_M was analysed by *Tree* alone, *Date* being omitted because of insufficient data points to create a balanced design (see Section 8.3.1.3 and subsections). The results of the one-way ANOVA of the complete data by F_V/F_M with respect to *Tree* (Tables 8.5 and 8.6, and Figures 8.4 and 8.6) show varied and depressed mean F_V/F_M levels when compared with the generally accepted benchmark F_V/F_M value of 0.800 units. This indicates that the *FCFK* technique as performed by the *PSM* functioned properly, and suggested that further analysis of the data for

potential causal relationships for the depressed levels of mean F_V/F_M values would be worthwhile.

The extent of F_V/F_M depression between trees was able to be correlated directly with visual differences in the canopies of each of the respective sample trees. The trees with the highest F_V/F_M values (*L44* and *AGN*) were those which defoliated least (as opposed to having a normally sparse canopy - as in the case of *L44*) throughout the study period. Of these two trees, *AGN*, which had the higher F_V/F_M value, had more leaves per unit volume than had *L44*. *AGN*'s leaves were also of a darker green than those of *L44*. Interestingly, *L44* is situated less than 50 m from the western side of the Sea Wall at Farm Cove on Sydney Harbour, suggesting that proximity to the waterfront and a correspondingly highly saline environment (both air and soil) relative to the sites where the other four study trees were growing, had little effect on the health of the tree. This is brought into sharper relief by the facts that the tree is approximately 30 m in canopy diameter and 15–20 m in height, and has been growing there for an estimated 150 years. The bottom right-hand graph in Figure 8.4, mean F_V/F_M values for *MFG* and *L44* at the August-September study period, show that *L44*'s mean F_V/F_M values are higher than those of *MFG*, the most cosseted Moreton Bay fig in the Royal Botanic Gardens/Domain precinct. The variation in F_V/F_M values is lower for *L44* than for *MFG*. This indicates that *L44* was in better condition than *MFG* - and also that this state of good health was maintained throughout the twelve months of the study, a period which also included a psyllid *plague* and geographically-widespread defoliation event. The health of *L44* may be due to the very high moisture content of the soil in which it was growing.

All trees showed the same general pattern of rising and falling mean F_V/F_M value over the five sample periods (Figures 8.4 and 8.5), indicating repeatability (i.e., good precision) of measurement. These results engender confidence in the reliability of the *PSM* in accurate diagnosis of *F. macrophylla* health.

8.4.1.2.3 F_V/F_M with respect to *Date*

This analysis also produced a statistically significant result, and potentially detected seasonal influences in between-tree mean F_V/F_M values (Tables 8.5 and 8.6). The graphs in Figure 8.5 show the differences in mean F_V/F_M value between trees, especially from the October-November period onwards. Figure 8.6 (right hand side) shows the different overall mean F_V/F_M

values for each of the five study periods. The general increase in F_v/F_M to a peak in summer, falling off again into winter, is consistent with effects of increasing temperature, solar declination and daylength on plant metabolism, photosynthesis and hence chlorophyll fluorescence in general, the actual health status of particular trees notwithstanding. Indeed, this cyclic pattern should be expected as growing conditions approach and recede from the optimum. The leaves on all study trees from October onwards had a much smaller psyllid population (egg, lerp or adult) resident on them as a result of the massive defoliation event that took place in mid to late September 1996, thus allowing the trees some time to recover from any effects induced by the psyllid plague. The statistical detection of a component in the overall depression of F_v/F_M means for these trees that could be attributed to the psyllid is one of the objects of the analyses discussed below.

The smallest amount of variation around mean F_v/F_M value in the right-hand graph in Figure 8.6 occurs in December 1996. The sampling period was during the early summer, and generally before the weather had become excessively warm for the region near the Harbour foreshore (i.e., hotter than 30 °C for extended periods). The variation about the mean F_v/F_M value appears to be inversely proportional to the value of mean F_v/F_M value (and which is also, implicitly, inversely proportional to increasing seasonal temperature and gross solar irradiance) can be seen across the five columns of the right-hand graph in Figure 8.6. This suggests that future monitoring of *F. macrophylla* using fast chlorophyll fluorescence analysis would benefit by incorporating a more precise indication of temperature for use in fitting revised models – as would F_v/F_M data collection on *F. macrophylla* specimens without psyllid populations, a task currently possible only in Adelaide (see also Chapter 7).

8.4.1.3 Alterations to data set

8.4.1.3.1 First alteration: removal of data

The reduction in numbers of variables by removing duplicate information made the landscape cleaner for further exploration of the data. The overall view was perforce made narrower by restrictions imposed by missing data. The latter resulted in the pruning of all *AS97* and *BTC* data, effectively shortening the length of time over which the F_v/F_M data could be studied if the maximum number of study trees was to be retained in the following analyses. The alternative of maximising with respect to *Date* meant eliminating data from *HRB* and *AGN*, as well as *BTC*, and since *AGN* and *HRB* represented the extremes of the range with respect to F_v/F_M

values, and therefore much of the interest in the data, I decided to sacrifice the data for *MFG* and *L44* within *AGS*, and those arising from *BTC* measurements.

8.4.1.4 Second major alteration to data set: *Plague/Non-plague* variable partitioning

The decision to split the data into the Plague status subsets *Plague* and *Non-plague* was made in the context of the reduction of information, and a perceived need to maximise the utility of the remaining data (654 F_V/F_M data points over the two subsets). Accordingly, it should be emphasised that the splitting of the data into the two sets was not seen at any time during the subsequent analyses as acting as a replacement for lost information.

8.4.1.4.1 One-way ANOVA of F_V/F_M with respect to *Plague status*

The analysis successfully correlated major differences in mean F_V/F_M value with the presence and absence of psylloids in plague population sizes (Table 8.10). The results indicated that the mean F_V/F_M value for the *Nonplague* period (0.710 mean units) was significantly higher than the value for the *Plague* period (0.674 mean units: see Table 8.11), with a difference between the two of just under 5.1%. The variation about the mean is also less for the *Non-plague* subset (at 0.0998) than for the *Plague* subset (at 0.115), a difference of about 13.1%. This difference in variation reflects the difference in mean F_V/F_M value discussed above. These results give sufficient justification for further statistical exploration of the *Non-plague/Plague* partitioning of the data.

The picture thus far presents reasonably sound evidence, *via Lerp (+/-)*, *Tree*, *Date* and *Plague status* variables, that the psylloid may be contributing to the depression of F_V/F_M values encountered in the data.

8.4.1.5 Three-way ANCOVA models of *Plague* and *Non-plague* data sets

8.4.1.5.1 *Non-plague* data

The model of best fit for the *Non-plague* F_V/F_M data retained *Tree* and *Date* as the independent variables, and *Leaf age* and *Sunlight* as covariates (Table 8.12, left-hand side). The significant independent interaction term, again consistent with previous analyses, was *Date* \times *Lerp* (+/-). *Lerp* (+/-) was not significant by itself. The R^2 value for the covariate component of the *Non-*

plague model was quite low, although the R^2 value for the *Plague* ANCOVA model was almost half that of the *Non-plague* model (see Section 8.4.1.5.2 immediately below).

8.4.1.5.2 *Plague* data

The best fit of the *Plague* F_V/F_M data was achieved with *Date* and *Tree* as the independent variables, and two covariates *Egg mass* and *Sunlight* (Table 8.12, right-hand side). The significant independent interaction terms were: *Tree* \times *Lerp* (+/-); and *Date* \times *Tree* \times *Lerp*(+/-). This is consistent with the previous analyses of variance discussed above. Interestingly, the *Lerp* (+/-) variable on its own was again not significant (probability of 8.47% at the 5% error level), although it does occur as part of the two- and three-way interaction terms: again consistent with previously reported results.

8.4.1.5.3 Comparison of *Non-plague* and *Plague* ANCOVA models

The *Non-plague/Plague* ANCOVA models suggest that the presence of the psylloid may have been contributing to the depression of mean F_V/F_M values. The common terms between the two models were *Date*, *Tree*, *Date* \times *Tree* and *Sunlight*. The *Plague* model additionally included *Lerp* (+/-) as an interaction component, with *Egg mass* and *Sunlight* as covariate components - which is consistent with the hypothesis of psylloid effect on F_V/F_M . The *Non-plague* model, additionally to the common factors named above, added *Leaf age*. The latter model also places more emphasis on the effects of *Date* and *Tree*, with the difference in *Tree* probabilities being five orders of magnitude in favour of the *Non-plague* factor, and a sevenfold difference in *Date* probabilities in favour of the *Non-plague* factor.

The *Plague* model contains *Egg mass* as a covariate, while the *Non-plague* model does not; and the *Non-plague* model contains *Leaf age*, while the *Plague* model does not. This reinforces the hypothesis of a psylloid-based model (psylloid activity adversely affecting *F. macrophylla* health) operating during psylloid-plague conditions, and a tree-based model (leaf age attributing to variation in F_V/F_M values) operating during non-plague conditions.

The inclusion of the *Sunlight* variable as the major covariate in both models is also consistent with general theory of photosynthesis, and current chlorophyll fluorescence theory in particular.

8.4.1.6 Multiple regression analysis of *Non-plague* and *Plague* data sets

8.4.1.6.1 Multiple Linear Regression models

Two possible ANCOVA models for *F. macrophylla* F_V/F_M with respect to the psylloid having been formulated, attention was turned to investigating the parameters in more detail using multiple linear regression. The R^2 values for both models, 0.14558601 for *Non-plague* and 0.15181768 for *Plague* (Table 8.13, left- and right-hand sides respectively), were not only higher than the corresponding values for the *Non-plague* and *Plague* ANCOVA models, but were comparable with each other, there being only about 4% difference between the two. It may be considered, therefore, that the multiple linear regression models of F_V/F_M with respect to the psylloid are more robust (and are consequently more valid) than their ANCOVA counterparts.

Egg mass, present in the *Plague* ANCOVA model, is absent from the *Plague* regression model; *Tree*, present in the *Non-plague* model, is likewise absent from the *Plague* regression model.

The presence of the *Lerp*s term in the *Plague* model (probability of 0.00065 of occurring by chance) is certainly what one would expect to see if the psylloid was to be having an effect on its host. The fact that the *Lerp* coefficient is negative (approximately -0.2) also fits the idea that the psylloid is having an adverse effect on the plant. The *Date* and *Tree* terms are seen to have a positive effect on F_V/F_M – of a similar quantity to the *Lerp* term, but opposite in sign. None of the additional ‘environmental’ variables was included in the *Plague* model, which suggests that either the *Lerp* effect is sufficiently large so as to mask the others, or that the other terms were present not in sufficient strength to be significantly noticeable. These two possibilities are not necessarily mutually exclusive, since, for example, sunlight is the fundamental limiting factor in photosynthesis and unlikely to be masked by the ‘psylloid effect’. The fact that the psylloid plague occurred at a time of year when solar declination is halfway towards its zenith means that solar radiation is not as strong (nor present for as long) as it would have been during the *Non-plague* period.

In the *Plague* model, by contrast, the *Sunlight* term was retained, with a negative coefficient (approximately -0.23), and about 18% stronger than the effect attributed to the *Lerps* term in the *Plague* model. The *Sunlight* term is in fact the strongest term in the model. Missing from the *Non-plague* model are the *Tree* and *Lerps* terms, with *Date* retained, but negative, suggesting that conditions pertaining on the day(s) of the samples were adversely affecting F_V/F_M values – which is to be expected, since the values of other variables such as the retained *Sunlight* term are directly correlated with *Date*. The remaining retained terms in the *Non-plague* model all have coefficients carrying a negative sign, and are between approximately 51% and 67% of the value of the *Sunlight* term coefficient. These terms are: *Egg mass*, *Predator*, and *Orientation*. *Orientation* is the easiest term to explain the effect of, since if a leaf is on a shaded part of the tree, it will probably have a better response to quenching excess energy than a leaf that has spent a good part of the day in full sunlight, with all *PS II* reaction centres, etc., fully reduced, and photoinhibition more probable. If the leaf sample population happened to be selected from leaves of the sunlit aspects of the tree because, for example, they were the only ones that were readily accessible, then the negative effect of *Orientation* (i.e., aspect), which is also correlated with *Sunlight*, would be apparent in the results of the F_V/F_M tests. *Necrosis* is also readily understandable in its negative effects, since the more dead leaf tissue there is on a leaf, the more that leaf is functioning below its overall capacity, and with a resultant depression of F_V/F_M . This is not a hard-and-fast rule, since leaves with a major proportion of their leaf blade missing or necrotised can have higher F_V/F_M readings than a leaf that looks perfectly healthy.

The ‘odd one out’ of the terms retained in the *Non-plague* regression model is *Predator*. It is hard to see how a predator, which normally one would consider as being beneficial to a plant by ridding it of its (the plant’s) natural enemies, could be indirectly deleterious to the tree by adversely affecting the photosynthetic apparatus of its leaves. No likely explanation for the negative sign on *Predator* has presented itself other than the possibility that the *presence* of predators may be correlated with *Date*, *Sunlight*, *Orientation*, etc. In other words, predators, for whatever reason, come out onto sunlit leaves on warm to hot days – the influence of *Predator* on the depression of F_V/F_M is most probably not causative, even though the numerical value of *Predator* (0.15) is the second largest (unsigned) value of all the retained terms in the *Plague* model. The putative influence of *Predator* may also have been an artifact of the way that the data was collected and set up (coded) for analysis.

8.4.3 Chlorophyll fluorescence study of five trees from the Adelaide population of *F. macrophylla*: differences between the Adelaide and Sydney populations

The mean F_v/F_M values (Table 8.14) from the significant ANOVA performed on the data from the study of the five Adelaide trees (Tables 8.14 and 8.15) reflected the conditions in which they were growing, almost directly. The tree with the highest mean F_v/F_M ratio values, and therefore in best condition, had the highest F_v/F_M ratio value of any tree sampled at any stage during the project. This can be directly attributed to the high degree of direct and indirect care in terms of water and nutrients that it received, and as such it can be considered as being effective comparison *F. macrophylla* specimen for the entire project, since it had no psyllid load (which distinction it also shared with all four other Adelaide *F. macrophylla* specimens studied.) Another notable feature of *ABG1* was the distinct lack of falling leaves - so much so that it was almost the first thing that was noticed about the tree. By contrast, *BP1*, growing in relatively harsh conditions (Figure 8.9), without direct irrigation or applied nutrients, was surrounded by a dense carpet of dead but intact leaves (Figure 8.10). This carpet is of a higher density than the shown under *MFG* in Figure 1.6 in Chapter 1. The *MFG* photograph was taken during the September 1996 psyllid plague outbreak. *BP1* had the second lowest mean F_v/F_M value of the five Adelaide specimens.

The Adelaide trees with the lowest readings were those which were existing in harsher conditions than the trees with higher readings. When comparing the overall mean F_v/F_M value for Adelaide *F. macrophylla* specimens in December 2000–January 2001, with those obtained from Sydney trees in December 1996 (Table 8.16), the Sydney mean is approximately 3% higher. This difference indicates that the trees in Adelaide were under greater stress than those in Sydney in 1996, and I suggest that the main reasons for this is that Adelaide typically has an overall drier climate than does Sydney, and that when the measurements were taken it was getting towards the middle of summer, which is relatively hot and dry in Adelaide anyway. Comparing the Adelaide mean F_v/F_M value in December 2000–January 2001 with the overall Sydney mean for all studies conducted in 1996 and 1997, the Adelaide mean is higher. I suggest that this difference can be directly attributed to the psyllid's presence in Sydney and absence in Adelaide, although a parallel study in both locations, over perhaps two or more years, would be needed to confirm or reject this statement with a reasonable degree of statistical certainty.



Figure 8.9: *BP1* – one of the four *CF* study trees sampled in Botanic Park,



Figure 8.10: Leaf carpet beneath *BP1* in early January 2001, looking outwards from trunk

The observations made in Adelaide (especially situations such as that shown in Figure 8.10) where the psyllid does not exist and therefore cannot be having an effect on the trees, and comparisons presented in Tables 8.17 and 8.18, however, suggest the possibility that water deficit stress resulting from a drier-than-normal environment than *F. macrophylla* has become adapted to (as a species) may be a major contributing factor to the massed defoliation events periodically occurring from the *F. macrophylla* trees growing in urban areas on the eastern seaboard – or at least far more significant than has hitherto been realised. The psyllid is probably contributory to stresses placed on the tree, either by removing nutrients and/or water (especially during plague conditions), or by the possible toxic effects of secondary metabolites injected by the psyllid into the plant as part of its feeding process (also potentially worse during psyllid plague conditions), or both. Although the psyllid certainly appears to be contributory to the depressed F_V/F_M ratios of *F. macrophylla* trees and their defoliation, it seems highly probable that it is not the only factor impinging on *F. macrophylla* health: it may well be that two other major factors are climate (temperature and rainfall) and growing conditions (soil compaction and lack of an adequate supply of water).

8.4.4 Comparisons of *F. macrophylla* specimens at different locations, with respect to F_V/F_M , psyllid presence and environmental conditions

The preceding remarks lead directly into the next part of the Discussion, namely, a closer look at the comparison tables of Tables 8.17 – 8.19. The creation of the *RT* ('simple tree + environmental conditions') index (Table 8.17) does allow a ready appreciation of the apparent conditions of a tree, although it is a light-weight 'statistic' and was not intended for any other use than as a quick means of scanning for differences in tree growing conditions. Having said that, the fairly close correlation between mean F_V/F_M ratios and the *RT* 'index' is interesting: the almost-correlation also brings the relatively low F_V/F_M mean for *MFG* into greater contrast, since this tree, as has already been remarked upon in this thesis, has what may be considered to be the best growing conditions of any *F. macrophylla* in the vicinity. *MFG* is not subjected to heavy pedestrian traffic around its immediate periphery; there are garden beds and turf surrounding it which are watered regularly, and which garden beds are given a good animal manure and plant cutting compost, also on a fairly regular basis (about every six months). The tree does not appear to have any visible signs of disease, is sheltered from salt spray and most high winds, is regularly maintained structurally (as a result of its proximity to the public) by qualified arborists, and has hessian overcoats on its aerial roots to encourage their growth.

MFG's counterpart at the Adelaide Botanic Gardens (*ABGI*), by contrast has many of the advantages that are bestowed on *MFG* – and had a mean F_V/F_M ratio above 0.800 (0.810, see Tables 8.15, 8.16 and 8.19 above). One major difference, and advantage, that *ABGI* enjoys over *MFG*, is the fact that epiphytes (e.g., bromeliads) are fixed onto the lower portion of the trunk, and to some of the lower limbs – and thus the tree gets watered far more often than *MFG* does.

The Sydney study trees which are *ABGI*'s peers with respect to a (relatively) high mean F_V/F_M ratio (Tables 8.16, 8.18 and 8.19) are *L44* (0.744) and *AGN* (0.736). The common denominator for all three trees is that each has an almost constant supply of water. *AGN* grows in a part of the Sydney Domain that happens to be immediately adjacent to the garden area outside the Art Gallery of NSW coffee shop. As a result of its highly prominent and visible location, this garden probably has more care apportioned to it than the area immediately surrounding *MFG* (the author actually cared for this garden just before starting work on the *M. fici* project). Part of that care includes an adequate application of water, and around (and before) the time that the chlorophyll fluorescence study was being conducted, the amount of pedestrian traffic past (and over the roots of) the tree was in the process of being minimised. The lawn underneath the tree was also fertilised at the usual regular intervals consistent with good turf care.

Conversely, *BP1* in Adelaide was heavily defoliating at the time that the Adelaide chlorophyll fluorescence sampling was conducted (Figure 8.10: cf Figure 8.9). This tree was also growing in one of the driest situations in which the author has seen an *F. macrophylla* growing in an urban situation. The soil in which the tree was growing was the 'Bay of Biscay' type of cracking clay that makes up the greater proportion of the upper soil horizons of the Adelaide Plains, and which was duly cracking wide open (by several centimetres) in the hot dry weather. The mean F_V/F_M value for *BP1* (0.641) was the second-lowest of the Adelaide study trees, the lowest (0.618) belonging to *BP2*, a tree growing in slightly damper conditions, but apparently infected with a plant pathogen, the effects of which were highly visible in the form of die-back in a couple of portions of the canopy on the northern and northwestern aspects of the tree. Despite the severity of conditions in which *BP1* was growing, however, its F_V/F_M value was not as low as one would have predicted, based on the healthy appearance of *MFG* (see Figure 1.1, Chapter 1, and Figure 6.1, Chapter 6) and the unusually low F_V/F_M value for a tree with its apparent advantages.

Of all the environmental factors already considered, apart from (or probably including) the psyllid, it is water that appears to be the one factor that is associated with the variation in F_V/F_M values: trees which received abundant quantities of water were the ones to have relatively high F_V/F_M ratios, compared with those trees whose water supply was lower. Since soil compaction is also a major factor preventing the infiltration of surface moisture further down into the soil profile and the rhizosphere, it is intimately bound with water deficit stress. Trees existing in areas with compacted soil, e.g., *BP1*, *BP2* and *BP4*, will almost automatically have a degree of water deficit stress, and this will in turn affect the overall health of the plant.

The observed between-tree difference in defoliation might also explain some of the between-tree differences in psyllid variation, since some trees may have been able to resist the psyllid better than others; or, some trees (not necessarily only putatively non-resistant trees) might have been more nutritious to the psyllid and therefore had a higher psyllid load as a consequence of heavier oviposition by preceding generations. Differential herbivory (Mopper and Simberloff, 1995; Mopper *et al.*, 1991) may be occurring as a result of differences between individual *F. macrophylla* trees' resistance to the psyllid (and therefore influence or even determine psyllid population size on a tree, and also the choice of tree by the psyllid). This hypothesised resistance may be a function of genotype (Maddox and Cappuccino, 1987; Maddox and Root, 1986) or an interaction between the plant genotype and the environment (Maddox and Cappuccino, 1987; Maddox and Root, 1986; Mopper *et al.*, 1991). Journet discusses this phenomenon with respect to the feeding activity of psyllids on *Eucalyptus blakelyi* M. (Journet, 1980; Journet, 1981), and with the environmental component of water availability (Journet, 1979). Mopper *et al.* (1991) discuss the reaction between host plants (*Pinus edulis*) growing on water-deficient and low-nutrient soils, their genotypic variation for resistance within and between patches, and their resistance to herbivorous insects. The case with *F. macrophylla* may be that the differences between water availability, differences in soil, and a possible genotypically-conferred resistance to the psyllid, are combining, in the Royal Botanic Gardens population at least, to produce the observed between-tree differences in both F_V/F_M ratio depression and defoliation response. The author is convinced of the fundamental role of water in the dynamics of the psyllid-tree interaction, as well as the tree defoliation events. There may also be a genetically-based tolerance to water deficit stress in *F. macrophylla* that has hitherto not been suspected, and this could be investigated in conjunction with potential psyllid resistance within the *F. macrophylla* population.

8.5 Conclusions

Separation of the Sydney part of the study data into *Plague* and *Non-plague* sets, with the result of correspondingly different components in their predictive models, was found to be an effective method of interpreting interactions between *M. fici* and *F. macrophylla*. The chlorophyll fluorescence study of the five chosen *F. macrophylla* specimens growing in the Royal Botanic Gardens and Domain, near Sydney Harbour was, based on the interpretation of the evidence presented here, able to detect the effects of *M. fici* on those Moreton Bay fig trees studied. The nature of that effect also appears to be a negative one. The *FCFK* technique was also useful for evaluating the general health of the selected Moreton Bay fig trees, as well as for comparing the relative states between the trees, and the trees and their growing conditions. The only reservation regarding the use of the technique is the current high cost of the equipment.

The results of analysis from the study of the five main *F. macrophylla* specimens (*MFG*, *L44*, *HRB*, *BTC* and *AGN*) indicated a strong correlation between fig psylloid number (as quantified by the number of lerps counted per leaf) and depressed F_v/F_M values for these trees, based on the division of the data into the separate *Plague* and *Non-plague* sets. The difference between the independent variables of the *Plague* and *Non-plague* Analysis of Variance models was consistent with the hypothesis of a negative effect due to the psylloid on *F. macrophylla* F_v/F_M . The results of the study, especially those from the Adelaide group of study trees, also suggest that lack of sufficient water, caused by drought and/or soil compaction, will also adversely affect the health of *F. macrophylla*. Thus, the psylloid, climate and growing conditions all contribute to *F. macrophylla* defoliation, since defoliation occurs even when the psylloid is not present, as in Adelaide.

Chapter 9. Conclusion

9.1 Major outcomes of the studies with respect to *Mycopsylla fici*

M. fici can be interpreted as being a *K*-selected 'mature-leaf'-feeding organism since it feeds on a *K*-selected host. It is more likely from the evidence (e.g., very large clutches of eggs laid per female), however, to be *r*-selected. This point of view is supported by the fact that the host periodically defoliates almost completely, thus, and rather suddenly, removing the psylloid's feeding and oviposition sites. *F. macrophylla* can therefore be interpreted as being a somewhat unpredictable environment for *M. fici*, and it is *r*-selected organisms that by definition succeed in such an environment. Large egg clutches could therefore be interpreted as the psylloid's *r*-selected response to the (most likely *K*-selected) tree's defoliation (i.e., the tree's attempts at unapparency *via* defoliation).

The psylloid is a selective feeder. It chooses a relatively narrow range of leaves on which to lay eggs, usually between the third and fifth fully expanded leaves back from the shoot (up to twelve leaves can be borne on the same branchlet). Occasionally, younger leaves will be chosen if more suitable egg-laying sites are not available as a result of competition (i.e., a psylloid plague). Older leaves than the fifth, however, appear never to be utilised by juvenile psylloids, although the older leaves will, of course, usually still carry the firmly inserted egg masses that they acquired when they were younger. That the psylloid avoids feeding on newly-unfurled leaves (and on young trees below a certain age) is evidence in favour of *M. fici* being *K*-selected. This is also consistent with *F. macrophylla* being an apparent plant (Feeny, 1976), since *K*-selected herbivores tend to exhibit a preference for (and a resistance to the phytoalexins of) *K*-selected plants. The new leaves produced by *F. macrophylla*, however, appear to be using what could be described as an *r*-selection strategy: the psylloid avoids new leaves except for egg-laying *in extremis*. As psylloid females also avoided laying eggs on leaves of saplings less than 1.5 m high or younger than about three years of age, it may be that a similar type of mechanism to that which is suggested as being used in new *F. macrophylla* leaves, is functioning to prevent the psylloid from colonising and harming the young plant. This in turn suggests that there may well be a plant toxin in the psylloid's salivary components.

M. fici was not susceptible to easy study as it was found not to be possible to rear it successfully in laboratory conditions. Adult female psylloids refused to lay eggs on small

potted *F. macrophylla*, either when the plants were placed in trees during the presence of adults, or placed in a psyllid-proof cage with 50 male and 50 female adult psyllids. Excised tree branches were not a practical proposition, since branches of suitable size to last an indefinite amount of time were too large to fit into the available glasshouse or laboratory space. Another difficulty in observing the psyllid was that it is obscured for most of its post-egg juvenile life by an opaque lerp, through which the juveniles could not readily be observed.

Throughout a survey of the eastern seaboard of Australia from Melbourne to Brisbane and North Stradbroke Island, *M. fici* was observed to occur in abundance in all urban areas visited. *M. fici* was, however, notable by its apparent absence in most of the stands of rainforest visited by the author in northern New South Wales and southern Queensland. *M. fici* does not exist naturally in Adelaide, and there is no evidence to suggest that it ever has - *F. macrophylla* is an introduced species in South Australia.

Despite the problems in observing the psyllid, considerable information on its life cycle was collected in the field, from direct observation and sticky trapping. Sufficient data was obtained to enable the development of life-tables for the psyllid. The data were also used to develop a set of degree days models for *M. fici* development from egg lay to adult eclosion. These models can be used in the prediction of outbreaks. Care will be needed in the application of these models, however, as the lower development threshold could not be defined in laboratory experiments. The Sydney degree days models were used to estimate the number of generations per year (assuming a 10 °C lower development threshold) at six other locations throughout the psyllid's range, plus a hypothetical Adelaide population.

The stadal durations of the juvenile psyllid instars were estimated for the purpose of constructing a degree days model for *M. fici*. In the process, the number of juvenile instars was confirmed as five, as per the usual Psylloidea plan, and juvenile growth was found to be approximately linear from first to fifth instars.

An interesting behaviour exhibited by *M. fici* hatchlings was their capacity for 'migrating': lerps appeared on leaves which had no egg masses laid upon them. A psyllid hatchling must

be able to travel at least the length of two leaf petioles plus the intervening branchlet distance between them to do so, since *M. fici* only appears to lay eggs on the leaf lamina. This behaviour greatly assists in a more even dispersal of the psyllid throughout the canopy, and may in fact be more advantageous to the tree since the psyllid load would be less concentrated on particular leaves. This would then reduce the likelihood of heavily infested leaves being stressed to the point of abscission. The individual psyllid would also gain from this situation by having more space and food available from the leaf.

The lerp is highly susceptible to atmospheric moisture, and was observed to swell and contract noticeably from day to day. The lerp's hygroscopic tendency is so marked that if the atmospheric moisture is high enough, younger lerps will flow across the leaf surface like water. Older lerps in such situations will soften, sag, and fall from the leaves, although most psyllids are left on the leaf surface. The psyllids, on loss of house, move to midvein and form a new lerp, presumably feeding from the midvein. This lerp can run almost the entire length of the leaf lamina if more than one lerp cover is lost (as is usual).

A third of all study eggs laid was found either to have been eaten, possibly by coccinellid or other predators, or to have failed to hatch. This was a major cause of mortality observed in the study population. No egg parasitisation was observed either in terms of female parasitoids stinging eggs, or adult parasitoid exit holes in egg cases.

The second highest cause of mortality in the cohort studied in detail in the field was not predators or parasitoids as was expected, but leaf fall, and this can possibly be interpreted as a defence mechanism on the part of the plant, since once the psyllids have fallen from the tree, it is highly likely that they would perish on the ground, from starvation or by being eaten.

Predators were observed to account for more psyllid mortality than parasitoids in the study cohort. Death by predation occurred at the beginning of the generation's life cycle; death from parasitisation occurred at the end of the juvenile life cycle stage (i.e., just before eclosion to adulthood). The overall chance of *M. fici* surviving from egg lay to adulthood was approximately 3.75% after egg and larval predation, lerp loss, leaf loss and parasitisation had taken their toll.

The guild structure of the free-living arthropods in an *F. macrophylla* canopy was determined from a fogging survey. Phytophages, predators, parasitoids, detritivores and mycovores were all found in the sample, with phytophages having the greatest representation. The relatively large numbers of other phytophages (predominantly thrips) on the tree does not appear to affect the psyllid with respect to food and space, or to affect the tree.

A stratification study of an *F. macrophylla* canopy with respect to the psyllid (eggs, lerps and adults) indicated a preference for the inside, top and north of the tree (adult) and lower down (eggs and lerps). Psyllid females were observed to lay eggs on other nearby fig trees of different species if *F. macrophylla* was already crowded to apparent capacity with other *M. fici* individuals.

9.2 Major outcomes of the studies with respect to natural enemies of *M. fici*.

Two size morphs of the parasitoid female were observed stinging into lerps during psyllid life history studies, and it was initially thought that the two females were two separate species of *Psyllaephagus*. Specimens were collected and sent to CSIRO Canberra for identification. The samples were studied by GAP Gibson, who concluded that they were two different size forms of the same species of *Psyllaephagus*. This species has yet to be formally described, although its existence has been known to science for the same length of time that *M. fici* has.

Psyllaephagus breathing tubes were observed, under the microscope, exiting juvenile psyllid host bodies. The tubes were blue or white in colour. As many as seven parasitoid larvae were identified, via their breathing tubes, in a single psyllid juvenile. This is evidence to suggest that *Psyllaephagus* sp. may be a polyembryonic encyrtid parasitoid: there are many documented examples of polyembryony in Family Encyrtidae. The possibility of polyembryony in *Psyllaephagus* gives a plausible mechanism for the existence of the two different size morphs of the *Psyllaephagus* sp. female, since polyembryony gives rise to clutches of smaller adults than would have been produced had the host been inhabited by a single parasitoid. It is not clear what the actual selection pressure might have been to induce polyembryony in *Psyllaephagus* sp., as one of the main advantages of polyembryony is the defence of a reproductive caste against other species of parasitoid or hyperparasitoid. There was no evidence of other such parasitoids, or higher trophic levels, associated with *M. fici*,

although the smaller form of the *Psyllaephagus* sp. female was initially viewed as possibly being such a candidate, until this was proven otherwise.

Parasitoids were observed to delay stinging until the psylloids were fully covered by a thick layer (more than 1.5 mm) of consolidated lerp material (wax and honeydew). The reason suggested for this is a delaying tactic on the part of the parasitoid mothers to prevent loss of host and egg by predation from neuropteran larvae, which attack psylloids under maturing lerps, and coleopteran larvae. Parasitoid females were also observed to eat parts of the lerp, although even under the microscope it was not clear whether the wasps were eating the wax tubes, the solidified honeydew, or both. Given that the type of food often consumed by parasitic wasps is nectar, it would seem more likely that it was the honeydew that the psylloid was observed to be eating. As a virtually inexhaustible source of food, the lerps would have enabled adult parasitoids to be quite long lived.

While leaf fall appeared to be one of the major causes of mortality in the psylloid cohorts studied, predation and parasitisation also played a part in limiting the psylloid population size, and it is therefore suggested that controlling psylloid numbers by pesticidal spraying would be unwise.

9.3 Major outcomes of the studies with respect to *Ficus macrophylla*

A new species of thrips, *Parabaliorthrips newmani*, was found by Peter Gillespie (Gillespie *et al.*, 2002) in the thysanopteran portion of the *F. macrophylla* canopy arthropod fauna surveying sample that was sent to him for identification. This thrips was found to be present in large numbers on specimens of *F. macrophylla* f. *columnaris* and *F. macrophylla* f. *macrophylla* in the Royal Botanic Gardens, Sydney. This and two other species of thrips did not appear to be outcompeting the psylloid for either space or food.

Chlorophyll fluorescence kinetics analysis was employed to detect the presence of an effect on *F. macrophylla* that was attributable to the psylloid. ANCOVA and multiple regression models of effects attributable to the psylloid on the photosynthetic apparatus of the *F. macrophylla* leaf indicated a partitioning in mean F_v/F_M value with respect to whether the tree was supporting a plague outbreak of psylloids or not. The major factor of negative influence on F_v/F_M value

depression in *F. macrophylla* leaves that the *Plague* regression model determined as significant was the presence of lerps (i.e., actively feeding juvenile psylloids), although there was also a noticeable effect from the difference between trees. The major factor of negative influence in the *Non-plague* regression model was sunlight, which is consistent with the fact that it is excess light energy within the light-harvesting side of the photosynthetic apparatus of plants that is most dangerous to its ongoing function and maintenances.

A subsample of the population of *F. macrophylla* growing in the Adelaide Botanic Gardens and adjacent Botanic Park was also subjected to chlorophyll fluorescence analysis. In the light of the fact that the psylloid does not occur in Adelaide, the Adelaide *F. macrophylla* trees may be considered to represent a control population. The results showed a clear trend towards higher values (healthier trees) as the growing conditions for the tree improved (i.e., more water and nutrients, and less soil compaction). The chlorophyll fluorescence study of Adelaide Botanic Gardens *F. macrophylla* specimens therefore supports the idea that appropriate management of *F. macrophylla* specimens may be the most practical factor in controlling psylloids. The Adelaide study trees that appeared to be the healthiest, both from their visual appearance and from the results of F_v/F_M studies, were those trees which had the most water and nutrients available to them; conversely, those Adelaide trees with least water and nutrients appeared to have lost as many leaves as some of the more severe cases of defoliation in Sydney. This suggests that the psylloid is not the only factor in the defoliation of *F. macrophylla*: the tree in general is an exceedingly robust and long lived plant, and it is possible, and even probable, that the impact of human activities, as well as climatic factors, may be more deleterious to the tree than those of the psylloid. Soil compaction and withholding of water are two areas that lie within our abilities to address satisfactorily, and by relatively simple means.

The chlorophyll studies of *F. macrophylla* in Adelaide and Sydney, and observation of *F. macrophylla* defoliation in both regions, suggest that defoliation may be associated with a threshold level of water loss or water stress within the tree, and it is hypothesized in the light of this project that there are several contributing factors to the attainment of this putative threshold. These factors are most likely to be: lower than average rainfall and atmospheric relative humidity; soil compaction reducing the uptake of water and nutrients to a level below that required for growth and general health; psylloid load reducing the amount of water and

nutrients in the plant, and also possibly inducing a phytoalexin response from the tree by injection of psyllid secondary metabolites (salivary compounds); and higher than average temperatures. The stress probably takes some time to build up, and may be alleviated by the reduction of any one (or more) of these putative stress factors. Conversely, the coincidence and maintenance of most or all of these stress factors over a number of seasons may exceed the hypothetical abscission trigger, resulting in the loss of most of the trees' leaf cover. It is also suggested that a large aggregation of factors occurs during severe *El Niño* events, and that *F. macrophylla* defoliation events may be expected to occur towards the end of a particularly severe example of this climate cycle.

The fact that there is no population of *M. fici* in Adelaide is interesting in itself, and the reasons for its absence there are worthy of further investigation. It seems most likely at this juncture that the psyllid simply has not been introduced there, since there are large climatic and geographical barriers to the psyllid between Adelaide and the eastern coast of Australia. The inability of the psyllid to live on small saplings would also have prevented the introduction of the psyllid into Adelaide. On the other hand, the psyllid is present in Melbourne and it can occur in extremely large numbers, and as Melbourne is also outside the natural range of the psyllid, the hypothesis of geographical isolation in the case of the psyllid's absence from Adelaide should be treated with a certain amount of circumspection.

9.4 Implications of research project findings on the future of *F. macrophylla* in an urban environment

While it is easy to apportion most of the blame to the psyllid with respect to the condition of *F. macrophylla* trees growing in the urban environment, it may be a good deal less convenient to accept that human activities may also have a deleterious effect on the trees. One of these activities is in fact an *inactivity* – neglect. If one accepts that we (as a society) have taken *F. macrophylla*, amongst many other things, more or less for granted, and also that there is 'no such thing as a free lunch' (Heinlein, 1966), then it becomes apparent that while we have benefited enormously from the characteristics of the tree that have made it so horticulturally popular, we have not done much in the way of returning the favour (Benson and Howell, 1990). It is perhaps time to look more closely at giving *F. macrophylla* more care and attention.

There are a number of choices facing those whose task it is to maintain and plant *F. macrophylla* specimens in Sydney and elsewhere up and down the eastern seaboard of Australia.

The first, and easiest, is to care for *F. macrophylla* specimens better: by keeping the public out from underneath the canopy and well away from the trees' rhizospheres to reduce deleterious soil compaction, and to increase levels of soil and atmospheric moisture for the trees. The former suggestion has been simply and easily effected at the Royal Botanic Gardens, Sydney, by placing fencing around the trees. Given that this then reduces the amenity value of the trees as sources of shade, the fencing about the trees could be rotated on, say, a two-yearly cycle, depending on the health of the tree and its location. Soil and atmospheric moisture can be increased by the installation of irrigation systems around the tree and in its canopy (Bates and Niemiera, 1994; Katsoulas *et al.*, 2001; Mata *et al.*, 1992). Canopy misting systems were installed with apparent effect in transplanted *F. macrophylla* specimens in the Sydney Domain and at the Homebush Bay Olympic Games complex.

Another alternative is the spraying of the *F. macrophylla* canopy with various pesticides. This approach has the inherent danger of causing indirect outbreaks of the psyllid by the removal of its various predators and parasitoids, and the induction of pesticide resistance in *M. fici*. There is also the added possible risk of inducing a completely new but previously innocuous pest on *F. macrophylla* or on other nearby plants as a result of the removal of predators from the system by the use of insecticides. None of the methods of pesticide application is cheap, and the opportunities for off-target pesticide drift and damage, and risk to public health, are high (Olkowski *et al.*, 1976; Olkowski *et al.*, 1978). Pesticidal petroleum spray oils are currently considered to be one of the safest general purpose insecticides that are available (Beattie *et al.*, 1995) and have been evaluated for use in the control of egg-laying by *Cacopsylla pyri* (pear psyllid) in pear orchards (Zwick and Westigard, 1978), and also with notable success in the control of *Phyllocnistis citrella*, the citrus leaf miner. *M. fici* has a distinct advantage over spray oils in that most of its life is spent under the protection of its communally-constructed lerp. Pesticidal spray oil could only be considered effective either during the presence of egg-laying adult female psyllids, or possibly during the first two weeks or so of the psyllid juvenile's life outside of its egg, while it is still vulnerable to 'external'

events (The psyllid, however, is still vulnerable even when under the lerp, but the danger is from other sources – first lacewing larvae, then *M. fici*'s *Psyllaephagus* sp. parasitoid.)

In any event, a public education campaign is necessary to explain to the general population what measures are being, and need to be, employed to rehabilitate and protect urban *F. macrophylla* populations, and this process has already been started at the Royal Botanic Gardens, Sydney, by the use of appropriate signage explaining why a number of the largest *F. macrophylla* specimens in the Gardens and Domain have been roped or fenced off from public access.

9.5 Suggested further avenues of research

There are a number of avenues of research that can be followed, leading on from various aspects of the studies done in the project. These can be grouped into the following somewhat overlapping categories:

- psyllid and parasitoid rearing;
- further psyllid population studies;
- detailed physiological studies of psyllid-fig tree interaction;
- research into possible interactions (e.g., at a biochemical level) between the thrips species *P. newmani*, *M. fici* and *F. macrophylla* with respect to defoliation triggers.

The suggestion mooted in the last point above, that of investigation into a possible interaction between the thrips species and the psyllid in the *F. macrophylla* leaves, could provide useful information in the control of defoliation.

Research to elucidate the reasons why *M. fici* will not thrive on *F. macrophylla* saplings less than 1.5 m high and/or less than three years of age may provide information likely to be useful in developing a relatively environmentally benign control of psyllid population sizes.

Detailed plant physiological studies of the psyllid-host interaction at the biochemical level are needed to determine what the psyllid's physiological effect on the tree is. Such methods as

pressure bomb testing (Tomos and Leigh, 1999) of leaves to determine if lerp flow or synthetically applied analogue(s) induces and/or promotes leaf senescence and abscission.

Research into the possible occurrence of psylloid-resistant and drought-tolerant genotypes of *F. macrophylla* may well pay great dividends if in fact they are found to exist. If one or both of these hypothesised resistances do in fact exist, the possibility exists for their manipulation and utilisation in producing a broader distribution of psylloid-resistant *F. macrophylla* trees in the urban environment.

Microsatellite DNA analysis of psylloid populations on different trees and in different regions could be employed to determine: large and small scale migration patterns; where the psylloid originally radiated from; and to develop an epidemiological model of psylloid reinfestation of trees from which the resident psylloid population has been removed.

Long term chlorophyll fluorescence studies allied with leaf boundary layer gas exchange analysis would provide useful information about the psylloid-fig system.

Experiments with methods to facilitate field study of *M. fici* under its lerp, such as the use of a synthetic pliable compound (such as 'Blue-Tak[®]' or similar) covers to replace the opaque lerps and also requiring the removal of honeydew and wax on a regular (daily or twice daily) basis, would be worthwhile. Such a method, if successful, would lead to more accurate and efficient observations of the juvenile stages of the psylloid.

The determination of methods of successfully mass rearing *M. fici* and *Psyllaephagus* sp. in the laboratory would be advantageous for more accurate and precise study of the psylloid's developmental phases, and would assist in the development of a possible cost-effective inundative release control program for the psylloid, if such a program was considered desirable.

Detailed study of egg development of *M. fici* would determine whether development within the egg is at a constant rate, or whether there are periods of egg diapause or quiescence. This would be useful in assisting to gain more accurate estimates of egg-lay to development time,

and may also offer further insights into the psylloid's overall biology. Investigations on the egg stage of the psylloid could also include looking at whether there are one or more 'winter' egg diapause or quiescence periods.

Nothing is known of psylloid and parasitoid courting and mating behaviours, and this type of study has now become standard in work on developing biological control systems, since a more or less complex web of interactions is involved, and attaining the broadest knowledge possible (and which is practicable) of the interacting species is necessary for predicting any problems that might arise from such systems. These studies might also include the investigation of any stridulatory behaviour by male psylloids when courting, to determine if psylloid mating disruption might be possible as a means of controlling the insect in urban areas where the tree is growing.

A longer term study of several geographically distinct psylloid populations under a single project is required to provide better data than we have at present. This is common practice in population studies.

Flight tunnel experiments could be conducted to determine useful attractants and confusants (semiochemicals) for *M. fici*. Such semiochemicals could be used as part of a biologically-based control program for the psylloid.

Determination of mean adult longevity, female fecundity, including r_m and r_c indices are additional pieces of information that are required to be able to predict psylloid population outbreaks on an accurate and reliable basis.

9.6 Closing remarks

The emphasis in this project has been to elucidate the population dynamics of *Mycopsylla fici*, the Moreton Bay fig psylloid, and thereby, to understanding how it might affect its sole host, *Ficus macrophylla*, the Moreton Bay fig. Studies were undertaken with these aims in mind, resulting in a large amount of field-collected data on both organisms gathered from a number of disparate studies, from which a picture of this insect-tree system was gradually assembled. I have also had the satisfaction of being able to conduct a body of scientific biological study in

an environment not usually associated with this type of activity. Such types of study are not particularly easy to undertake without interference or interruption. The assembled data have enabled me to develop several numerical models of the psyllid, the tree, and the psyllid-tree interaction, in a number of different contexts. These models have in turn enabled me to hypothesise climatic and cultural reasons for psyllid outbreaks resulting in marked defoliation events in *F. macrophylla* trees growing in urban areas. The conclusions at which I have arrived have also supported the evolving systems of management of *F. macrophylla* in urban areas. Development of these management systems was begun some years before I started my own work on the psyllid, but the conclusions which I have drawn have added greater weight to their implementation, from a scientific perspective. These management practices have been adopted partially, in a small section of the horticultural community centred largely around the Royal Botanic Gardens, Sydney, and its sphere of influence. For the long term viability of the Moreton Bay fig in urban areas, these management strategies for reducing psyllid and climate-induced defoliation events are of prime importance. My conclusions also have implications for the care of other species of plants used in urban amenity horticulture, since the life of any street or parkland tree is usually a difficult one, and the same principles apply to them all.

References

- Abeles FB. 1973. *Ethylene in Plant Biology*. Academic Press, Inc., New York. 302 pp.
- Abrams PA & Matsuda H. 1996. Fitness minimization and dynamic instability as a consequence of predator-prey coevolution. *Evolutionary Ecology* **10**, 167–186.
- Adams JB & van Emden HF. 1972. The Biological Properties of Aphids and their Host Plant Relationships. In *Aphid Technology with Special Reference to Aphids in the Field*. van Emden HF (Ed.), Academic Press, London & New York, 47–104.
- Adams WW & Demmig-Adams B. 1992. Carotenoid composition and down-regulation of Photosystem II in three conifer species during the winter. *Physiologia Plantarum* **92**(3), 451–458.
- Ahmed M, Jakupovic J, Bohlmann F & Niemeyer HM. 1991. Highly oxygenated furoeremophilane derivatives from *Senecio zoellneri*. *Phytochemistry* **30**(7), 2407–2409.
- Albertsson P-A, Hsu B-D, Tang G M-S & Arnon DI. 1983. Enhanced sensitivity to plastoquinone inhibitors of ferredoxin photoreduction by Photosystem II in inside-out chloroplast vesicles. *Biochemical & Biophysical Research Communications* **115**(2), 722–729.
- Albertsson P-A, Andreasson E, Svensson P & Yu S. 1992. The domains of the plant thylakoid membrane. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG & Medrano H (Eds), 45–57.
- Allen JC. 1976. A modified sine wave method for calculating degree days. *Environmental Entomology* **5**(3), 388–396.

- An M, Johnson IR & Lovett JV. 1996. Mathematical modelling of allelopathy. I. Phytotoxicity caused by plant residues during decomposition. *Allelopathy Journal* 3(1), 33–42.
- Anderson RM, May RM, Dawkins R & Krebs JR. 1990. A simulation model of the population dynamics and evolution of myxomatosis. *Ecological Monographs* 60(4), 423–447.
- Andersson B. 1992. Thylakoid membrane dynamics in relation to light stress and photoinhibition. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG & Medrano H (Eds), 71–86.
- Andrewartha HG & Birch LC. 1954. *The distribution and abundance of animals*. University of Chicago Press, Chicago. 782 p.
- Anholt BR & Werner EE. 1998. Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. *Evolutionary Ecology* 12(6), 729–738.
- Anonymous. 1913. In Royal Botanic Gardens Report 1913, p. 8.
- Anonymous. 1924. In Royal Botanic Gardens Report 1923–1924, p. 6.
- Anonymous. 1999a. Atacora/Donga/intoxication alimentaire. Trois morts et dix-sept personnes hospitalisées à Bassil. In *Le Matinal*, 17 August, 1999.
- Anonymous. 1999b. Consommation de gombo intoxiqué à Banikoara et Gogounou. Des victimes se font compter. *Le Matinal*, 10 September, 1999.
- Apori SO, Castro FB, Shand WJ & Orskov ER. 1998. Chemical composition, *in sacco* degradation and *in vitro* gas production of some Ghanaian browse plants. *Animal Feed Science & Technology* 76(1/2), 129–137.

- Appel HM & Martin MM. 1992. Significance of metabolic load in the evolution of host specificity of *Manduca sexta*. *Ecology* **73**(1), 216–228.
- Argandona VH, Luza JG, Niemeyer HM & Corcuera LJ. 1980. Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry (Oxford)* **19**(8), 1665–1668.
- Arnon DI. 1988. The discovery of ferredoxin the photosynthetic path. *Trends in Biochemical Sciences* **13**(1), 30–33.
- Arnon DI. 1992a. Unconventional pathways of photosynthetic electron transport: The autonomous oxygenic and anoxygenic photosystems. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG & Medrano H (Eds), 103–123.
- Arnon DI. 1992b. Conceptual evolution in photosynthesis: The quest for a common denominator. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG & Medrano H (Eds), 13–25.
- Arnon DI. 1995. Divergent pathways of photosynthetic electron transfer: The autonomous oxygenic and anoxygenic photosystems. *Photosynthesis Research*. **46**(1–2), 47–71.
- Arnon DI & Tang G M-S. 1988. Cytochrome b-559 and proton conductance in oxygenic photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **85**(24), 9524–9528.
- Arnon DI, Tsujimoto HY & Tang GM-S. 1981a. Oxygenic photoreduction of ferredoxin independently of the membrane bound iron sulfur centers of Photosystem I. *Biochemical & Biophysical Research Communications* **99**(3), 936–945.
- Arnon DI, Tsujimoto HY & Tang GM-S. 1981b. Proton transport in photo oxidation of water a new perspective on photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **78**(5), 2942–2946.

- Arnon DI, Tsujimoto HY & Tang GM-S. 1982. Different roles of plastoquinone in the photoreduction of ferredoxin and of membrane bound iron sulfur centers of chloroplasts. *Biochemical & Biophysical Research Communications* **106**(2), 450–457.
- Asada, K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 601–639.
- Asquith A & Kido M. 1994. Native Hawaiian insects attracted to the semiochemical methyl eugenol, used for male annihilation of the Oriental fruit fly (Diptera: Tephritidae). *Environmental Entomology* **23**(6), 1397–1408.
- Avinent L, Llacer G, Almacella SJ & Tora R. 1997. Pear decline in Spain. *Plant Pathology* **46**(5), 694–698.
- Aunger M & Bernays EA. 1998. Plant defence signals and Batesian mimicry. *Evolutionary Ecology* **12**, 667–679.
- Azcón-Bieto J. 1992. Relationship between photosynthesis and respiration in the dark in plants. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG, Medrano H (Eds), 241–253.
- Azcón-Bieto J, Lambers H, Day DA. 1983. Respiratory properties of developing bean and pea leaves. *Australian Journal of Plant Physiology* **10**, 237–245.
- Baldassari N, Baronio P, Rocchetta G & Salvaterra I. 1996. Research on the development of *Cacopsylla pyri* (L.) (Hemiptera Psyllidae) on different mutants and selections of pear. *Bollettino Dell'Istituto di Entomologia 'Guido Grandi' della Universita Degli Studi di Bologna* **50**(0), 201–213.

- Ball MC, Butterworth JA, Roden JS, Christian R & Egerton JJG. 1995. Applications of chlorophyll fluorescence to forest ecology. *Australian Journal of Plant Physiology* **22**, 311–319.
- Balota M & Lichtenthaler HK. 1999. Red chlorophyll fluorescence as an ecological method to assess the behaviour of wheat genotypes under drought and heat. *Cereal Research Communications* **27**(1–2), 179–187.
- Bar, D & Gerling D. 1985. Cannibalism in *Chrysoperla carnea* (Stephens) (Neuroptera, Chrysopidae). *Israel Journal of Entomology* **19**, 13–22.
- Barber J. 1992. Composition, structure and dynamics of the isolated reaction centre of Photosystem Two. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG & Medrano H (Eds), 29–43.
- Barria BN, Copaja SV & Niemeyer HM. 1992. Occurrence of DIBOA in wild *Hordeum* spp. and its relation to aphid resistance. *Phytochemistry (Oxford)* **31**(1), 89–92.
- Baskerville GL & Emin P. 1969. Rapid estimation of heat accumulations from maximum and minimum temperatures. *Ecology* **50**(3), 514–517.
- Basset Y. 1992. Aggregation and synecology of arboreal arthropods associated with an overstorey rain forest tree in Australia. *Journal of Tropical Ecology* **8**(3), 317–327.
- Basset Y & Arthington, AH. 1992. The arthropod community of an Australian rainforest tree: Abundance of component taxa, species richness and guild structure. *Australian Journal of Ecology* **17**, 89–98.
- Basset Y & Kitching RL. 1991. Species number, species abundance and body length of arboreal arthropods associated with an Australian rainforest tree. *Ecological Entomology* **16**, 391–402.

- Bates RM & Niemiera AX. 1994. Mist irrigation reduces post-transplant desiccation of bare-root trees. *Journal of Environmental Horticulture* **12**(1), 1–3.
- Bauer H, Plattner K, Volgger W. 2000. Photosynthesis in Norway spruce seedlings infected by the needle rust *Chrysomyxa rhododendri*. *Tree Physiology* **20**(3), 211–216.
- Beardsley JW & Uchida GK. 1990. Parasites associated with the *Leucaena* psyllid, *Heteropsylla cubana* Crawford, in Hawaii. *Proceedings of the Hawaiian Entomological Society* **30**, 155–156.
- Beattie GAC, Liu ZM, Watson DM, Clift AD & Jiang L. 1995. Evaluation of petroleum spray oils and polysaccharides for control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *Journal of the Australian Entomological Society* **34**(4), 349–353.
- Beattie GAC, Weir RG, Clift AD & Jiang L. 1990. Effect of nutrients on the growth and phenology of *Gascardia destructor* (Newstead) and *Ceroplastes sinensis* Del Guercio (Hemiptera: Coccidae) infesting citrus. *Journal of the Australian Entomological Society* **29**, 199–203.
- Benson D & Howell J. 1990. *Taken for Granted: The Bushland of Sydney and its Suburbs*. Kangaroo Press, Sydney, NSW. 160 pp.
- Bentley R. 1990. The shikimate pathway – a metabolic tree with many branches. *Critical Reviews in Biochemistry & Molecular Biology* **25**(5), 307–384.
- Berenbaum MR. 1988. Allelochemicals in insect-microbe-plant interactions; agents provocateurs in the coevolutionary arms race. In *Novel aspects of insect-plant interactions*. Barbosa P & Letourneau DK (Eds), J. Wiley, New York. p. 97–123.

- Berenbaum MR & Feeny P. 1981. Toxicity of angular furano-coumarins to swallowtail butterflies – Escalation in a co-evolutionary arms race. *Science (Washington D C)* **212(4497)**, 927–929.
- Berenbaum MR & Zangerl AR. 1988. Stalemates in the coevolutionary arms race: syntheses, synergisms, and sundry other sins. In *Chemical mediation of coevolution*. Spencer KC (Ed.), Academic Press, San Diego. p. 113–132.
- Berenbaum MR & Zangerl AR. 1993. Furanocoumarin metabolism in *Papilio polyxenes*: Biochemistry, genetic variability, and ecological significance. *Oecologia (Heidelberg)* **95(3)**, 370–375.
- Berenbaum MR & Zangerl AR. 1998. Chemical phenotype matching between a plant and its insect herbivore. *Proceedings of the National Academy of Sciences of the United States of America* **95(23)**, 13743–13748.
- Bellows TR, Van Driesche RG & Elkinton JS. 1992. Life table construction and analysis in the evaluation of natural enemies. *Annual Review of Entomology* **37**, 587–614.
- Beyer EM Jr., Morgan PW & Yang SF. 1984. Ethylene. In *Advanced Plant Physiology*. Wilkins MB (Ed.), Pitman Publishing Limited, London, 111–126.
- Binder WD, Fielder P, Mohammed GH & L'Hirondelle SJ. 1997. Applications of chlorophyll fluorescence for stock quality assessment with different types of fluorometers. *New Forests* **13(1–3)**, 63–89.
- Björkman O & Demmig B. 1987. Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta* **170**, 489–504.
- Blanco L. 1994. *Aphid host plant interactions*. PhD thesis, Macquarie University, Sydney, 122 pp.

- Blomquist CL & Kirkpatrick BC. 1997. Infectivity of pear psylla (*Cacopsylla pyricola* Foerster) collected from pear decline infected trees. *Phytopathology* **87**(6 Suppl.), S9.
- Blomquist CL & Kirkpatrick BC. 2002. Frequency and seasonal distribution of pear psylla infected with the pear decline phytoplasma in California pear orchards. *Phytopathology* **92**(11), 1218–1226.
- Blossey B, Eberts D, Morrison E & Hunt TR. 2000. Mass rearing the weevil *Hylobius transversovittatus* (Coleoptera: Curculionidae), biological control agent of *Lythrum salicaria*, on semiartificial diet. *Journal of Economic Entomology* **93**(6), 1644–1656.
- Blundell AG & Peart DR. 2000. High abscission rates of damaged expanding leaves: Field evidence from seedlings of a Bornean rain forest tree. *American Journal of Botany* **87**(11), 1693–1698.
- Bohlár-Nordenkamp HR, Long SP, Baker NR, Öquist G, Schreiber U & Lechner EG. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Functional Ecology* **3**, 497–514.
- Bohlár-Nordenkamp HR, Critchley CH, Haumann J, Ludlow MM, Postl W & Syme AJ. 1994. Can chlorophyll fluorescence and *P700* absorbtion changes detect environmental stress. In *Plant Production on the Threshold of a New Century*. Stuik *et al.* (Eds), Kluwer Academic Publishers, Amsterdam, 295–302.
- Bongi G & Long S. 1987. Light dependent damage to photosynthesis in olive leaves during chilling and high temperature stress. *Plant, Cell and Environment* **10**, 241–249.
- Booth SR & Riedl H. 1996. Diflubenzuron-based management of the pear pest complex in commercial orchards of the Hood River Valley in Oregon. *Journal of Economic Entomology* **89**(3), 621–630.

- van den Bosch R. 1971. The melancholy addiction of Ol' King Cotton. *Natural History* **80**(10), 86–90.
- van den Bosch R. 1972. The cost of poisons. *Environment (Washington D C)* **14**(7), 18–22.
- van den Bosch, R. 1980. The pesticide mafia. *Ecologist* **10**(3) 78–82.
- Bowers, MD & Puttick GM. 1988. Response of generalist and specialist insects to qualitative allelochemical variation. *Journal of Chemical Ecology* **14**(1), 319–334.
- Brandon NJ & Shelton HM. 1997. Factors affecting the early growth of *Leucaena leucocephala* L. Effects of nitrogen, phosphorus, lime and irrigation at three sites in south-east Queensland. *Australian Journal of Experimental Agriculture* **37**(1), 27–34.
- Buckner JS, Freeman TP, Ruud RL, Chu CC & Henneberry TJ. 2002. Characterization and functions of the whitefly egg pedicel. *Archives of Insect Biochemistry & Physiology* **49**(1), 22–33.
- Bues R, Toubon J, Rieux R & D'Arcier FF. 1994. *Cacopsylla pyri* (L.) (Homoptera: Psyllidae) esterase polymorphism and insecticide resistance. *Comptes Rendus de l'Academie des Sciences – Serie III, Sciences de la Vie* **317**(12), 1115–1121.
- Bues R, Boudinhon L, Toubon JF & D'Arcier F. 1999. Geographic and seasonal variability of resistance to insecticides in *Cacopsylla pyri* L. (Hom., Psyllidae). *Journal of Applied Entomology* **123**(5), 289–297.
- Buonaccorsi JP, Harrington LC & Edman JD. 2003. Estimation and comparison of mosquito survival rates with release-recapture-removal data. *Journal of Medical Entomology* **40**(1), 6–17.

- Byrne DN, Cohen AC & Draeger EA. 1990. Water uptake from plant tissue by the egg pedicel of the greenhouse whitefly, *Trialeurodes vaporariorum*. *Canadian Journal of Zoology* **68**(6), 1193–1195.
- Cameron PJ, Walker GP, Herman TJB & Wallace AR. 2001. Development of economic thresholds and monitoring systems for *Helicoverpa armigera* (Lepidoptera: Noctuidae) in tomatoes. *Journal of Economic Entomology* **94**(5), 1104–1112.
- Campbell KG. 1992. The biology and population ecology of two species of *Cardiaspina* (Hemiptera: Psyllidae) in plague numbers on *Eucalyptus grandis* in New South Wales. *Proceedings of the Linnean Society of New South Wales* **113**(1–4), 135–150.
- Carisey N & Bauce E. 1997. Balsam fir foliar chemistry in middle and lower crowns and spruce budworm growth, development, food and nitrogen utilization. *Journal of Chemical Ecology* **23**(8), 1963–1978.
- Carpenter JE & Greany PD. 1998. Comparative development and performance of artificially reared versus host-reared *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) wasps. *Biological Control* **11**(3), 203–208.
- Carraro L, Osler R, Loi N, Ermacora P & Refatti E. 2000. Current status of experimental transmission of fruit tree phytoplasmas in north-eastern Italy. *Journal of Plant Pathology* **82**(1), 69.
- Carraro L, Loi N & Ermacora P. 2001. The ‘life cycle’ of pear decline phytoplasma in the vector *Cacopsylla pyri*. *Journal of Plant Pathology* **83**(2), 87–90.
- Carson RL. 1962. *Silent Spring*. Penguin Books in association with Hamish Hamilton, Harmondsworth, England. 317 p.

- Carson WP & Root R. 2000. Herbivory and plant species coexistence: Community regulation by an outbreaking phytophagous insect. *Ecological Monographs* **70**(1), 73–99.
- Chase WR, Nair MG & Putnam AR. 1991. 2,2' oxo-1,1'-azobenzene selective toxicity of rye (*Secale cereale* L.) allelochemicals to weed and crop species II. *Journal of Chemical Ecology* **17**(1), 9–20.
- Chew FS & Courtney SP. 1991. Plant apparency and evolutionary escape from insect herbivory. *American Naturalist* **138**(3), 729–750.
- Chi H. 1994. Periodic mass rearing and harvesting based on the theories of both the age-specific life table and the age-stage, two-sex life table. *Environmental Entomology* **23**(3), 535–542.
- Chou CH, Waller GR, Cheng CS, Yang CF. & Kim D. 1995. Allelochemical activity of naturally occurring compounds from mungbean (*Vigna radiata* L.) plants and their surrounding soil. *Botanical Bulletin of Academia Sinica* **36**(1), 9–18.
- Clark LR. 1962. The general biology of *Cardiaspina albitextura* (Psyllidae) and its abundance in relation to weather and parasitism. *Australian Journal of Zoology* **10**, 537–586.
- Clark LR & Dallwitz MJ. 1975. The life system of *Cardiaspina albitextura* Psyllidae 1950–1974. *Australian Journal of Zoology* **23**(4), 523–561.
- Cleland RE. 1988. Molecular events of photoinhibitory inactivation in the reaction centre of Photosystem II. *Australian Journal of Plant Physiology* **15**, 135–150.
- Cobbinah JR. 1986. Factors affecting the distribution and abundance of *Phytolyma lata* (Homoptera: Psyllidae). *Insect Science and its Application* **7**(1), 111–116.

- Coelho JR. 1997. Sexual size dimorphism and flight behavior in cicada killers, *Sphecius speciosus*. *Oikos* **79**(2), 371–375.
- Colegrave N. 1995. The cost of exploitation competition in *Callosobruchus* beetles. *Functional Ecology* **9**, 191–196.
- Collett N. 2001. Biology and control of psyllids, and the possible causes for defoliation of *Eucalyptus camaldulensis* Dehnh. (river red gum) in south-eastern Australia – a review. *Australian Forestry* **64**(2), 88–95.
- Constant B, Grenier S & Bonnot G. 1994. Analysis of some morphological and biochemical characterisation of the egg of the predaceous bug *Macrolophus caliginosus* (Het.: Miridae) during embryogenesis. *Entomophaga* **39**(2), 189–198.
- Copaja SV, Barria BN & Niemeyer HM. 1991. Hydroxamic acid content of perennial Triciceae. *Phytochemistry (Oxford)* **30**(5), 1531–1534.
- Cox DR. 1959. The analysis of exponentially distributed life-times with two types of failures. *Journal of the Royal Statistical Society* **26**, 103–110.
- Cox DR. 1972. Regression models and life tables. *Journal of the Royal Statistical Society* **34**, 187–220.
- Cox DR & Oakes D. 1984. *Analysis of survival data*. Chapman and Hall, London & New York. viii+201 pp. Illus.
- Crawford SA & Wilkens S. 1996. Ultrastructural aspects of damage to leaves of *Eucalyptus camaldulensis* by the psyllid *Cardiaspina retator*. *Micron* **27**(5), 359–366.
- CRC Industries (Aust) Pty Ltd. 2003. MSDS for CRC 5-56® (Product No. 5005, 5027, 5028; U.N. Number 1950). 2 pp.

- Cregg BM, Rowe DB, Fernandez RT, Duck MW & Rios CM. 2001. Evaluating conifer nutrient status via chlorophyll fluorescence. *Hortscience* **36**(3), 542.
- Crouchley R & Pickles A. 1993. A specification test for univariate and multivariate proportional hazards models. *Biometrics* **49**(4), 1067–1076.
- Cruz YP. 1981. A sterile defender morph in a poly-embryonic hymenopterous parasite. *Nature* **294**(5840), 446–447.
- Cruz YP. 1986a. Development of the polyembryonic parasite *Copidosomopsis tanytmenus* (Hymenoptera: Encyrtidae). *Annals of the Entomological Society of America* **79**(1), 121–127.
- Cruz YP. 1986b. The defender role of the precocious larvae of *Copidosomopsis tanytmenus* (Encyrtidae: Hymenoptera). *Journal of Experimental Zoology* **237**(3), 309–318.
- Cruz YP, Oelhaf RC Jr & Jockusch EL. 1990. Polymorphic precocious larvae in the polyembryonic parasitoid *Copidosomopsis tanytmema* (Hymenoptera: Encyrtidae). *Annals of the Entomological Society of America* **83**(3), 549–554.
- Das PK, Singh RN, Brahmachari BN, Sharan SK & Sengupta K. 1988. Seasonal intensity of infestation of the gall insect *Trioza fletcheri minor* Crawford (Homoptera: Psyllidae) on *Terminalia tomentosa* and *Terminalia arjuna* A & W, and its control through systemic insecticides. *Indian Journal of Sericulture* **27**(2), 117–121.
- Davies DL & Eyre S. 1996. Detection of phytoplasmas associated with pear decline in pear psyllids by polymerase chain reaction. In *Diagnostics in crop production* (65), Marshall, G (Ed.), British Crop Protection Council Monograph, 67–72.

- Dayton PK, Tegner MJ, Parnell PE & Edwards PB. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp forest community. *Ecological Monographs* **62**(3), 421–445.
- Demmig B & Winter K. 1988. Light response of CO₂ assimilation, reduction status of *Q*, and radiationless energy dissipation in intact leaves. *Australian Journal of Plant Physiology* **15**, 151–62.
- Demmig B & Winter K. 1993. Characterisation of three components of non-photochemical fluorescence quenching and their response to photoinhibition. *Australian Journal of Plant Physiology* **15**, 163–177.
- Denno RF & Benrey B. 1997. Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology* **22**(2), 133–141.
- Diaz J, Ros-Barcelo A & Merino-DeCaceres F. 1997. Changes in shikimate dehydrogenase and the end products of the shikimate pathway, chlorogenic acid and lignins, during the early development of seedlings of *Capsicum annum*. *New Phytologist* **136**(2), 183–188.
- Dixon DJ. 2001a. A chequered history: The taxonomy of *Ficus platypoda* and *F. leucotricha* (Moraceae: Urostigma sect. Malvanthera) unravelled. *Australian Systematic Botany* **14**(4), 535–563.
- Dixon DJ. 2001b. Figs, wasps and species concepts: A re-evaluation of the infraspecific taxa of *Ficus macrophylla* (Moraceae: Urostigma sect. Malvanthera). *Australian Systematic Botany* **14**(1), 125–132.
- Dixon DJ. 2002. Short note: *Ficus rubiginosa* f. *glabrescens* (F.M.Bailey) D.J. Dixon: A new taxonomic status for an old Bailey name. *Australian Systematic Botany* **15**(2), p. 245.

- Dixon DJ, Jackes BR & Bielig LM. 2001. Figuring out the figs: The *Ficus obliqua*- *Ficus rubiginosa* complex (Moraceae: Urostigma sect. Malvanthera). *Australian Systematic Botany* **14**(1), 133–154.
- Dupré S, Grenz M, Jakupovic J, Bohlmann F & Niemeyer HM. 1991. Eremophilane, germacrane and shikimic acid derivatives from Chilean *Senecio* species. *Phytochemistry* **30**(4), 1211–1220.
- Edwards PB & Wanjura WJ. 1989. Eucalypt-feeding insects bite off more than they can chew: Sabotage of induced defenses. *Oikos* **54**(2), 246–248.
- Edwards PB & Wanjura WJ. 1991. Physical attributes of eucalypt leaves and the host range of chrysomelid beetles. *Symposia Biologica Hungarica, Vol. 39. Insects-Plants '89; 7th International Symposium on Insect-Plant Relationships, Budapest, Hungary, July 3–8, 1989*. Szentesi A & Jermy T (Eds), Akademiai Kiado, Budapest, Hungary, xiii+577pp., illus. p. 227–236.
- Edwards PB, Wanjura WJ & Brown WV. 1993. Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* **95**, 551–557.
- Edwards OR & Hoy MA. 1998. Biology of *Ageniaspis citricola* (Hymenoptera: Encyrtidae), a parasitoid of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) *Annals of the Entomological Society of America* **91**(5), 654–660.
- Efron B. 1982. *The jackknife, the bootstrap, and other resampling plans*. Philadelphia Society for Industrial and Applied Mathematics. Philadelphia, PA.
- Ellers J & Jervis M. 2003. Body size and timing of egg production in parasitoid wasps. *Oikos* **102**, 164–172.

- Erwin, TL. 1983. Beetles and other insects of tropical forest canopies at Manaus, Brazil, sampled by insecticidal fogging. In *British Ecological Society Special Publications Series, Vol. 2. Tropical Rain Forest: Ecology and Management; Symposium, Leeds, England, Apr., 1982*. Blackwell Scientific Publications, Palo Alto, London, xiii+498 pp., 59–75.
- Eveleens KG. 1983. Cotton-insect control in the Sudan Gezira: analysis of a crisis. *Crop Protection* **2**(3), 273–287.
- Eveleens KG, Van Den Bosch R & Ehler LE. 1973. Secondary outbreak induction of beet armyworm by experimental insecticide applications in cotton in California. *Environmental Entomology* **2**(4), 497–503.
- Facchini PJ, Huber-ach KL & Tari LW. 2000. Plant aromatic *L*-amino acid decarboxylases: evolution, biochemistry, regulation, and metabolic engineering applications. *Phytochemistry* **54**(2), 121–138.
- Feeny, PP. 1976. Plant apparency and chemical defense. In *Biochemical interaction between plants and insects*. Wallace JW and Mansell RL (Eds), Plenum Press, New York, 1–40.
- Floss HG. 1985. The shikimate pathway – an overview. In *The shikimic acid pathway. Recent Advances in Phytochemistry* (Conn EE, Ed.) **20**, 13–55.
- Froggatt WW. 1901a. Australian Psyllidae. Part I. *Proceedings of the Linnean Society of New South Wales* **25**, 250–302; plates 11–14.
- Froggatt WW. 1901b. Australian Psyllidae. Part II. *Proceedings of the Linnean Society of New South Wales* **26**, 242–298; plates 14–16.
- Froggatt WW. 1903. Australian Psyllidae. Part III. *Proceedings of the Linnean Society of New South Wales* **28**, 315–337; plates 4–5.

- Froggatt WW. 1907. *Australian Insects*. William Brooks & Company, Limited, Sydney 1907, 361–367.
- Froggatt WW. 1909. *Report on parasitic and injurious insects*. WA Gullick, Government Printer, Sydney, 115 pp.
- Froggatt WW. 1923. *Forest Insects of Australia*. AJ Kent, Government Printer, Sydney, 146–148.
- Fuentes-Contreras E & Niemeyer HM. 1988. DIMBOA glucoside, a wheat chemical defense, affects host acceptance and suitability of *Sitobion avenae* to the cereal aphid parasitoid *Aphidius rhopalosiphi*. *Journal of Chemical Ecology* **24**(2), 371–381.
- Gianoli E & Niemeyer HM. 1997. Characteristics of hydroxamic acid induction in wheat triggered by aphid infestation. *Journal of Chemical Ecology* **23**(12), 2695–2705.
- Gianoli E, Rios JM & Niemeyer HM. 1999. Within-plant allocation of a chemical defense in *Secale cereale*. Is concentration the appropriate currency of allocation? *Chemoecology* **9**(3), 113–117.
- Gibbs RD. 1974. *Chemotaxonomy of Flowering Plants*. McGill-Queen's Univ. Press, Montreal and London.
- Gilby AR, McKellar JW & Beaton CD. 1976. The structure of lerps: carbohydrate, lipid, and protein components. *Journal of Insect Physiology* **22**(5), 689–696.
- Givovich A & Niemeyer HM. 1995. Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. *Entomologia Experimentalis et Applicata* **74**(2), 115–119.
- Gilbert, LE. 1975. In *Coevolution of Animals and Plants*. Gilbert LE and Raven PH (Eds), pp. 210–240. Univ. Texas Press, Austin and London.

- Gillespie PS, Mound LA & Wang C-L. 2002. Austral-oriental genus *ParabaliOTHrips* Priesner (Thysanoptera: Thripidae), with a new Australian species forming male aggregations. *Australian Journal of Entomology* **41**, 111–17.
- Glasser M. 1967. Exponential survival with covariance. *Journal of the American Statistical Association* **62**, 561–568.
- Gonzales WL, Ramirez CC, Olea N & Niemeyer HM. 2002. Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth. *Entomologia Experimentalis et Applicata* **103**(2), 107–113.
- Govindjee and Papageorgiou G. 1971. Chlorophyll Fluorescence and Photosynthesis: Fluorescence Transients. In *Photophysiology*. Giese AC (Ed.), Academic Press, New York, NY, USA. 6, 1–46.
- Grbic M, Rivers D & Strand MR. 1997. Caste formation in the polyembryonic wasp *Copidosoma floridanum* (Hymenoptera: Encyrtidae): *in vivo* and *in vitro* analysis. *Journal of Insect Physiology* **43**(6), 553–565.
- Grbic M, Nagy LM & Strand MR. 1998. Development of polyembryonic insects: a major departure from typical insect embryogenesis. *Development Genes & Evolution* **208**(2), 69–81.
- Grbic M & Strand MR. 1991. Intersexual variation in the precocious larvae of the polyembryonic wasp *Copidosoma floridanum*. *Colloques de L'Inra* **56**, 25–28.
- Grbic M, Ode PJ & Strand MR. 1992. Sibling rivalry and brood sex ratios in polyembryonic wasps. *Nature* **360**(6401), 254–256.
- Greenaway P & Linton SM. 1995. Dietary assimilation and food retention time in the herbivorous terrestrial crab *Geracoidea natalis*. *Physiological Zoology* **68**(6), 1006–1028.

- Grove AJ & Ghosh CC. 1914. The life history of *Psylla isitis* Buckt. (*Psyllopa punctipennis*, Crawford): The "Psylla" disease of Indigo. *Memoirs of the Department of Agriculture in India Entomological Series, Vol. IV, No. 6*. Thacker, Spink & Co., Calcutta, 329–357.
- Guo SW & Lin DY. 1994. Regression analysis of multivariate grouped survival data. *Biometrics* **50**(3), 632–639.
- Gupta HK & Balaraman N. 1992. Tannins in relation to top feeds of eastern Himalayan region. *Indian Journal of Animal Nutrition* **9**(1), 55–58.
- Halperin J. 1990. Mass breeding of egg parasitoids (Hymenoptera: Chalcidoidea) of *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Thaumetopoeidae). *Journal of Applied Entomology* **109**(4), 336–340.
- Harden GJ. 1990. Moraceae. In *The Flora of New South Wales, Volume 3*. NSW University Press, Kensington, N.S.W. 4 volumes: illustrated, 342–347.
- Hardy ICW. 1996. Precocious larvae in the polyembryonic parasitoid *Copidosoma sosares* (Hymenoptera: Encyrtidae). *Entomologische Berichten* **56**(5), 88–92.
- Hardy ICW, Ode PJ & Strand MR. 1993. Factors influencing brood sex ratios in polyembryonic Hymenoptera. *Oecologia* **93**(3), 343–348.
- Harvey JA, Corley LS & Strand MR. 2000. Competition induces adaptive shifts in caste ratios of a polyembryonic wasp. *Nature* **406**(6792), 183–186.
- Hegazy AK, Amer WM & Khedr AA. 2001. Allelopathic effect of *Nymphaea lotus* L. on growth and yield of cultivated rice around Lake Manzala (Nile Delta). *Hydrobiologia* **464**, 133–142.
- Heinlein RA. 1966. *The moon is a harsh mistress*. New English Library, London, UK.

- Hemptinne JL, Dixon AFG & Adam B. 2001. Do Males and Females of the Two-Spot Ladybird, *Adalia bipunctata* (L.), Differ in When They Mature Sexually? *Journal of Insect Behavior* **14**(3), 411–419.
- Herrmann KM. 1995a. The shikimate pathway as an entry to aromatic secondary metabolism. *Plant Physiology* **107**(1), 7–12.
- Herrmann KM. 1995b. The shikimate pathway: early steps in the biosynthesis of aromatic compounds. *Plant Cell* **7**(7), 907–919.
- Herrmann KM, Zhao J, Pinto JEBP, Weaver L & Henstrand JM. 1992. Regulation of carbon flow into the shikimate pathway. *Current Topics in Plant Physiology* **7**, 12–18.
- Herrmann, KM & Weaver, LM. 1999. The shikimate pathway. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 473–503.
- Hetherington SE, Smillie RM, Hardacre AK & Eagles HA. 1983. Using chlorophyll fluorescence *in vivo* to measure the chilling tolerances of different populations of maize. *Australian Journal of Plant Physiology* **10**, 247–256.
- Hilker M & Dicke M. 2003. Induced plant responses towards herbivory. *Basic and Applied Ecology* **4**, 1–2.
- Hill R & Bendall F. 1960. Function of the two cytochrome components in chloroplasts: a working hypothesis.. *Nature* **186**, 136–137.
- Hodge S, Keesing VF & Wratten SD. 2000. Leaf damage does not affect leaf loss or chlorophyll content in the New Zealand pepper tree, kawakawa (*Macropiper excelsum*). *New Zealand Journal of Ecology* **24**(1), 87–89.

- Hodkinson ID. 1974. The biology of the Psylloidea (Homoptera): a review. *Bulletin of Entomological Research* **64**, 325–339.
- Hollis D & Broomfield PS. 1989. Ficus-feeding psyllids (Homoptera), with special reference to the Homotomidae. *Bulletin of the British Museum of Natural History (Ent.)* **58(2)**, 131–83.
- Honda K & Hayashi N. 1995. A flavonoid glucoside, phellamurin, regulates differential oviposition on a rutaceous plant, *Phellodendron amurense*, by two sympatric swallowtail butterflies, *Papilio protenor* and *P. xuthus*: The front line of a coevolutionary arms race. *Journal of Chemical Ecology* **21(10)**, 1513–1539.
- Hori K & Miles PW. 1993. The etiology of damage to lucerne by the green mirid, *Creontiades dilutus* (Stål). *Australian Journal of Experimental Agriculture* **33(3)**, 327–331.
- Hornung M & Reynolds B. 1995. The effects of natural and anthropogenic environmental changes on ecosystem processes at the catchment scale. *Trends in Ecology and Evolution* **10(11)**, 443–449.
- Horton DR, Broers DA, Hinojosa T & Lewis TM. 1998. Ovarian development in overwintering pear psylla, *Cacopsylla pyricola* (Homoptera: Psyllidae): Seasonality and effects of photoperiod. *Canadian Entomologist* **130(6)**, 859–867.
- Horton DR & Lewis TM. 1996. Tethered flight activity of pear psylla, *Cacopsylla pyricola*: Seasonal, host, and morphotypic effects. *Entomologia Experimentalis et Applicata* **78(1)**, 39–49.
- Inoue T, Yumoto, T, Hamid AA, Seng LH & Ogino, 1995. K. Construction of a canopy observation system in a tropical rainforest of Sarawak. *Selbyana* **16(1)**, 24–35.
- Ito Y. 1998. Role of escape from predators in periodical cicada (Homoptera: Cicadidae) cycles. *Annals of the Entomological Society of America* **91(5)**, 493–496.

- Ivanova-Kasas OM. 1972. Polyembryony in insects. In *Developmental Systems: Insects*, vol. 2. Counce SJ & Waddington CH (Eds), Academic Press, London. 234–271
- Jaksic FM. 1998. The multiple facets of *El Niño*/Southern oscillation in Chile. *Revista Chilena de Historia Natural* **71**(2), 121–131.
- Jaksic FM. 2001. Ecological effects of *El Niño* in terrestrial ecosystems of western South America. *Ecography* **24**(3), 241–250.
- Janzen DH. 1968. Host plants as islands in evolutionary and contemporary time. *American Naturalist* **102**, 592–595.
- Janzen DH. 1987. Insect diversity of a Costa Rican dry forest: Why keep it and how? *Biological Journal of the Linnean Society* **30**(4), 343–356.
- Jayaraj S, Mohanasundaram M, Parameswaran S & Pillayarsamy K. 1989. Occurrence of *Mycopsylla fici* (Tryon) (Psyllidae: Homoptera) as a pest of *Ficus amplissima* (Smith) in Coimbatore. *South Indian Journal of Horticulture* **37**(4), 253.
- Jayaraman KS. 2001. Illicit GM cotton sparks corporate fury. *Nature* **413**(6856), 555, October 11, 2001.
- Jervis M, Ferns PN & Heimpel GE. 2003. Body size and timing of egg production in parasitoid wasps: a comparative analysis. *Functional Ecology* **17**, 375–383.
- Jones MJ & Crowley JA 1989. A general class of nonparametric tests for survival analysis. *Biometrics* **45**(1), 157–170.
- Joshi, L. 1991. The leucaena psyllid; an experience from Nepal. *PAC Occasional Paper No. 9*. Pakhribas Agricultural Centre. v+10 pp.

- Journet ARP. 1979. The water status of *Eucalyptus blakelyi* M. under forest and woodland field conditions in Australia. *Annals of Botany* **44**(1), 125–228.
- Journet ARP. 1980. Intraspecific variation in food plant favourability to phytophagous insects: psyllids on *Eucalyptus blakelyi* M. *Ecological Entomology* **5**(3), 249–261.
- Journet ARP. 1981. Insect herbivory on the Australian woodland eucalypt, *Eucalyptus blakelyi* M. *Australian Journal of Ecology* **6**(2), 135–138.
- Kalra RL, Kaur H, Sharma S, Kapoor SK, Chakraborty SS, Kshirasagar RB, Vaidya RC, Sagade RB, Shirolkar SB, Dikshith TSS, Raizada RB, Srivastava MK, Singh V, Nagarja KV, Appaiah KM, Srinivasa MA, Usha RM, Rama RSN, Toteja GS, Dasgupta J, Ghosh PK & Saxena BN. 1999. DDT and HCH residues in dairy milk samples collected from different geographical regions of India: A multicentre study. *Food Additives & Contaminants* **16**(10), 411–417.
- Kalra RL, Singh B & Battu RS. 1994. Organochlorine pesticide residues in human milk in Punjab, India. *Environmental Pollution* **85**(2), 147–151.
- Kapatos ET & Stratopoulou ET. 1996. Demographic study of the reproductive potential of pear psylla, *Cacopsylla pyri*. *Entomologia Experimentalis et Applicata* **80**(3), 497–502.
- Kaplan EL & Meier P. 1958. Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association* **53**, 457–481.
- Karim S, Nasreen Z, Malik K & Riazuddin S. 1998. A simple and fast method for mass rearing of sweet potato whitefly (*Bemisia tabaci*) and cotton aphid (*Aphis gossypii*) in contained conditions. *Asia Life Sciences* **7**(1), 103–108.
- Katsoulas N, Baille A & Kittas C. 2001a. Effect of misting on transpiration and conductances of a greenhouse rose canopy. *Agricultural & Forest Meteorology* **106**(3), 233–247.

- Katsoulas N, Kittas C & Baille A. 2001b. Estimating transpiration rate and canopy resistance of a rose crop in a fan-ventilated greenhouse. *Acta Horticulturae* **548**, 303-309.
- Kato-Noguchi H, Kosemura S, Yamamura S, Mizutani J & Hasegawa K. 1994a. Allelopathy of oats. I. Assessment of allelopathic potential of extract of oat shoots and identification of an allelochemical. *Journal of Chemical Ecology* **20**(2), 309-314.
- Kato-Noguchi H, Mizutani J & Hasegawa K. Allelopathy of oats. 1994b. II. Allelochemical effect of *L*-tryptophan and its concentration in oat root exudates. *Journal of Chemical Ecology* **20**(2), 315-319.
- Kato-Noguchi H. 2001. Effects of lemon balm (*Melissa officinalis* L.) extract on germination and seedling growth of six plants. *Acta Physiologiae Plantarum* **23**(1), 49-53.
- Katsoulas N, Baille A & Kittas C. 2001. Effect of misting on transpiration and conductances of a greenhouse rose canopy. *Agricultural & Forest Meteorology* **106**(3), 233-247
- Kelley RB, Seiber JN, Jones AD, Segall HJ & Brower LP. 1987. Pyrrolizidine alkaloids in overwintering monarch butterflies *Danaus plexippus* from Mexico. *Experientia (Basel)* **43**(8), 943-946.
- Khatta VK, Tewatia BS & Gupta PC. 1999. Nutritional evaluation of tree leaves of humid subtropical region of Himachal Pradesh. *Indian Journal of Animal Nutrition* **16**(3), 220-223.
- Kim MY & Xue, X. 2002. The analysis of multivariate interval-censored survival data. *Statistics in Medicine* **21**(23), 3715-3726.
- Kishor NM. 1992. Pesticide Externalities, Comparative Advantage, and Commodity Trade; Cotton in Andhra Pradesh, India. *Report written for the World Bank, Country Economics Department*. World Bank, Washington, DC, U.S.A.

- Klein K & Blum U. 1990. Inhibition of cucumber leaf expansion by ferulic acid in split-root experiments. *Journal of Chemical Ecology* **16**(2), 455–463.
- Knipling EF. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology* **48**, 902–904.
- Knipling EF. 1985. Sterile insect technique as a screwworm control measure: The concept and its development. *Symposium on eradication of the screwworm from the United States and Mexico. Miscellaneous Publications of The Entomological Society of America* **62**, 4–7.
- Komae K, Komae A & Misaki A. 1990. A 4,5-unsaturated low molecular oligogalacturonide as a potent phytoalexin-elicitor isolated from polygalacturonide of *Ficus awkeotsang*. *Agricultural & Biological Chemistry* **54**(6), 1477–1484.
- van Kouten O & Snel JFH. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research* **25**, 147–150.
- Krause GH. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum* **74**, 566–574.
- Kuno E. 1991. Sampling and analysis of insect populations. *Annual Review of Entomology* **36**, 285–304.
- Laing JE & Eden GM. 1990. Inundative release of the egg parasitoid *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) against forest insect pests such as the Spruce Budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae): The Ontario Project 1982-1986 2.0. Mass production of *Trichogramma minutum* Riley on factitious host eggs. *Memoirs of the Entomological Society of Canada* **153**, 10–24.

- Lancelot R, Lesnoff M & McDermott JJ. 2002. Use of Akaike information criteria for model selection and inference. An application to assess prevention of gastrointestinal parasitism and respiratory mortality of Guinean goats in Kolda, Senegal. *Preventive Veterinary Medicine* **55**(4), 217–240.
- Laughlin R. 1965. Capacity for increase: a useful population statistic. *Journal of Animal Ecology* **34**, 77–91.
- Lee J, Yoshizawa C, Wilkens L, & Lee HP. 1992. Covariance adjustment of survival curves based on Cox's Proportional Hazards regression model. *Computer Applications in the Biosciences* **8**(1), 23–27.
- Lee RE(Jr), Litzgus JD, Mugnano JA, Lee MR, Horton DR & Dunley J. 1999. Evaluation of ice-nucleating microorganisms for reducing the supercooling capacity and cold-hardiness of *Cacopsylla pyricola* (Hemiptera: Psyllidae). *Canadian Entomologist* **131**(6), 715–723.
- Leszczynski B, Wright LC & Bakowski T. 1989. Effect of secondary plant substances on winter wheat resistance to grain aphid. *Entomologia Experimentalis et Applicata* **52**(2), 135–140.
- Leverenz JW & Öquist G. 1987. Quantum yields of photosynthesis at temperatures between -2 and 35 in a cold tolerant C3 plant (*Pinus sylvestris*) during the course of one year. *Plant, Cell and Environment* **10**, 287–295.
- Levitt, J. 1972. *Responses of Plants to Environmental Stresses*. Academic Press, Inc., New York and London.
- Levitt, J. 1980. *Responses of Plants to Environmental Stresses, 2nd Edition, Vol. I and II*. Academic Press, Inc., New York and London.

- Li D, Zhang C; Su H & Xu G. 1992. Studies on the biological characters of the Chinese pear sucker *Psylla chinensis*. *Forest Research* 5(3), 278–283.
- Li D, Zhang C, Su H & Xu G. 1994. Studies on the damage and control of *Psylla chinensis*. *Forest Research* 7(6), 666–676.
- Lichtenthaler HK. 1988. Applications of chlorophyll fluorescence. In *Photosynthesis research, stress physiology, hydrobiology, and remote sensing*. Lichtenthaler HK (Ed.), Kluwer Academic Publishers. Dordrecht, Netherlands; Boston USA. x, 366 p.: ill.
- Lichtenthaler HK. 1996. Vegetation stress: An introduction to the stress concept in plants. *Journal of Plant Physiology* 148, 4–14.
- Lichtenthaler HK & Miehe JA. 1997. Fluorescence imaging as a diagnostic tool for plant stress. *Trends in Plant Science* 2(8), 315–320.
- Lieberman M. 1979. Biosynthesis and of action of ethylene. *Annual Review of Plant Physiology* 30, 533–589.
- Limburg DD & Rosenheim JA. 2001. Extrafloral nectar consumption and its influence on survival and development of an omnivorous predator, larval *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). *Environmental Entomology* 30(3), 595–604.
- Lindquist AW. 1955. The use of gamma radiation for control or eradication of the screwworm. *Journal of Economic Entomology* 48, 467–469.
- Liu YH & Tsai JH. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera : Psyllidae). *Annals of Applied Biology* 137(3), 201–206.

- Losada M. 1992. Sequential energy transduction in photosynthesis. In *Trends in Photosynthesis Research*. Barber J, Guerrero MG & Medrano H (Eds), 87–103.
- Lowman MD, Kitching RL & Carruthers G. 1996. Arthropod sampling in Australian subtropical rain forests – how accurate are some of the more common techniques? *Selbyana* 17, 36–42.
- Lowman MD & Wittman PK. 1996. Forest canopies: Methods, hypotheses, and future directions. *Annual Review of Ecology and Systematics* 27, 55–81.
- Lu C & Zhang J. 1998. Effects of water stress on photosynthesis, chlorophyll fluorescence and photoinhibition in wheat plants. *Australian Journal of Plant Physiology* 25, 883–892.
- Luck RF, van den Bosch R & Garcia R. 1977. Chemical insect control: A troubled pest management strategy. *Bioscience* 27(9), 606–612.
- Ludlow MM & Powles SB. 1988. Effects of photoinhibition induced by water stress on growth and yield of grain sorghum. *Australian Journal of Plant Physiology* 15, 179–194.
- Luft PA, Paine TD & Walker GP. 2001. Interactions of colonisation density and leaf environments on survival of *Trioza eugeniae* nymphs. *Ecological Entomology* 26(3), 263–270.
- Lym RG. 1992. Propagation of *Euphorbia esula* for leafy spurge biocontrol agents. *Weed Science* 40(2), 326–332.
- MacArthur RH and Wilson EO. 1967. *The theory of island biogeography*. Princeton University Press, Princeton, New Jersey, USA. 203 pp.
- Mackauer M. 1996. Sexual size dimorphism in solitary parasitoid wasps: influence of host quality. *Oikos* 76(2), 265–272.

- McCauley SW, Melis A, Tang G M-S & Arnon DI. 1987. Protonophores induce plastoquinol oxidation and quench chloroplast fluorescence: Evidence for a cyclic proton-conducting pathway in oxygenic photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **84**(23), 8424–8428.
- McFarland CD & Hoy MA. 2001. Survival of *Diaphorina citri* (Homoptera : Psyllidae), and its two parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), under different relative humidities and temperature regimes. *Florida Entomologist* **84**(2), 227–233.
- MacNally RC. 1988. On the statistical significance of the Hutchinson size-ratio parameter. *Ecology* **96**(6), 1974–1982.
- Madden JL & Stone C. 1984. Induction and formation of pouch and emergence galls in *Eucalyptus pulchella*. *Australian Journal of Botany* **32**(1), 33–42.
- Maddox GD & Cappuccino N. 1986. Genetic determination of plant susceptibility to an herbivorous insect depends on environmental context. *Evolution* **40**, 863–866.
- Maddox GD & Root RB. 1987. Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia* **72**, 8–14.
- Malcolm SB & Zalucki MP. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomologia Experimentalis et Applicata* **80**(1), 193–196.
- Malcolm SB, Cockrell BJ & Brower LP. 1989. Cardenolide fingerprint of Monarch Butterflies reared on common milkweed (*Asclepias syriaca* L.). *Journal of Chemical Ecology* **15**(3), 819–854.

- Manickavasagam S & Kanagarajan R. 2003. Host and brood size of an egg-larval polyembryonic parasitoid, *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae) on *Helicoverpa armigera* (Hübner) from India. *Journal of Biological Control* **17**(1), 85–86.
- Mansour MH. 1984. Antharufin as an allelochemic affecting the larval development and utilization of food of the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Bulletin de la Société Entomologique D’Egypte 1980–1981, publ. 1984* **63**, 89–93.
- Marks EN. 1991. Chapter 8: Biographical History. In *The Insects of Australia: a Textbook for Students and Research Workers, 2nd Edition*. The Division of Entomology, Commonwealth Scientific and Industrial Research Organization. Melbourne University Press, Carlton South, Victoria, 205–206.
- Marshall DC. 2001. Periodical cicada (Homoptera: Cicadidae) life-cycle variations, the historical emergence record, and the geographic stability of brood distributions. *Annals of the Entomological Society of America* **94**(3), 386–399.
- Marshall DC & Cooley JR. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. *Evolution* **54**(4), 1313–1325.
- Martin RA & Lynch SP. 1988. Cardenolide content and thin-layer chromatography profiles of monarch butterflies *Danaus plexippus* L. and their larval host-plant milkweed *Asclepias asperula* ssp. *capricornu* Woods in north central Texas USA. *Journal of Chemical Ecology* **14**(1), 295–318.
- Mata MM, Matthews MA, Paw-u KT & Snyder RL. 1992. A field misting system for reducing canopy temperatures. *American Journal of Enology & Viticulture* **43**(3), 311.
- Matile P, Hörtensteiner S & Thomas H. 1999. Chlorophyll degradation. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 67–95.

- Matsuda H & Abrams P. 1994. Plant-herbivore interactions and theory of coevolution. *Plant Species Biology* **9**(3), 155–161.
- Mayr E. 1963. *Animal species and evolution*. Belknap Press, imprint of Harvard University Press, Cambridge, Massachusetts. 811 pp.
- Meats A & Fay HAC. 2000. Distribution of mating frequency among males of the Queensland fruit fly, *Bactrocera tryoni*, in relation to temperature, acclimation and chance. *General and Applied Entomology* **29**, 7–30.
- Meats A. 1983. Strategies for maximizing the potential of the sterile insect release method experiments with *Dacus tryoni*. In *Fruit Flies of Economic Importance; Proceedings of the CEC/IOBC International Symposium, Athens, Greece, Nov. 16–19, 1982*. Cavalloro R (Ed.), fxii+642p. Illus. AAA Balkema, Rotterdam, Netherlands, 371–377.
- Memmott J, Godfray HCJ & Gault ID. 1994. The structure of a tropical host-parasitoid community. *Journal of Animal Ecology* **63**, 521–540.
- Mensah RK & Madden JL. 1991a. Resistance and susceptibility of *Boronia megastigma* cultivars to infestations by the psyllid *Ctenarytaina thysanura*. *Entomologia Experimentalis et Applicata* **61**, 189–198.
- Mensah RK & Madden JL. 1991b. Technique for mass rearing *Ctenarytaina thysanura* (Ferris and Klyver) (Hemiptera: Psyllidae). *Journal of the Australian Entomological Society* **30**, 267–268.
- Mensah RK & Madden JL. 1992. Field studies on colour preferences of *Ctenarytaina thysanura* in Tasmanian boronia farms. *Entomologia Experimentalis et Applicata* **64**, 111–115.

- Mensah RK & Madden JL. 1993a. Life history and biology of *Ctenarytaina thysanura* Ferris and Klyver (Hemiptera: Psyllidae) on *Boronia megastigma* Nees ex Bartl. (Rutaceae) in Tasmania. *Journal of the Australian Entomological Society* **32**(4), 327–337.
- Mensah RK & Madden JL. 1993b. Development and application of an integrated pest management programme for the psyllid, *Ctenarytaina thysanura* on *Boronia megastigma* in Tasmania. *Entomologia Experimentalis et Applicata* **66**(1), 59–74.
- Mensah RK & Madden JL. 1994. Life table and key factor studies of *Ctenarytaina thysanura* Ferris and Klyver (Hemiptera: Psyllidae) on *Boronia megastigma* Nees. ex Bartl. (Rutaceae) in Tasmania. *Journal of the Australian Entomological Society* **33**(4), 353–361.
- Messenger PS, Biliotti E & Van Den Bosch R. 1976. The importance of natural enemies in integrated control. In *Theory and Practice of Biological Control* Huffaker CB & Messenger PS (Eds), xxi+788p. Illus. Academic Press New York, NY, USA & London, England, 543–563.
- Metcalf RL & Luckman WH. 1994. *Introduction to insect pest management 3rd edition*. Wiley, New York, NY, USA. xiii, 650 p. : ill., maps.
- Michener CD & Houston TF. 1991. Chapter 42: Hymenoptera. (Naumann ID ,Ed.) In *The Insects of Australia: a Textbook for Students and Research Workers, 2nd Edition*. The Division of Entomology, Commonwealth Scientific and Industrial Research Organization. 993–1000. Melbourne University Press, Carlton South, Victoria.
- Milbrath LR, Tauber MJ & Tauber CA. 1993. Prey specificity in *Chrysopa*: An interspecific comparison of larval feeding and defensive behavior. *Ecology* **74**(5), 1384–1393.
- Miles PW. 1999. Aphid saliva. *Biological Reviews (Cambridge)* **74**(1), 41–85.

- Miles PW & Oertli JJ. 1993. The significance of antioxidants in the aphid–plant interaction: The redox hypothesis. *Entomologia Experimentalis et Applicata* **67**(3), 275–283.
- Milevoj L & Kroselj M. 1995. Effects of some insecticides on pear psylla (*Cacopsylla pyri* L.) in laboratory conditions. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent* **60**(3B), 1023–1026.
- Misirli A, Gulcan R and Topuz F. 1998. Determination of phenolic compounds in male and female figs. In *Proceedings of the First International Symposium on Fig, Izmir, Turkey, 24–28 June 1997. Acta Horticulturae* **480**, 253–258.
- Miyatake Y. 1994. Further knowledge on the distribution and biology of two species of the genus *Celtisaspis* (Homoptera: Psylloidea: Spondyliaspidae). *Bulletin of the Osaka Museum of Natural History* **0**(48), 27–30.
- Mopper S, Mitton JB, Whitham TG, Cobb NS & Christensen KM. 1991. Genetic differentiation and heterozygosity in pinyon pine associated with herbivory and environmental stress. *Evolution* **45**, 989–999.
- Mopper S & Simberloff D. 1995. Differential herbivory in an oak population: the role of plant phenology and insect performance. *Ecology* **76**(4), 1233–1241.
- Morgan, FD. 1984. *The Psylloidea of South Australia*. In series *Handbook of the Flora and Fauna of South Australia*. SA Government Printer. 136 pp.
- Mohammed GH, Binder WD & Gilles SL. 1995. Chlorophyll fluorescence: a review of its practical forestry applications and instrumentation. *Scandinavian Journal of Forest Research* **10**, 383–410.

- Morallo-Rejesus B, Buctuanon EM & Rejesus RS. 1990. Defining the economic threshold determinants for the Asian corn borer, *Ostrinia furnacalis* (Guenée) in the Philippines. *Tropical Pest Management* **36**(2), 114–121.
- Moreno-Murillo B, Arango-Bonilla, AI, Gonzalez-Gonzalez J & Niemeyer HM. 1995. Studies of the potential insecticide activity of some native species. 1. Evaluation of extracts of *Brunfelsia pauciflora* (H.B.K.). *Revista Colombiana de Entomologia* **21**(1), 45–50.
- Morris RF & Fulton WC. 1970. Models for the development and survival of *Hyphantria cunea* in relation to temperature and humidity. *The Memoirs of the Entomological Society of Canada* **70**, 1–60.
- Morse DR, Stork NE & Lawton JH. 1988. Species number, species abundance and body length relationships of arboreal beetles in Bornean lowland rain forest trees. *Ecological Entomology* **13**, 25–37.
- Mound LA. 1971. The feeding apparatus of thrips. *Bulletin of Entomological Research* **60**, 547–548.
- Mound LA & Kibby G. 1998. *Thysanoptera: an identification guide*, 2nd edition. CAB International, Wallingford, Oxford, UK & New York, NY, USA. vi+70 p. ill.
- Mowry, T.M. 2001. Green peach aphid (Homoptera: Aphididae) action thresholds for controlling the spread of potato leafroll virus in Idaho. *Journal of Economic Entomology* **94**(6), 1332–1339.
- Muller WH. 1986. Allelochemical mechanisms in the inhibition of herbs by chaparral shrubs. In *The Science of Allelopathy*. John Wiley & Sons Inc., New York, USA. p. 189–199.

- Mulrooney JE, Hedin PA, Parrott WL & Jenkins JN. 1985. Effects of PIX, a plant growth regulator, on allelochemical content of cotton and growth of tobacco budworm larvae (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **78**(5), 1100–1104.
- Mullen BF, Gabunada F, Shelton HM, Stur WW & Napompeth B. 1998. Psyllid resistance in *Leucaena*. *ACIAR Proceedings Series* **86**, 51–60.
- Mullen BF, Gabunada F, Shelton HM & Stur WW. 2003a. Agronomic evaluation of *Leucaena*. Part 2. Productivity of the genus for forage production in subtropical Australia and humid-tropical Philippines. *Agroforestry Systems* **58**(2), 93–107.
- Mullen BF, Gabunada F, Shelton HM & Stur WW. 2003b. Psyllid resistance in *Leucaena*. Part 1. Genetic resistance in subtropical Australia and humid-tropical Philippines. *Agroforestry Systems* **58**(3), 149–161.
- Mullen BF & Shelton HM. 2003. Psyllid resistance in *Leucaena*. Part 2. Quantification of production losses from psyllid damage. *Agroforestry Systems* **58**(3), 163–171.
- Mullen BF, Shelton HM, Gutteridge RC & Basford KE. 2003. Agronomic evaluation of *Leucaena*. Part 1. Adaptation to environmental challenges in multi-environment trials. *Agroforestry Systems* **58**(2), 77–92.
- Muller C. 2002. Variation in the effectiveness of abdominal shields of cassidine larvae against predators. *Entomologia Experimentalis et Applicata* **102** (2), 191–198.
- Nakahara LM & Funasaki GY. 1987. Release of *Psyllaephagus* sp. nr. *rotundiformis* (Howard) (Hymenoptera: Encyrtidae) for the biological control of *Heteropsylla cubana* Crawford (Homoptera: Psyllidae) and other species in Hawaii. *Leucaena Research Reports* **8**, 11–13.

- Nagamine W, Matayoshi S, Kumashiro B & Nakahara LM. 1987. Biological control program of the leucaena psyllid *Heteropsylla cubana* Crawford (Homoptera: Psyllidae) in Hawaii. Proceedings of a workshop on biological and genetic control strategies for the leucaena psyllid, November 3–7 1986. *Leucaena Research Reports* 7(2), Withington D & Brewbaker JL (Eds), Mokakai and Honolulu, Hawaii, 39–44.
- Nelson CJ. 1993. A model for cardenolide and cardenolide glycoside storage by the monarch butterfly. *Natural History Museum of Los Angeles County Science Series* 0(38), 83–90.
- Nenon JP. 1976. Variations in the rate of polyembryony of the parasitoid *Ageniaspis fuscicollis* Dalm. (Hymenoptera, Encyrtidae) according to the temperature at which its hosts develop. *Annales de Zoologie Ecologie Animale* 8(2), 161–175.
- Nenon JP. 1978. Modulation of the polyembryonic rate of *Ageniaspis fuscicollis* Dalm. (Hymenoptera, Encyrtidae) according to the hosts chosen for development. *Annales de Zoologie Ecologie Animale* 10, 441–442.
- Nicol CMY & Mackauer M. 1999. The scaling of body size and mass in a host- parasitoid association: Influence of host species and stage. *Entomologia Experimentalis et Applicata* 90(1), 83–92.
- Niemeyer HM. 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. *Phytochemistry* 27(11), 3349–3358.
- Niemeyer HM. 1992. Ecological and molecular aspects of the interaction between aphids and their host plants. *Revista Chilena de Historia Natural* 65(1), 103–114.
- Niemeyer HM. 1995. Biologically active compounds from Chilean medicinal plants. In *Phytochemistry of medicinal plants. Recent advances in phytochemistry* 29, 137–159.

- Niemeyer HM, Copaja SV & Barria BN. 1992. The Triticeae as sources of hydroxamic acids: Secondary metabolites in wheat conferring resistance against aphids. *Hereditas (Lund)* **116**(3), 295–299.
- Noyes JS. 1989. *Copidosoma graminis* sp. n. (Hymenoptera: Encyrtidae), a polyembryonic parasitoid of armyworms (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research* **79**(3), 367–371.
- Noyes JS. 1990. A new encyrtid (Hymenoptera) parasitoid of the leucaena psyllid (Homoptera: Psyllidae) from Mexico, Central America and the Caribbean. *Bulletin of Entomological Research* **80**(1), 37–42.
- Ode PJ & Strand MR. 1995. Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. *Journal of Animal Ecology* **64**, 213–224.
- Öquist G, Chow WS & Anderson JM. 1992. Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of Photosystem II. *Planta* **186**, 450–460.
- Öquist G & Wass R. 1988. A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. *Physiologia Plantarum* **73**, 211–217.
- Olkowski W, Olkowski H, van den Bosch R & Hom R. 1976. Ecosystem management: A framework for urban pest control. *Bioscience* **26**(6), 384–389.
- Olkowski W, Olkowski H, Kaplan AI & van den Bosch R. 1978. The potential for biological control in urban areas: shade tree insect pests. In *Perspectives in Urban Entomology. Symposium held at the XV International Congress of Entomology*. Washington, DC, USA. 1976. Frankie GW & Koehler CS (Eds), xvi+417pp. Illus. Maps. Academic Press, Inc., New York, NY, USA, 311–347.

- Ossipov V, Salminen J-P, Ossipova S, Haukioja E & Pihlaja K. 2003. Gallic acid and hydrolysable tannins are formed in birch leaves from an intermediate compound of the shikimate pathway. *Biochemical Systematics & Ecology* **31**(1), 3–16.
- Paarmann W & Stork NE. 1987. Canopy fogging, a method of collecting living insects for investigations of life history strategies. *Journal of Natural History* **21**, 563–566.
- Pan W & Chappell R. 2002. Estimation in the Cox proportional hazards model with left-truncated and interval-censored data. *Biometrics* **58**(1), 64–70.
- Papageorgiou, G. 1975. Chlorophyll fluorescence: An intrinsic probe of photosynthesis. In *Bioenergetics of Photosynthesis*. Govindjee (Ed.), Academic Press, New York, USA, 319–371.
- Patil NG, Baker PS & Pollard GV. 1994. Life history parameters of the leucaena psyllid *Heteropsylla cubana* (Crawford) (Homoptera: Psyllidae) under various temperature and relative humidity regimes. *Insect Science and its Application* **15**(3), 293–299.
- van Pelt R & North MP. 1999. Testing a ground-based canopy model using the Wind River canopy crane. *Selbyana* **20**, 357–362.
- Persons MH & Uetz GW. 1999. Age and Sex-Based Differences in the Use of Prey Sensory Cues in Wolf Spiders (Araneae: Lycosidae). *Journal of Insect Behavior* **12**(6), 723–736.
- Petersen J & Adolph SC. 2001. Lizards in a fluctuating environment: A stochastic population model for the desert night lizard *Xantusia vigilis*. *American Zoologist* **41**(6), 1553.
- Pianka ER. 1970. On *r*- and *K*-selection. *American Naturalist* **104**, 592–597.
- Pianka ER. 1972. *r*- and *K*-selection, or *b*- and *d*-selection? *American Naturalist* **106**, 581–588.

- Prentice RL & Hsu L. 1997. Regression on hazard ratios and cross ratios in multivariate failure time analysis. *Biometrika* **84**(2), 349–363.
- Press Trust of India. 1997. Pesticide use in India trebled in 8 years. In *The Financial Express*, Monday, December 08 1997. Indian Express Newspapers (Bombay) Ltd. Bombay, India. Published on the Internet.
- Price, PW. 1997. *Insect Ecology, 3rd Edition*. John Wiley & Sons, Inc., New York, NY, USA. 874 pp.
- Puterka GJ. 1997. Intraspecific variation in pear psylla (Psyllidae: Homoptera): nymphal survival and development on resistant and susceptible pear. *Environmental Entomology* **26**(3), 552–558.
- Ramirez CC & Niemeyer HM. 1999. Salivation into sieve elements in relation to plant chemistry: the case of the aphid *Sitobion fragariae* and the wheat, *Triticum aestivum*. *Entomologia Experimentalis et Applicata* **91**(1), 111–114.
- Reeve JP & Fairbairn DJ. 1999. Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* **83**(6), 697–706.
- Reeve JD, Cronin JT, Strong, DR. 1994. Parasitoid aggregation and the stabilization of a salt marsh host-parasitoid system. *Ecology* **75**(2), 288–295.
- Reis R (Jr), Lima ER, Vilela EF & Barros RS. 2000. Method for maintenance of coffee leaves in vitro for mass rearing of *Leucoptera coffeellum* (Guerin-Meneville) (Lepidoptera: Lyonetiidae). *Anais Da Sociedade Entomologica do Brasil* **29**(4), 849–854.
- Reynolds V, Plumptre AJ, Greenham J and Harborne J. 1998. Condensed tannins and sugars in the diet of chimpanzees (*Pan troglodytes schweinfurthii*) in the Budongo Forest, Uganda. *Oecologia* **115**(3), 331–336.

- Richardson BJ, Azarbayjani FF, Burgin S & Richardson S. 1999. Arboreal arthropod biodiversity in woodlands: Effect of collection procedures and geographic distance on estimates of diversity found on two species of *Melaleuca*. *Australian Journal of Ecology* **25**, 544–554.
- Ring RA & Winchester NN. 1996. Coastal temperate rainforest canopy access systems in British Columbia, Canada. *Selbyana* **17**, 22–26.
- Roff DA. 1992. *The evolution of life histories: Theory and Analysis*. Chapman and Hall, New York. xii+535 pp.
- Rosenheim JA, Wilhoit LR & Armer CA. 1993. Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. *Oecologia* **96**(3), 439–449.
- Root RB. 1973. Organization of a plant arthropod association in simple and diverse habitats the fauna of collards (*Brassica oleracea*). *Ecological Monographs* **43**(1), 95–124.
- Russell GB, Bowers WS, Keesing V, Niemeyer HM, Sevenet T, Vasanthaverni S & Wratten SD. 2000. Patterns of bioactivity and herbivory on *Nothofagus* species from Chile and New Zealand. *Journal of Chemical Ecology* **26** (1), 41–56.
- Ruuhola TM, Sipura M, Nousiainen O & Tahvanainen J. 2001. Systemic induction of salicylates in *Salix myrsinifolia* (Salisb.). *Annals of Botany* **88**(3), 483–497.
- Salminen J-P & Lempa K. 2002. Effects of hydrolysable tannins on a herbivorous insect: Fate of individual tannins in insect digestive tract. *Chemoecology* **12**(4), 203–211.
- Sands DPS. 1984. Dissolving wax from scale insects: a method for assessing parasitism and determining instars of *Ceroplasts* spp. and *Gascardia destructor* (Newstead) (Homoptera: Coccidae). *Journal of the Australian Entomological Society* **23**, 295–296.

- Satoh A, Narita Y, Endo N & Nishimura H. 1996. Potent allelochemical falcacindiol from *Glehnia littoralis* F. Schm. *Bioscience, Biotechnology & Biochemistry* **60**(1), 152–153.
- Schmid J & Amrhein N. 1995. Molecular organization of the shikimate pathway in higher plants. *Phytochemistry* **39**(4), 737–749.
- Schreiber U & Bilger W. 1987. Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. In *Plant Response to Stress: Functional analysis in Mediterranean ecosystems*. Tenhunen JD (Ed.), Springer-Verlag, Berlin & Heidelberg, 27–53.
- Schroeder LA. 1986. Changes in tree leaf quality and growth performance and lepidopteran larvae. *Ecology* **67**(6), 1628–1636.
- Schoch G, Goepfert S, Morant M, Hehn A, Meyer D, Ullmann P & Werck-Reichhart D. 2001. CYP98A3 from *Arabidopsis thaliana* is a 3'-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. *Journal of Biological Chemistry* **276**(39), 36566–36574.
- Shimazaki K-I, Igarashi T & Kondo N. 1988. Protection by the epidermis of photosynthesis against UV-c radiation estimated by chlorophyll *a* fluorescence. *Physiologia Plantarum* **74**(1), 34–38.
- Shirata A & Takahashi K. 1983. Detection and production of anti microbial substances in leaves of Mulberry and other Moraceae tree plants. *Bulletin of the Seri cultural Experiment Station* **28**(5), 707–718.
- Singh, M. 1977. Effect of pipal leaves' tannin and tannic acid on volatile fatty acids (VFA) production in goat's rumen. *Indian Veterinary Journal* **54**(7), 553–557.
- Singh M & Roy LN. 1981. Effect of Bargad leaves tannin and tannic acid on volatile fatty acids (VFA) production in goat's rumen. *Indian Veterinary Journal* **58**(8), 837–840.

- Singh S. 1996. Two new species of *Psyllaephagus* Ashmead (Hymenoptera: Chalcidoidea: Encyrtidae) attacking *Mycopsylla* sp. (Homoptera: Psyllidae) infesting *Ficus religiosa* in Mizoram, India. *Oriental Insects* **30(0)**, 155–166.
- Singhal RK and Saharia HS. 1980. Chemical examination of *Ficus glomerata*. *Herba Hungarica* **19(2)**, 17–20.
- Smith CJ. 1996. Tansley Review No. 86. Accumulation of phytoalexins: defence mechanism and stimulus response system. *New Phytologist* **132**, 1–45.
- Souliotis C & Broumas T. 1998. Study of the population dynamics of the *Cacopsylla pyri* L. (Homoptera: Psyllidae) and its predators in Eastern Attiki. *Annales de L'Institut Phytopathologique Benaki* **18(2)**, 97–109.
- Southwood TRE. 1977. Habitat: the template for ecological strategies. Presidential address to the British Ecological Society January 5 1977. *Journal of Animal Ecology* **46(2)**, 337–365.
- Southwood TRE. 1987. *Ecological Methods: With Particular Reference to the Study of Insect Populations*. xxiv+524p. Routledge, Chapman and Hall, Ltd.: New York, USA and London, England, UK. Illus. xxiv+524p.
- Southwood TRE, Moran VC & Kennedy CEJ. 1982a. The assessment of arboreal insect fauna: comparisons of knockdown sampling and faunal lists. *Ecological Entomology* **7**, 331–340.
- Southwood TRE, Moran VC & Kennedy CEJ. 1982b. The richness, abundance and biomass of the arthropod communities on trees. *Journal of Animal Ecology* **51**, 635–649.
- Srivastava A, Juttner F & Strasser RJ. 1998. Action of the allelochemical, fischerellin A, on photosystem II. *Biochimica et Biophysica Acta* **1364(3)**, 326–336.

- Stamp NE. 1994. Interactive effects of rutin and constant versus alternating temperatures on performance of *Manduca sexta* caterpillars. *Entomologia Experimentalis et Applicata* **72**(2), 125–133.
- Stamp NE & Osier TL. 1997. Combined effects of night-time temperature and allelochemicals on performance of a Solanaceae specialist herbivore. *Ecoscience* **4**(3), 286–295.
- Stamp NE & Yang Y. 1996. Response of insect herbivores to multiple allelochemicals under different thermal regimes. *Ecology* **77**(4), 1088–1102.
- Statsoft Inc. 1998. *STATISTICA Version 5.1m for Windows* computer program. StatSoft, Inc. Tulsa, OK USA.
- Steel RGD & Torre JH. 1980. *Principles and Procedures of Statistics 2nd Edition*. McGraw-Hill, New York, NY. 633 pp.
- Stone C. 1993. Fertilizer and insecticide effects on tree growth and psyllid infestation of young *Eucalyptus grandis* and *E. dunnii* plantations in northern New South Wales. *Australian Forestry* **56**(3), 257–263.
- Stone C. 1996. The role of psyllids (Hemiptera, Psyllidae) and bell miners (*Manorina melanophrys*) in canopy dieback of Sydney Blue Gum (*Eucalyptus saligna* Sm.). *Australian Journal of Ecology* **21**(4), 450–458.
- Stone C. 1999. Assessment and monitoring of decline and dieback of forest eucalypts in relation to ecologically sustainable forest management: a review with a case study. *Australian Forestry* **62**(1), 51–58.
- Stone, C. 2000. Reducing the impact of insect herbivory in plantation eucalypts through management of extrinsic influences on tree vigour. *Austral Ecology* **26**, 482–488.

- Stone C & Bacon PE. 1994. Insect herbivory in a river red gum (*Eucalyptus camaldulensis* Dehnh.) forest in southern New South Wales. *Journal of the Australian Entomological Society* **33**(1), 51–56.
- Stone C & Bacon PE. 1995a. Influence of insect herbivory on the decline of black box (*Eucalyptus largiflorens*). *Australian Journal of Botany* **43**(6), 555–564.
- Stone C & Bacon PE. 1995b. Leaf dynamics and insect herbivory in a *Eucalyptus camaldulensis* forest under moisture stress. *Australian Journal of Ecology* **20**(4), 473–481.
- Stone C, Chisholm L & Coops N. 2001. Spectral reflectance characteristics of eucalypt foliage damaged by insects. *Australian Journal of Botany* **49**(6), 687–698.
- Stone C & Clarke AR. 1998. A phytocentric approach towards future pest management in Australian Eucalypt plantations – an alternative to classical IPM. In *Pest Management – Future Changes (1998). Proceedings of the 6th Australasian Applied Research Conference. Brisbane, Australia 29 Sept.–2 Oct.* Zalucki MP, Drew RAI & White GG (Eds), 144–152.
- Stone C, Simpson JA & Eldridge RH. 1998. Insect and fungal damage to young eucalypt trial plantings in northern New South Wales. *Australian Forestry* **61**(1), 7–20.
- Stork NE. 1987a. Arthropod faunal similarity of Bornean rain forest trees. *Ecological Entomology* **12**(2), 219–226.
- Stork NE. 1987b. Guild structure of arthropods from Bornean rain forest trees. *Ecological Entomology* **12**(1), 69–80.
- Stork NE. 1988. Insect diversity: facts, fiction and speculation. *Biological Journal of the Linnean Society* **35**, 321–337.

- Strickler JD & Walker ED. 1993. Seasonal abundance and species diversity of adult Tabanidae (Diptera) at Lake Lansing Park-North, Michigan. *Great Lakes Entomologist* **26**(2), 107–112.
- Sutherst RW & Maywald GF. 1991. Climate modelling and pest establishment. Climate-matching for quarantine, using *CLIMEX*. *Plant Protection Quarterly* **6**(1), 3–7.
- Takagi S & Miyatake Y. 1993. SEM observations on two lerp-forming psylloids (Homoptera). *Insecta Matsumurana* **49**, 69–104.
- Tang CS & Zhang B. 1986. Qualitative and quantitative determination of the allelochemical sphere of germinating mung bean. In *The science of allelopathy*. John Wiley & Sons Inc., New York, USA. p. 229–242.
- Taylor GS. 1987. The gall forming Psylloidea of *Eucalyptus obliqua* in the Mount Lofty Ranges of South Australia. *Journal of the Australian Entomological Society* **26**(3), 223–228.
- Taylor GS & Miles PW. 1994. Composition and variability of the saliva of coreids in relation to phytotoxicoses and other aspects of the salivary physiology of phytophagous Heteroptera. *Entomologia Experimentalis et Applicata* **73**(3), 265–277.
- Taylor KL. 1976. Records of *Psylla dodonaeae* Tuthill (Homiptera: Psyllidae) in Australia, with notes on its biology. *Journal of the Australian Entomological Society* **15**(3), 347.
- Taylor KL. 1984a. A new genus and three new species of Spondyliaspidae (Homoptera: Psylloidea) on *Eucalyptus*. *Journal of the Australian Entomological Society* **23**(1), 13–19.
- Taylor KL. 1984b. Two new species of *Cecidopsylla* Kieffer (Homoptera: Psylloidea) from Australia forming pit galls on *Banksia* and *Geijera*. *Journal of the Australian Entomological Society* **23**(4), 277–283.

- Taylor KL. 1985a. Australian psyllids: a new genus of Ctenarytainini (Homoptera: Psylloidea) on *Eucalyptus*, with new species. *Journal of the Australian Entomological Society* **24**(1), 17–30.
- Taylor KL. 1985b. Possible stridulatory organ in some Psyllidae (Homoptera). *Journal of the Australian Entomological Society* **24**(1), 77–80.
- Taylor KL. 1987a. Revision of *Eucalyptolyma* Froggatt (Homoptera: Psylloidea) with two new genera of Australian Psyllids. *Journal of the Australian Entomological Society* **26**(2), 97–127.
- Taylor KL. 1987b. *Ctenarytaina longicauda*: new species (Homoptera: Psylloidea) from *Lophostemon confertus* R. Brown in Australia and California USA. *Journal of the Australian Entomological Society* **26**(3), 229–233.
- Taylor KL. 1987c. *Platyobria*: new genus of Aphalaridae (Homoptera: Psylloidea) from Australia. *Journal of the Australian Entomological Society* **26**(3), 253–264.
- Taylor KL. 1989. Three new species of *Cardiaspina* Crawford (Homoptera: Psylloidea) from Australian eucalypts. *Journal of the Australian Entomological Society* **28**(1), 45–52.
- Taylor KL. 1990a. The tribe Ctenarytainini (Homoptera: Psylloidea): a key to known Australian genera, with new species and two new genera. *Invertebrate Taxonomy* **4**(1), 95–122.
- Taylor KL. 1990b. Redescription of *Phyllolyma* Scott (Homoptera: Psylloidea) and description of three new species. *Journal of the Australian Entomological Society* **29**(4), 313–318.
- Taylor KL. 1992a. *Bleopharocosta*: New genus of Australian Spondyliaspidae (Homoptera: Psylloidea). *Invertebrate Taxonomy* **6**(1), 131–141.

- Taylor KL. 1992b. Two new species of *Cardiaspina* Crawford (Hemiptera: Psyllidae) from Western Australia and Tasmania. *Journal of the Australian Entomological Society* **31**(2), 171–175.
- Taylor KL. 1997a. Revision of *Syncarpiolyma* Froggatt (Hemiptera: Psyllidae) and description of a new species. *Australian Journal of Entomology* **36**(2), 109–111.
- Taylor KL. 1997b. A new Australian species of *Ctenarytaina* Ferris and Klyver (Hemiptera: Psyllidae: Spondyliaspidae) established in three other countries. *Australian Journal of Entomology* **36**(2), 113–115.
- Taylor KL & Carver M. 1991. Chapter 30: Hemiptera. (Carver M, Gross GF, & Woodward TE, Eds.) In *The Insects of Australia: a Textbook for Students and Research Workers, 2nd Edition*. The Division of Entomology, Commonwealth Scientific and Industrial Research Organization. 443–448. Melbourne University Press, Carlton South, Victoria.
- Taylor RW. 1991. Chapter 42: Hymenoptera. (Naumann ID, Ed.) In *The Insects of Australia: a Textbook for Students and Research Workers, 2nd Edition*. The Division of Entomology, Commonwealth Scientific and Industrial Research Organization. 980–989. Melbourne University Press, Carlton South, Victoria.
- Tiller KJ, Gurrin LC, Cookson W, Musk AW & Palmer LJ. 1999. Genetic variance components analysis for binary phenotypes using generalized linear mixed models (GLMMs) and Gibbs sampling. *Genetic Epidemiology* **17**(2), 118–140.
- Timmins WA & Reynolds SE. 1992. Azadirachtin inhibits secretion of trypsin in midgut of *Manduca sexta* caterpillars: Reduced growth due to impaired protein digestion. *Entomologia Experimentalis et Applicata* **63**(1), 47–54.
- Tomos, AD & Leigh, RA. 1999. The pressure probe: a versatile tool in plant cell physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 447–452.

- de la Torre-Bueno JR. 1989. *The Torre-Bueno glossary of entomology. Including Supplement A by George S. Tulloch*. Nichols SW (Comp. Ed.), Schuh RT (Man. Ed.), New York Entomological Society/American Museum of Natural History, New York, NY, USA, xvii+840 p.
- Tuthill LD & Taylor KL. 1955. Australian genera of the Family Psyllidae (Homiptera, Homoptera). *Australian Journal of Zoology* **3**(2), 227–257.
- Uchida GK, Beardsley JW, Reimer NJ & Wheeler RA. 1992. Comparison of the populations of *Heteropsylla cubana* Crawford (Homoptera: Psyllidae) and its parasitoids on seven accessions of *Leucaena* (Leguminosae: Mimosoideae) in Hawaii. *Proceedings of the Hawaiian Entomological Society* **31**, 97–108.
- Ueno T. 1998. Adaptiveness of sex ratio control by the pupal parasitoid *Itopectis naranyae* (Hymenoptera: Ichneumonidae) in response to host size. *Evolutionary Ecology* **12**, 643–654.
- Utsunomiya A & Iwabuchi K. 2002. Interspecific competition between the polyembryonic wasp *Copidosoma floridanum* and the gregarious endoparasitoid *Glyptapanteles pallipes*. *Entomologia Experimentalis et Applicata* **104**(2–3), 353–362.
- Vaithiyanathan S & Kumar R. 1993. Relationship between protein-precipitating capacity of fodder tree leaves and their tannin content. *Animal feed Science and Technology* **44**(3–4), 281–287.
- Vance HD & Francko DA. 1997. Allelopathic potential of *Nelumbo lutea* (Willd.) Pers. to alter growth of *Myriophyllum spicatum* L. and *Potamogeton pectinatus* L. *Journal of Freshwater Ecology* **12**(3), 405–409.
- Varma PN, Manisha SV & Rajat R. 1993. Histopharmacognostic evaluation of *Ficus benghalensis* aerial roots. *Journal of Plant Anatomy and Morphology* **6**(1), 67–71.

- Voinovich ND & Sugonyaev ES. 1993. Peculiarities of parasitism of chalcids (Hymenoptera, Chalcidoidea) on scale-insects (Homoptera: Coccoidea). V. Biology and preimaginal stages of *Pseudorhopus testaceus* Ratzeburg: A parasite of *Physokermes hemicryphus* Dalman. *Entomologicheskoe Obozrenie* 72(3), 549–557, 729.
- Walker DA. 2002. The Z-scheme: Down hill all the way. *Trends in Plant Science* 7(4), 183–185.
- Wald A. 1949. Note on the consistency of the maximum likelihood estimator. *Annals of Mathematical Statistics* 20, 595–601.
- Walter GH & Clarke AR. 1992. Unisexual broods and sex ratios in a polyembryonic encyrtid parasitoid (*Copidosoma* sp.: Hymenoptera). *Oecologia* 89(1), 147–149.
- Wang T & Laing JE. 1989. Polyembryony in *Holcothorax testaceipes* (Hymenoptera: Encyrtidae). *Annals of the Entomological Society of America* 82(6), 725–729.
- Watmough RH. 1968. Population studies on two species of Psyllidae (Homoptera, Sternorrhyncha) on broom (*Sarothamnus scoparius* (L.) Wimmer). *Journal of Animal Ecology* 37(2), 283–314.
- Watson DM & Beattie GAC. 1995. Effect of weather station logging interval on the precision of degree-day estimates. *Australian Journal of Experimental Agriculture* 35(6), 795–805.
- Watson DM & Beattie GAC. 1996. Degree-day models in New South Wales: Climatic variation in the accuracy of different algorithms and geographical bias correction procedures. *Australian Journal of Experimental Agriculture* 36(6), 717–729.
- Weaver LM & Herrmann KM. 1997. Dynamics of the shikimate pathway in plants. *Trends in Plant Science* 2(9), p 346–351.

- Weibull J & Niemeyer HM. 1995. Changes in dihydroxymethoxybenzoxazinone glycoside content in wheat plants infected by three plant pathogenic fungi. *Physiological and Molecular Plant Pathology* **47**(3), 201–212.
- Weiss U. 1985. Early research on the shikimate pathway: some personal remarks and reminiscences. In *The shikimic acid pathway. Recent Advances in Phytochemistry* (Conn EE, Ed.) **20**, 1–12.
- Wendeln MC, Runkle JR & Kalko EKV. 2000. Nutritional values of 14 fig species and bat feeding preferences in Panama. *Biotropica* **32**(3), 489–501.
- White TCR. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* **50**(5), 905–909.
- White TCR. 1970a. Some aspects of the life history, host selection, dispersal, and oviposition of adult *Cardiaspina densitexta* (Homoptera: Psyllidae). *Australia Journal of Zoology* **18**, 107–117.
- White TCR. 1970b. The nymphal stage of *Cardiaspina densitexta* (Homoptera: Psyllidae) on leaves of *Eucalyptus fasciculosa*. *Australian Journal of Zoology* **18**, 273–93.
- White TCR. 1971. Lerp insects (Homoptera: Psyllidae) on red gum (*E. camaldulensis*) in South Australia. *South Australian Naturalist* **46**, 20–23.
- White TCR. 1972. The production of amylose in the faeces of psyllid larvae with special reference to the lerps of *Cardiaspina densitexta*. *Journal of Insect Physiology* **18**, 2359–2367.
- White TCR. 1973. Aerial dispersal of adult *Cardiaspina densitexta* (Homoptera: Psyllidae) in South Australia. *Transactions of the Royal Society of South Australia* **97**, 29–31.

- White, T.C.R. 1978. A method of sampling populations of psyllids (*Cardiaspina densitexta*) living on the leaves of *Eucalyptus fasciculosa* trees in South Australia. *Pacific Insects* **19**(1), 39–44.
- White TCR. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* **63**, 90–105.
- White TCR. 1993. *The Inadequate Environment – Nitrogen and the abundance of animals*. Springer-Verlag, Berlin & Heidelberg. 425 p.
- Whittaker RH and Feeny PP. 1971. Allelochemicals: chemical interactions between species. *Science* **171**, 757–770.
- Wigglesworth VB. 1973. The significance of “apolysis” in the moulting of insects. *Journal of Entomology* **47**(2), 141–149.
- Wilde, WHA. 1962. Bionomics of the Pear Psylla, *Psylla pyricola* Förster in Pear Orchards of the Kootenay Valley of British Columbia, 1960. *The Canadian Entomologist* **94**, 845–849.
- Wilson EO. 1971. *The insect societies*. Belknap Press (Harvard University Press), Cambridge, MA. 548 p., illus.
- Wilson DJ, Patton S, Florova G, Hale V & Reynolds KA. 1998. The shikimic acid pathway and polyketide biosynthesis. **20**(5), 299–303.
- Winchester NN & Ring RA. 1999. The biodiversity of arthropods from northern temperate ancient coastal rainforests: conservation lessons from the high canopy. *Selbyana* **20**(2), 268–275.
- Woinarski JCZ & Cullen JM. 1984. Distribution of invertebrates on foliage in forests of southeastern Australia. *Australian Journal of Ecology* **9**(3), 207–232.

- Wood CD, Tiwari BN, Plub VE, Powell CJ, Roberts BT, Sirimane VDP, Rossiter JT & Gill M. 1994. Interspecies differences and variability with time of protein precipitation activity of extractable tannins, crude protein, ash, and dry matter content of leaves from 13 species of Nepalese fodder trees. *Journal of Chemical Ecology* **20**(12), 3149–3162.
- Wratten SD, Edwards PJ & Winder L. 1988. Insect herbivory in relation to dynamic changes in host quality. *Biological Journal of the Linnean Society* **35**, 339–350.
- Xu R & Adak S. 2002. Survival analysis with time-varying regression effects using a tree-based approach. *Biometrics* **58**(2), 305–315.
- Yang C-K & Li F. 1981. The Pear Psylla (Homoptera) of China with descriptions of 7 new species. *Entomotaxonomia* **3**(1), 35–47.
- Yang C-K & Li, F. 1982. Descriptions of the new genus *Celtisaspis* and five new species from China (Homoptera: Psyllidae). *Entomotaxonomia* **4**(3), 183–198.
- Zar JH. 1984. *Biostatistical Analysis 2nd Edition*. Prentice-Hall Inc., Englewood Cliffs, NJ. xiv+718 pp.
- Zdero C, Bohlmann F & Niemeyer HM. 1988. Diterpenes and 5-methyl coumarin derivatives from *Gypothamnium pinifolium* and *Plazia daphnoides*. *Phytochemistry* **27**(9), 2953–2959.
- Zdero C, Bohlmann F & Niemeyer HM. 1990a. Diterpenes and umbelliferone derivatives from *Haplopappus deserticola*. *Phytochemistry* **29**(1), 326–329.
- Zdero C, Bohlmann F & Niemeyer HM. 1990b. Seco-labdanes and other constituents from *Ophryosporus floribundus*. *Phytochemistry* **29**(10), 3247–3253.
- Zdero C, Bohlmann F & Niemeyer HM. 1991. An unusual dimeric sesquiterpene and other constituents from Chilean *Baccharis* species. *Phytochemistry* **30**(5), 1597–1601.

- Zeng RS, Luo SM, Shi YH, Shi MB & Tu CY. 2001. Physiological and biochemical mechanism of allelopathy of secalonic acid *F* on higher plants. *Agronomy Journal* **93**(1), 72–79.
- Zucker WV. 1983. Tannins: does structure determine function? An ecological perspective. *American Naturalist* **121**(3), 335–365.
- Zwick P. 1999. Egg diapause, egg swelling and mother-child size relationships in Plecoptera (Insecta). *Ergebnisse der Limnologie* **0**(54), 373–386.
- Zwick RW & Westigard PH. 1978. Prebloom petroleum oil applications for delaying Pear Psylla (Homoptera: Psyllidae) oviposition. *Canadian Entomologist* **110**(3), 225–236.