WHY DO TOMATO SEEDS PRIME?

Physiological Investigations into the Control of Tomato Seed Germination and Priming

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Summary

It is the aim of this thesis to examine hypotheses regarding the prevention of radicle emergence during priming and of the enhancement of germination following priming. This work should identify the control sites in the processes of radicle emergence and their modification during priming.

For the most part these hypotheses are underlain by an analysis of germination as a special case of plant cell expansion. A study of cell expansion requires an understanding of the water relations of the tissues involved. Therefore, the sequence of events during the germination of tomato seeds was first examined through a water relations study then the changes caused by priming were examined.

The study of the water relations of germinating tomato seeds revealed that the seeds came into Ψ equilibrium with the imbibitional solution, whereas, the embryo was measured at much lower Ψ. There was no evidence of a lowering of embryo Ψ_π nor of an increase in embryo Ψ_p prior to radicle emergence. The embryo Ψ measurements need to be interpreted with caution for they are *ex situ* measurements and thus do not directly measure these properties in the seed. It is unlikely that a large $\Delta\Psi$ could be maintained within the seed during imbibition. Thus the existence of a large seed to embryo Ψ_p can be inferred from these measurements. The moisture release isotherm of the excised embryo confirmed this inference. The endosperm tissue enclosing the embryo was found to restrict the hydration level of the embryo

prior to its emergence. As the embryo was capable of expansive growth prior to radicle emergence, it was concluded that the weakening of the endosperm controlled radicle emergence in tomato seeds.

During priming the tomato seeds were in Ψ equilibrium with the priming solution, but the embryo was not. As the embryo was capable of growth after 2 days of the 6 day priming treatment, it was concluded that radicle emergence was prevented by the maintenance of the endosperm restraint. Germinating primed seeds did not display a marked plateau during imbibition. Both seed and embryo water contents were higher than those of non-primed seeds. However, embryo Ψ and Ψ_π were lower than those of embryos from non-primed seeds, eventhough embryo Ψ_π measurements during priming had not revealed significant lowering. The relative growth rate of seedlings from primed seeds was higher than that of non-primed seeds for the first 12 h after radicle emergence.

The endosperm of tomato seeds consisted of two distinct cell types found in separate locations within the seed. At the micropylar end of the seed the endosperm cells had thin walls, whereas those in the rest of the seed had thickened walls. The outer walls of outermost endosperm cells in the rest of the seed had massively thickened walls whereas these were lacking from the outer cells of the micropylar region.

All cells, except those of the root cap, contained protein bodies. The protein bodies of the micropylar region endosperm cells were seen to breakdown to form vacuoles

prior to radicle emergence. The protein bodies in other cells did not appear to change prior to this time. During priming protein body breakdown was more extensive in the micropylar region endosperm cells and vacuole formation also occurred in the radicle. After radicle emergence the cells of the radicles from primed seeds were found to be about 50% larger than those of the radicles from non-primed seeds.

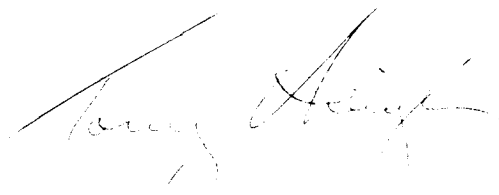
Endosperm weakening preceded radicle emergence in tomato seeds. Slower germinating seeds within the population had higher values for endosperm resistance. Endosperm weakening during priming resulted in values for endosperm resistance which were lower than those measured from a population of germinating non-primed seeds. Germinating primed seeds had resistances which were similar to those of priming seeds. It was concluded that a final rapid endosperm weakening step may be necessary for radicle emergence to occur.

These studies have shown that tomato seeds prime because the endosperm does not weaken sufficiently to permit expansion of the radicle. The mechanism by which some endosperm weakening was permitted, but the final weakening for radicle emergence was prevented was not identifiable. Priming advanced the timing of radicle emergence by improving the rate of water uptake by the seeds; by eliminating the time necessary for the loosening of embryo cell walls and by permitting the completion of the first step of the endosperm weakening process. Embryos from primed seeds had improved cell wall extensibilities which

permitted higher relative growth rates during the first 12 h after radicle emergence.

Certificate

I hereby declare that this work has not been submitted for a higher degree to any other university or institution.



Anthony Mark Haigh

Dedicated to my wife

Helen Jane Bailey

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General Introduction to Seed Priming

1.1 What is Seed Priming?

Seed priming involves the soaking of seeds in a solution of appropriate osmotic potential to permit the preliminary processes of germination but not radicle emergence (Heydecker and Coolbear 1975). Originally the technique was developed in response to difficulties experienced with the repeatability of the seed hardening (hydration-dehydration) treatments of Henckel (1964). Determination of suitable priming solutions has been empirically based following work with K_2HPO_4 and KNO_3 solutions (Ells 1963) and polyethylene glycol (Heydecker 1974). As priming solutions vary between cultivars and seedlots (Heydecker 1977), considerable effort is required to optimise priming conditions for commercial operations. A clearer understanding of the mechanisms involved in priming may lead to improvements in the technique and prediction of which types of seeds may benefit from priming.

This thesis seeks to identify the mechanisms involved in seed priming and the subsequent accelerated germination. There is a large empirical literature on seed priming, but the differences between solutions and techniques described in this literature will not be discussed in this study. As specific priming treatments vary with species, cultivar and seed lot the reasons for these differences are not apparent from a review of the treatments. As Bradford (1986) stated, clearer understanding of the mechanisms which enhance

subsequent germination may provide a basis for developing priming treatments.

Priming treatments affect only the processes of germination and seedling emergence and as a consequence may affect crop yield. The claims of yield improvements made by Henckel (1964) have been substantiated for a number of crops in which vegetative organs are harvested; carrot (Szafirowska *et al.*, 1981), and carrot, celery, onion and leek (Brocklehurst and Dearman 1983; Brocklehurst *et al.*, 1984). Mixed results have been achieved with the yield of primed tomato seeds, in several cases priming led to earlier field emergence which could result in earlier maturation of the crop (Wolfe and Sims 1982; Barlow and Haigh 1987; Leskovar and Sims 1987); while in one case priming resulted only in earlier development prior to flowering (Alvarado *et al.*, 1987). For all crops the growth advantage caused by priming could be attributed entirely to the earliness of emergence of the primed seeds.

While seed priming is gaining increased usage as a means of improving the speed of emergence of field crops, the mechanisms which permit seeds to prime and hasten subsequent germination are largely unknown. Usually priming modifies the processes of germination to reduce the time to radicle emergence by about half. However, before it is possible to determine what these modifications may be, it is first necessary to understand the normal sequence of the processes of germination.

1.2 Physiology of Germination

Germination is essentially the transition of a seed (or propagule) from a quiescent dry state to the beginning of growth of a new plant. This subject has been extensively reviewed recently (Bewley and Black 1985; Bewley and Black 1978, 1982; Khan 1977, 1982).

The processes of germination are controlled to a large extent by the level of hydration of the seed. Germination begins with water uptake and a subsequent massive increase in metabolism. Seed water uptake is generally a triphasic process, beginning with hydration of the tissues (imbibition). This is followed by a period of little or no water uptake during which the cellular contents are reorganized from their desiccated state to that of normal hydrated functionality. The third phase is one of rapid uptake coincident with the growth of the embryo at radicle emergence (Bewley and Black 1985).

The induction of a number of cellular events has been described for a range of germinating seeds. Respiration, enzyme and organelle activity, RNA and protein synthesis have been found to be essential for germination. Not surprisingly most research into the physiology and biochemistry of germination has concentrated on these events (Bewley and Black 1985).

Respiration increases with water content during germination in a triphasic pattern similar to water uptake. The hydrolysis of sucrose and the raffinose-series oligosaccharides within the embryo is the main source of

respiratory substrates during germination with major reserves not being hydrolysed until after radicle emergence. Mitochondria in the dry seed are poorly differentiated internally and are therefore deficient in their capacity to utilize NADH and conduct oxidative phosphorylation. Mitochondrial activity increases with time from imbibition (Bewley and Black 1985).

Protein synthesis begins within minutes of imbibition and follows the pattern of water uptake during germination (Marcus *et al.*, 1966). The mRNA for early protein synthesis is long-lived mRNA from the developing seed. This mRNA can be residual mRNA produced and translated during seed development or it can be stored mRNA laid down during development, but translated during germination. Some proteins translated from these latter mRNA are thought to be essential for immediate metabolism and are ubiquitous among growing plants, while other proteins translated from these mRNA have been found to be unique to germinating seeds (Payne *et al.*, 1978). Synthesis of new mRNA begins within a few hours of the beginning of imbibition (Sen *et al.*, 1975). Although much work has been conducted in this field, the proteins which may be essential for the initiation of radicle emergence have yet to be identified (Bewley and Black 1985).

DNA synthesis and cell division are generally found after radicle emergence for most seeds (Bewley and Black 1985), although in some seeds embryo growth and cell

division (and presumably DNA synthesis) occur prior to germination as in carrot (Jacobsen and Pressman 1979).

The sequence of events which control radicle emergence is not clearly understood. An examination of the process of radicle emergence as a special case of the general phenomenon of plant cell expansion may indicate possible control mechanisms. Radicle emergence inevitably involves expansion of the embryo.

Current theories of plant cell expansive growth are based upon the ideas proposed by Lockhart (1965). Cell expansion involves two interrelated physical processes: water absorption and cell wall yielding. Steady state cell expansion can be described by the equation:

$$dV/dt = m\ (\Psi_p - Y)$$

where V is cell volume, t is time, m is a cell wall extensibility coefficient, Ψ_p is cell turgor, and Y is cell wall yield threshold. Water uptake by a cell is a consequence of the gradient in osmotic potential ($\Delta\Psi_\pi$) between the cell and the water supply and tissue conductance (L_p). The rate of water uptake for cell expansion can be described by the equation:

$$dV/dt = L_p\ (\sigma\Delta\Psi_\pi - \Psi_p)$$

where σ is a solute reflection coefficient. Combination of these two equations results in:

$$dV/dt = (mL_p)\ (m + L_p)^{-1}\ (\sigma\Delta\Psi_\pi - Y)$$

Cell expansion is initiated with a yielding of the cell wall and continues under the effects of Ψ_p. Wall relaxation reduces the cell water potential (Ψ) by the dissipation of

Ψ_p and resulting in a water influx, which in turn increases cell volume (Cosgrove 1986).

Cosgrove (1986) concluded that tissue hydraulic conductance was always sufficiently large so as to have no effect on water uptake by expanding cells. In radicle tissue hydraulic conductance would be expected to be large because of the root-like nature of the tissue and the exposure of the radicle to the imbibing water. The solute reflection coefficient would be non-existent for laboratory germination studies using distilled water, but not for priming solutions.

Control of radicle emergence may lie in the initiation of cell expansion by the yielding of radicle cell walls. While it is generally accepted that cell expansion is initiated when load-bearing elements in the wall yield (Cosgrove 1986), the mechanisms which control this process of yielding are not well understood. To date, two factors have been identified as affecting wall loosening. Considerable evidence has been presented that H^+ extrusion is one of these (Taiz 1984). Ca^{2+} has also been suggested as a wall loosening factor, but less evidence has been presented for its involvement (Cleland 1986). The mechanism of initiation of radicle cell wall yielding has not been studied.

Heydecker (1974) originally proposed that the metabolic processes during seed priming essentially followed those of seeds in water except that radicle emergence was inhibited.

This hypothesis may need reevaluation. Priming may modify any or all of the processes of germination. The mechanisms which permit seeds to prime have not been identified nor have those which hasten germination following priming. This may be the result of the limited number of studies and the range of species used.

1.3 Physiology of Seed Priming

Priming causes a change in the nature of the metabolic activities of seeds. Seeds do not just follow the preliminary processes of germination and stop at the brink of cell expansion as was proposed by Heydecker (1974). Some preliminary germinative processes are reduced and others are enhanced during priming. For example, the respiration of carrot seeds during priming was lower than that of germinating non-primed seeds (Hegarty 1977), while accumulation of nucleic acids, mainly as ribosomal RNA, occurred during priming of tomato seeds (Coolbear and Grierson 1979).

Germination of primed seeds is accompanied by higher rates of metabolic activity than are found in germinating non-primed seeds. Respiration of germinating primed seeds was higher than that of control seeds (Woodstock 1969). Primed tomato seeds continued to increase their nucleic acid content during germination (Coolbear and Grierson 1979). Similarly, the synthesis of total RNA and poly A (+) RNA was correlated with the improvement in germination of primed *Chenopodium bonus-henricus* seeds (Khan and Karssen 1981).

Germinating primed lettuce seeds had higher rates of RNA and protein synthesis and activities of certain enzymes than during germination of non-primed seeds (Khan *et al.*, 1978).

Processes additional to those normally occurring during germination would be expected to occur during seed priming. The response of seeds to priming may be similar to those of a more mature plant to osmotic stress. Osmotic and drought stresses have been shown to modify metabolism by inducing the synthesis of specific stress proteins, and the relief of stress is often accompanied by accelerated growth (Bradford and Hsiao 1982). However, little connection between drought and osmotic stresses and seed priming has been made to date.

Analysis of the effects of priming on the germination of carrot seeds has resulted in the claim that embryo growth during priming was the main cause of the advancement of germination (Austin *et al.*, 1969; Weibe and Tiessen 1979). Cell division associated with embryo growth did not occur during the priming of tomato seeds because their DNA content was unchanged during priming (Coolbear and Grierson 1979). These apparent differences in response to priming may reflect fundamental differences in seed development. The embryo of carrot and other umbelliferae is small at seed maturity (Martin 1946; Borthwick 1931) and increases considerably in size before radicle emergence (Stokes 1952; Jacobsen and Pressman 1979). In contrast, the embryo of the tomato seed is fully developed at seed maturity (Smith 1935; Martin 1946) and there would appear to be no embryo growth before radicle emergence.

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Priming clearly modifies the normal processes of germination; however, to identify those changes which permit seeds to prime or which hasten subsequent germination it may be beneficial to frame our questions in light of the processes involved in the control of radicle expansion as outlined previously (Sect. 1.2).

1.4 **Why do Seeds Prime?**

During seed priming radicle emergence is prevented. Control of either water uptake or cell wall loosening may prevent radicle emergence during priming.

1.4.1 *Water Potential Gradients*

Prevention of cell expansion in the radicle during priming may result from the lack of a water potential gradient ($\Delta\Psi$) between the priming seed and the solution. As water moves along water potential gradients, seeds imbibed in solutions of low osmotic potential (Ψ_π) may come to equilibrium with that solution and thus be unable to take up further water necessary for radicle expansion without lowering their osmotic potential.

1.4.2 *Embryo Cell Wall Loosening*

Failure to loosen the cell walls of the radicle and enable cell expansion may prevent germination during priming. For cells to expand water uptake and cell wall loosening must both occur. Primary cell walls are composed largely of cellulose microfibrils bound together by other molecules into a rigid structure. Control over cell wall

loosening has been suggested to lie in the action of auxin and through H^+ extrusion; however, the exact mechanism is not well understood (Cleland 1986).

1.4.3 *Endosperm/Seed Coat Restraint*

In seeds such as tomato the embryo is enclosed by layers of endosperm and seed coat tissues. These tissues present a barrier to germination. Control of germination during priming may be achieved through the prevention of the decrease in endosperm/seed coat mechanical restraint. This mechanical resistance decreases prior to radicle emergence in tomato seeds and the decrease was mediated through gibberellic acid (GA) translocated from the embryo (Groot and Karssen 1987).

1.4.4 *Embryo Turgor*

While endosperm restraint decreases prior to radicle emergence it should be noted that there was some mechanical restraint remaining at the time of radicle protrusion (Groot and Karssen 1987). Although the restraining layers are weakened it is still necessary for the radicle to develop sufficient turgor to overcome some restraint. The embryo may be unable to overcome the endosperm restraint through insufficient development of turgor during priming. Turgor development would be associated with solute accumulation to lower embryo osmotic potential to permit water uptake.

In summary, a seed may be prevented from germinating in a priming solution by a number of mechanisms: (1) prevention

of water uptake for growth; (2) by lack of embryo cell wall yielding; (3) prevention of endosperm/seed coat weakening; or (4) insufficient embryo turgor to overcome the endosperm/seed coat restraint. Before examining these hypotheses of priming it is essential to first understand what is involved in the normal processes of germination.

1.5 Why do Primed Seeds Germinate Rapidly?

Enhancement of the speed at which germination occurs could result from changes in either the rate of water uptake, the rate of cell wall loosening or the rate of endosperm weakening. Increased rates of water uptake during germination could result from solute accumulation during priming. A number of authors have suggested that the rapid germination of primed seeds may be due to solute accumulation in the embryo during priming (Hegarty 1978; Khan and Karssen 1981; Khan and Samimy 1982; Liptay and Schopfer 1983; Cantliffe *et al.*, 1984; Bradford 1986); however, direct evidence of solute accumulation is lacking. As lowering of embryo Ψ_π by solute accumulation would result in higher embryo Ψ_p at a given Ψ equilibrium, then implicit in this hypothesis is the assumption that germination is prevented by maintenance of high embryo wall yield threshold or by the maintenance of high resistances of endosperm and seed coat tissues in those seeds where the embryo is enclosed by endosperm and seed coat.

Preliminary events in the process of cell wall loosening may occur during priming; however, the nature of

these events is not known. Cell wall loosening may be initiated during priming when germination is prevented by other mechanisms. The process of cell wall loosening may not have to be repeated during subsequent germination and radicle emergence may be accelerated.

Endosperm weakening during priming would also result in more rapid germination if this process did not have to be repeated. Endosperm weakening has been associated with the induction of certain enzyme activities (Halmer *et al.*, 1978) which may proceed more rapidly during subsequent germination. Removal of the restriction of radicle emergence by the endosperm of lettuce seeds during priming has been suggested as the mechanism by which thermodormancy was overcome (Guedes *et al.*, 1981).

Embryo growth during priming has also been claimed to be the cause of the observed effects of seed priming (Austin *et al.*, 1969; Weibe and Tiessen 1979); however, this does not seem to be the case with tomato seeds (Coolbear and Grierson 1979).

1.6 **Aims of the Thesis**

It is the aim of this thesis to examine hypotheses regarding the prevention of radicle emergence during priming and of the enhancement of germination following priming. This work should identify the control sites in the processes of radicle emergence and their modification during priming.

For the most part these hypotheses are underlain by an analysis of germination as a special case of plant cell

expansion. A study of cell expansion requires an understanding of the water relations of the tissues involved. Therefore, the sequence of events during the germination of tomato seeds will first be examined through a water relations study then the changes caused by priming will be examined.

Water Relations of Tomato Seed Germination

2.1 Introduction

Although seed germination is a much studied phenomenon, few studies of water uptake and the water relations of germinating seeds have been reported. Water uptake is generally triphasic (Bewley and Black 1985). Phase I is rapid due to the large water potential gradient ($\Delta\Psi$) between the seed and the environment as the water potential (Ψ) of the air dry seeds can be as low as -100 MPa (Shull 1913). Phase II, the plateau phase, is a period of varying duration, when little or no change occurs in seed water content. The cause of this plateau is not well understood and the magnitude of the $\Delta\Psi$ between the seed and the environment during phase II is not clear. Two hypotheses exist to explain this plateau; both are supported by some data.

Bewley and Black (1985) suggested that there may be a large $\Delta\Psi$ of about -1.0 MPa between the seed and imbibitional solution. Shaykewich's (1973) reports of the "swelling pressure" of imbibing wheat, corn and rapeseed have been interpreted as indicating seed Ψ about -1.0 MPa prior to germination. However, it was not clear that this was what Shaykewich (1973) meant. Shaykewich and Williams (1971) stated that the moisture characteristic (water potential-water content relationship) was meaningful only for seed water contents lower than that required for germination. It so happened that this was at Ψ lower

than -1.0 MPa. However, the Ψ of *Brassica napus* seeds prior to radicle growth was -1.2 MPa (Schopfer and Plachy 1985).

Bradford (1986) argued that Ψ equilibrium existed between the seed and the environment during phase II. McDonough (1975) found the Ψ of germinating seeds of 11 species to be near 0 MPa prior to radicle emergence. Tomato seed Ψ was also near 0 MPa prior to radicle emergence (Liptay and Schopfer 1983). Examination of the pattern of water uptake and associated changes in Ψ of germinating seeds may lead to a clearer interpretation of these differing observations.

The water uptake pattern for *B. napus* showed a short plateau, then breaking of the testa, and finally irreversible radicle growth (Schopfer and Plachy 1984). The Ψ continued to rise as the water content increased and the radicle grew (Schopfer and Plachy 1985). The low Ψ of *B. napus* seeds in water during phase II may result from the slow hydration of the storage material in the cotyledons. Cotyledons of *Salicornia bigelovii* had a Ψ of -2.0 MPa 4 days after the start of imbibition (Stumpf *et al.* 1986).

The 0 MPa Ψ values for the 11 species investigated by McDonough (1975) and for tomato seeds (Liptay and Schopfer 1983) indicated that equilibrium had been established with the environment. Unfortunately the water uptake pattern was not reported for any of these species.

Phase III of water uptake is one of rapid increase associated with radicle emergence (Bewley and Black 1985).

For radicle emergence to occur there must be expansion of the embryo (Sect. 1.2). Cell expansion involves two interrelated processes: water absorption and cell wall yielding. Growth is initiated with a yielding of the cell wall and continues under the effects of turgor. Wall relaxation reduces the cell water potential by the dissipation of Ψ_p giving rise to water uptake, which in turn increases cell volume (Cosgrove 1986). Although the onset of phase III of water uptake has been attributed to the lowering of embryo Ψ_π due to solute accumulation and an associated increase in embryo Ψ_p resulting in the expansion necessary for radicle emergence (Bewley and Black 1985; Bradford 1986), the evidence in support of this accumulation is not overwhelming.

Solute accumulation has generally been inferred from studies of dormancy breaking. The difference in Ψ_π of solutions of mannitol or polyethylene glycol which prevented the growth of dormant and treated embryos (or germination of seeds) has been interpreted as evidence of solute accumulation (Carpita and Nabors 1981; Carpita *et al.* 1979*a* 1979*b* 1979*c*; Nabors and Lang 1971*a* 1971*b*; Takeba 1980*a* 1980*b* 1980*c* 1980*d*; Takeba and Matsubara 1979; Thanos 1984). Changes in lettuce embryo Ψ have been measured, but the difference between red- and far-red-treated embryos was never more than 0.2 MPa and thus less than the 0.57 MPa difference in Ψ_π of solutions which prevented embryo growth (Carpita *et al.* 1979*a*). Similarly, Thanos (1984) found that amino acid concentration changes in watermelon

radicles resulted in a Ψ difference of only 0.15 MPa between treated and untreated radicles, whereas a 0.75 MPa difference in solutions was required to prevent germination. The extent of changes in embryo Ψ_π during phase II and at the onset of phase III requires further investigation.

In some seeds mechanical barriers to radicle emergence prevent or retard germination (Esashi and Leopold 1968; Hsiao *et al.* 1983; Ikuma and Thimann 1963; Liptay and Schopfer 1983; Watkins and Cantliffe 1983). In *Capsicum annuum* seeds the endosperm restraint lessened prior to germination (Watkins and Cantliffe 1983). In lettuce seeds degradative changes in the endosperm seem to occur after radicle growth has commenced (Georghiou *et al.* 1983; Halmer and Bewley 1979). Bradford (1986) argued that these seeds would require a lowering of Ψ_π to increase water uptake and generate sufficient turgor (Ψ_p) to overcome this restraint. The existence of large Ψ_p prior to radicle emergence has yet to be documented.

In this chapter I have investigated the hypotheses that (1) a Ψ gradient exists between the seed and its environment during phase II; and (2) the lowering of embryo Ψ_π with concurrent buildup of Ψ_p is necessary to permit radicle emergence by measuring the water relations of tomato seeds and their embryos throughout germination.

2.2 Materials and Methods

2.2.1 *Water Uptake by Tomato Seeds*

Seeds (UC 82B, Yates Seeds, Sydney, Australia) were imbibed in water at 25°C until germination. For imbibition, 10 seeds were placed on filter papers (Ekwip U70, Industrial Equipment, Sydney, Australia) moistened with approximately 16 ml of water in 90 mm glass Petri dishes. At every measurement time each seed was surface dried between layers of tissue paper before weighing on a CAHN 25 microbalance (Ventron Instruments Corporation, Cerritos, CA, U.S.A.) then dried at 80°C for 72 h for dry weight (DWt).

2.2.2 *Water Relations of Tomato Seeds*

Approximately 200 seeds were imbibed as above and seed Ψ and Ψ_π were measured in Peltier type thermocouple psychrometers (constructed at Macquarie University, Barlow *et al.* 1980) at regular intervals until germination; 15 seeds were placed in each chamber (volume 0.25 ml) and five replicate chambers were used. Seeds were equilibrated in the chambers in a temperature-stabilised water bath at 25°C before measurement; 5 hours equilibration resulted in stable Ψ measurements. Osmotic potential was measured after liquid N_2 freezing and thawing in the chamber, *i.e.*, the tissue was not removed from the chambers.

2.2.3 *Water Uptake and Water Relations of the Embryo within the Seed*

Seeds were imbibed as above. At frequent intervals seeds were removed from the Petri dishes and the embryo excised. Embryos were weighed then dried at 80°C for 24 h before reweighing for dry weight. The embryo Ψ and Ψ_π were

measured regularly, from imbibition until 3 days after germination, in thermocouple psychrometers (Wescor HR-33T dew point microvoltmeter with C52 sample chambers). Embryos were excised by cutting through the seed with a razor blade at the radicle end and also at a point judged to be near the junction of the cotyledons and the embryonic axis. Embryos so excised consisted of hypocotyl and radicle tissue without meristematic regions. Although capable of expansion (see below) these excised embryos did not develop further. For determination of embryo Ψ, 6 to 10 embryos were excised and loaded into the psychrometer chamber (volume 0.04 ml) for each measurement. Preliminary investigations showed that more than 3 embryos were required for vapour equilibration in the sample chamber without significant change in embryo water content. Between the excision and placement of each embryo the chamber was covered with a glass cover slip to reduce moisture loss and all manipulations were carried out within a high humidity enclosure (>90% relative humidity). Excision and placement was completed within 40 s per embryo. Although 10 min was found to be necessary for equilibration, all Ψ measurements were made after 30 min equilibration to ensure thermal and vapour equilibrium had been achieved after handling of the chambers. At least three replications were made of each measurement. Osmotic potential was measured on the same tissue after removal from the psychrometer and freezing inside aluminium foil packets placed in liquid N_2. Frozen tissue was returned to the

chambers to thaw before measurement.

In an additional experiment, seeds after 12 h imbibition, were cut through at a position judged to be about the end of the cotyledons to permit water flow directly to the embryo. The water relations of the embryos of these seeds were measured as above.

2.2.4 *Water Uptake and Water Relations of Excised Embryos*

Seeds were imbibed as above. At a number of times after the start of imbibition, embryos were excised from seeds, transferred to fresh water-moistened papers and their water uptake and water relations were measured. Least squares regression equations were fitted individually to all data sets when they showed linear rates of increase in fresh weight (ΔFWt).

2.2.5 *Embryo Water-Release Curve*

The water relations characteristics of tomato embryos were further investigated by constructing a water-release isotherm, or $1/\Psi$ v. relative water content (RWC) diagram for embryos excised from seeds which had been imbibed for 20 h at 25^{o}C: 5 embryos were excised into water for 2 h and then weighed, and Ψ measured. After Ψ measurement the embryos were reweighed and Ψ remeasured. This procedure was followed until a full range of Ψ measurements was obtained.

2.3 **Results**

2.3.1 *Seed Water Uptake and Water Relations*

Tomato seed water uptake was triphasic. Water content

rose rapidly for 10 h then plateaued at a level of about 80% of dry weight before increasing rapidly after radicle emergence in phase III. No evidence of a change in water content was found prior to radicle emergence in later germinating seeds (Fig. 2.1). Seed Ψ rose rapidly during phase I. The Ψ of the seeds during phase II was in equilibrium with that of the imbibitional solution and seed Ψ_π was about 0.5 MPa lower. No change in seed Ψ_π occurred following germination; both germinated and non-germinated seeds had the same Ψ_π at this time. As a consequence of the unchanging seed Ψ_π and 0 MPa Ψ, seed Ψ_p remained constant throughout phases II and III at 0.5 MPa (Fig. 2.2).

2.3.2 *Embryo Water Uptake and Water Relations within the Seed*

Embryo water uptake within the seed was also triphasic. The initial rise continued over a longer period than that of the whole seed. After 10 h the embryo water content was 45% DWt and increased slowly to 60% DWt prior to radicle emergence, after which water content increased rapidly. In only one case was it noted that embryo water content rose prior to radicle emergence. All other measurements of embryo water content of non-germinated seeds showed no change in water content at this time (Fig. 2.3).

The embryo Ψ of seeds in water continued to rise until 24 h after the start of imbibition, then plateaued at about -1.5 MPa. The embryo Ψ_π followed a similar course, but about 0.3 MPa lower, thus Ψ_p was about 0.3 MPa until 27 h

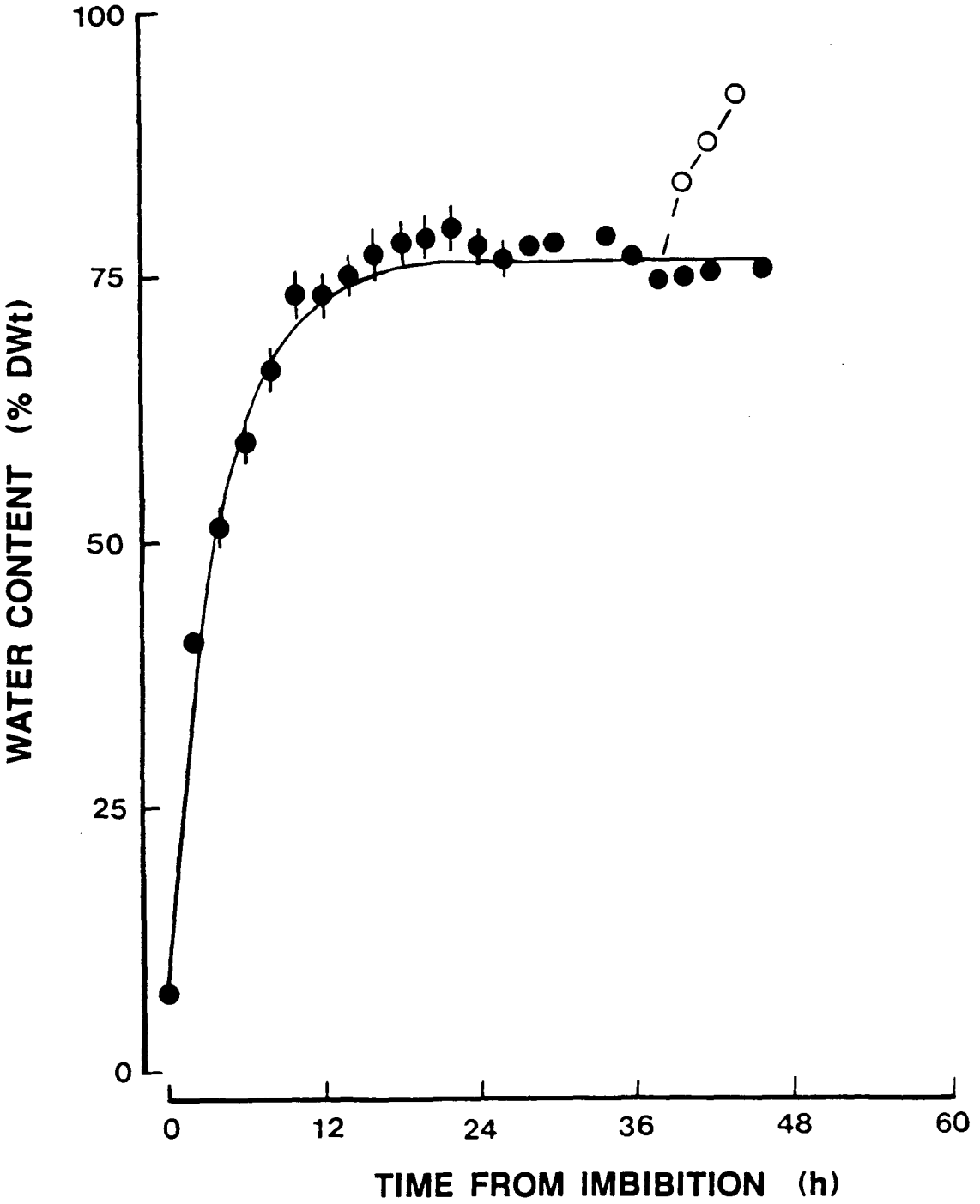

Fig. 2.1. Water uptake pattern of tomato seeds during germination. Vertical bars show s.e. of the means of 10 seeds. Open symbols are for germinated seeds. Lines are drawn by eye.

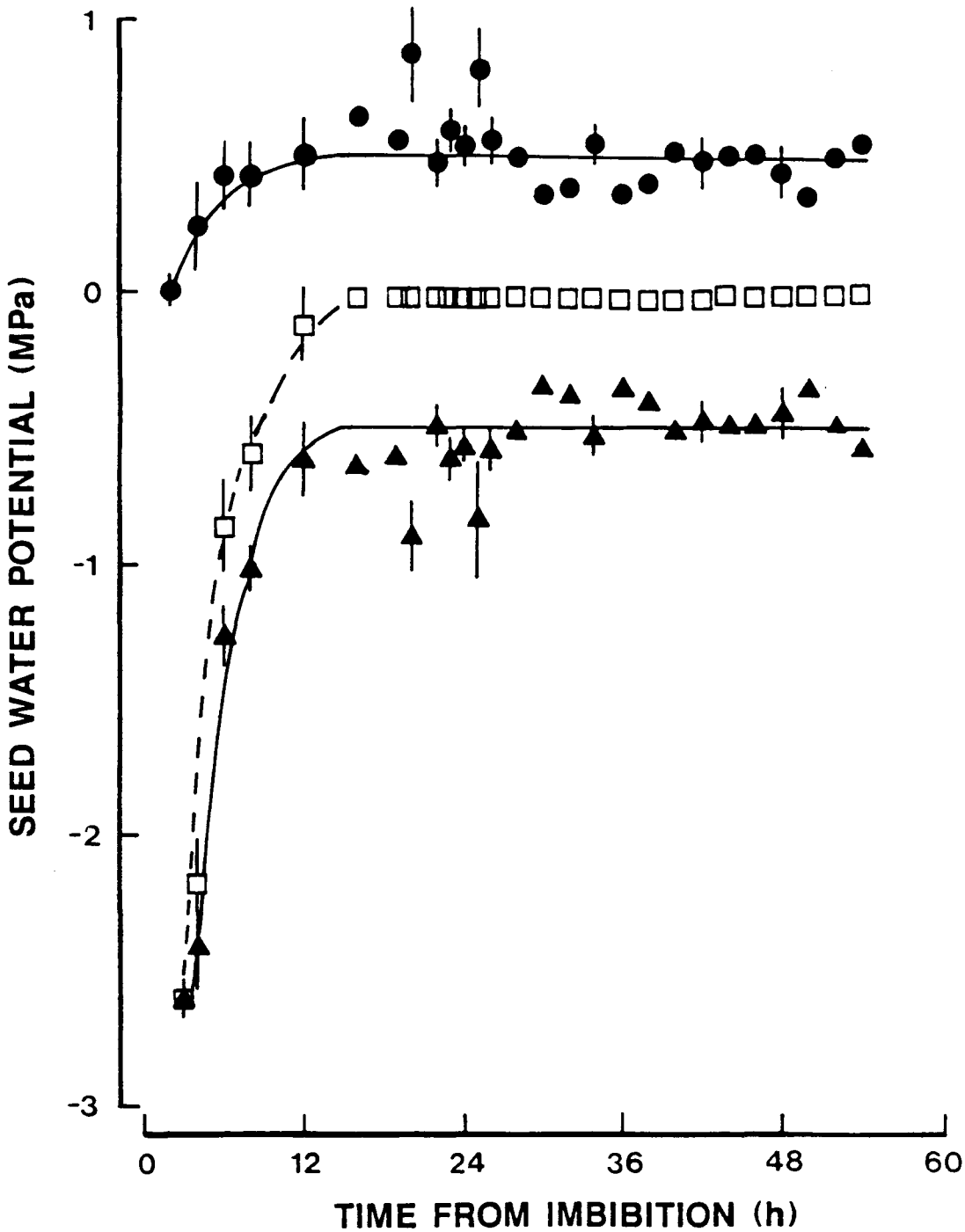

Fig. 2.2. Ψ (□), Ψ_π (▲), Ψ_p (●) of tomato seeds during germination. Vertical bars show s.e. of the means of 5 replicates of 15 seeds each. Lines are drawn by eye.

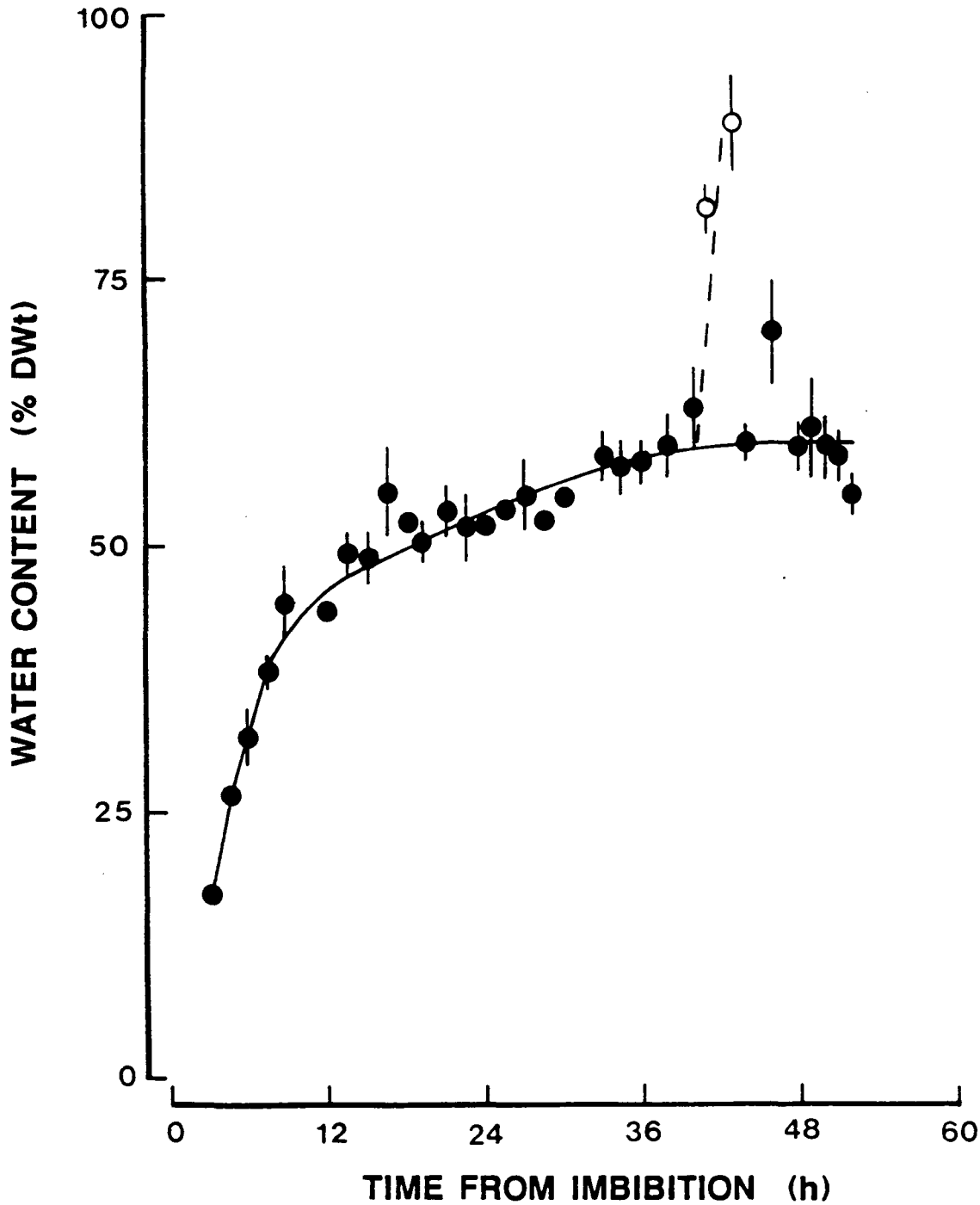

Fig. 2.3. Water uptake pattern of embryos within tomato seeds during germination. Vertical bars show s.e. of the means of 5 embryos. Open symbols are for embryos post radicle emergence. Lines are drawn by eye.

after which it became variable. Ψ_p did not appear to increase prior to radicle emergence nor was there evidence of a lowering in Ψ_π prior to radicle emergence (Fig. 2.4).

The Ψ of the embryo at radicle emergence was -0.84 MPa. However, the Ψ of the embryo from non-germinated seeds imbibed for the same time remained at the levels measured prior to the beginning of germination in the population of seeds. As the radicle grew its Ψ rose to -0.3 MPa (Fig. 2.4).

Cutting the testa, endosperm and cotyledons during imbibition permitted the Ψ of the embryo to rise rapidly to -1.0 MPa. No further rise was detected prior to radicle protrusion (Fig. 2.5). The cotyledons were seen to protrude from the cut testa within 3 to 8 h after the incision was made. In those cut seeds from which no cotyledon protrusion occurred within 10 h, the embryo Ψ was the same as for intact seeds.

2.3.3 *Water Uptake and Water Relations of Excised Embryos*

Embryos when excised from seeds and placed in water immediately increased their FWt by about 20% and remained at this water content for varying amounts of time depending on the time at which they had been excised (Fig. 2.6). As an indication of the time at which the linear phase of water uptake (fresh weight growth) began, the time at which 25% ΔFWt was reached was determined from regression equations fitted to the linear portion of the water uptake curve. The

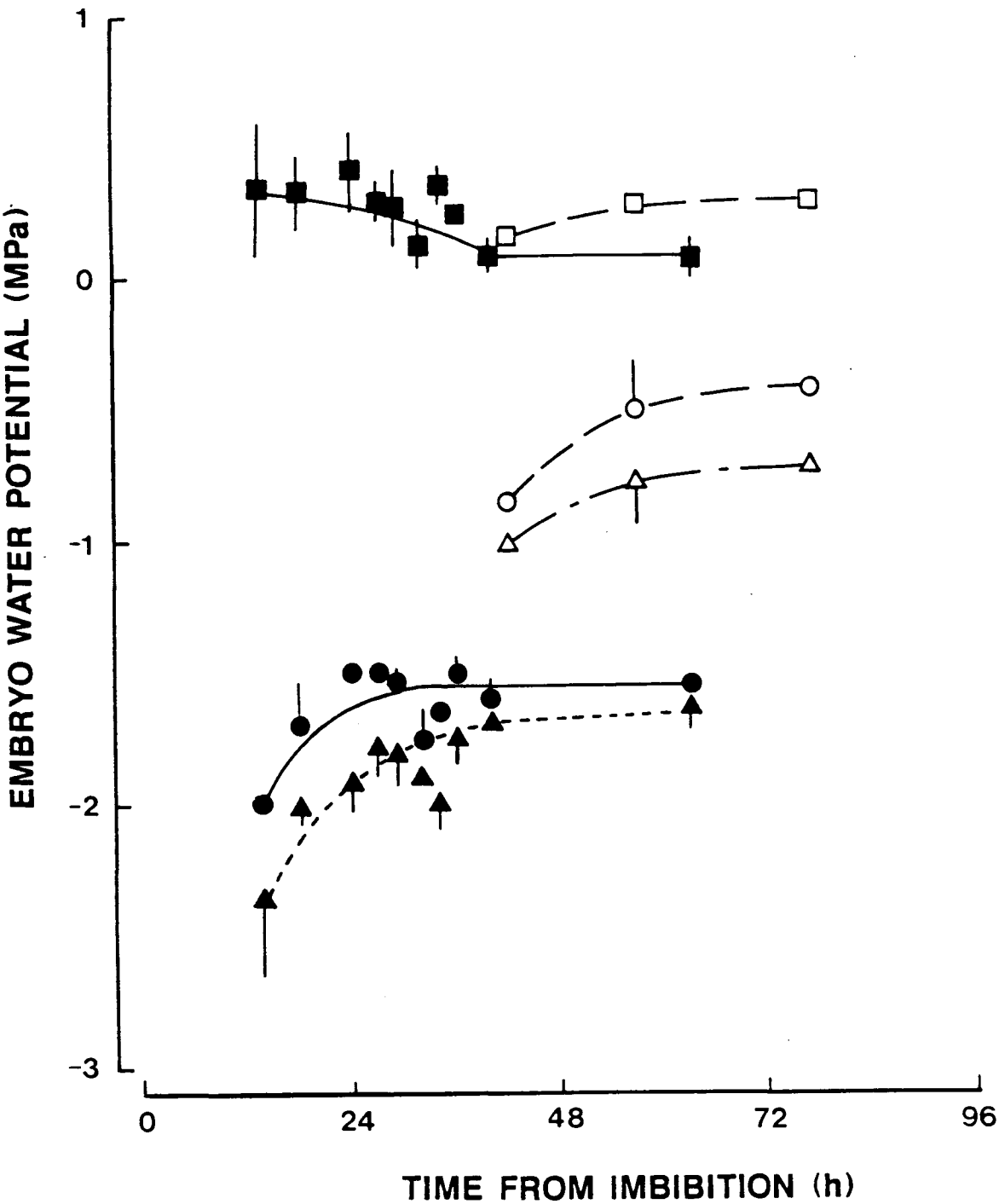

Fig. 2.4. Ψ (○, ●), Ψ_π (△, ▲), Ψ_p (□, ■) of the embryo during germination of tomato seeds. Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. Open symbols are for embryos post radicle emergence. Lines are drawn by eye.

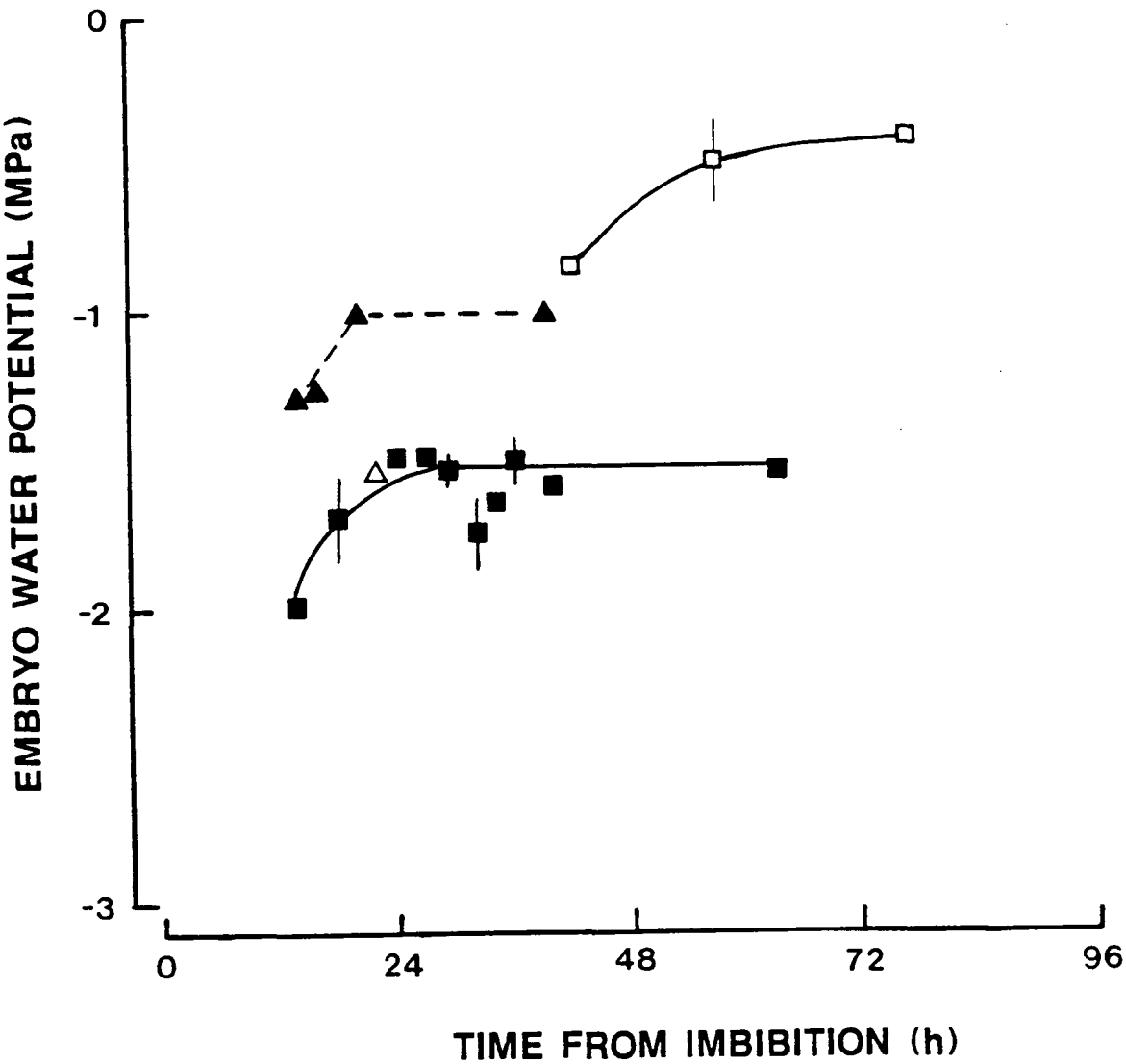

Fig. 2.5. The Ψ of embryos during the germination of tomato seeds which were cut 12 h after the start of imbibition (△, ▲) and from intact seeds (□, ■). Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. Open symbols (□) are for embryos from intact seeds post radicle emergence and (△) for embryos from cut seeds from which the cotyledons had not emerged at this time. Lines are drawn by eye.

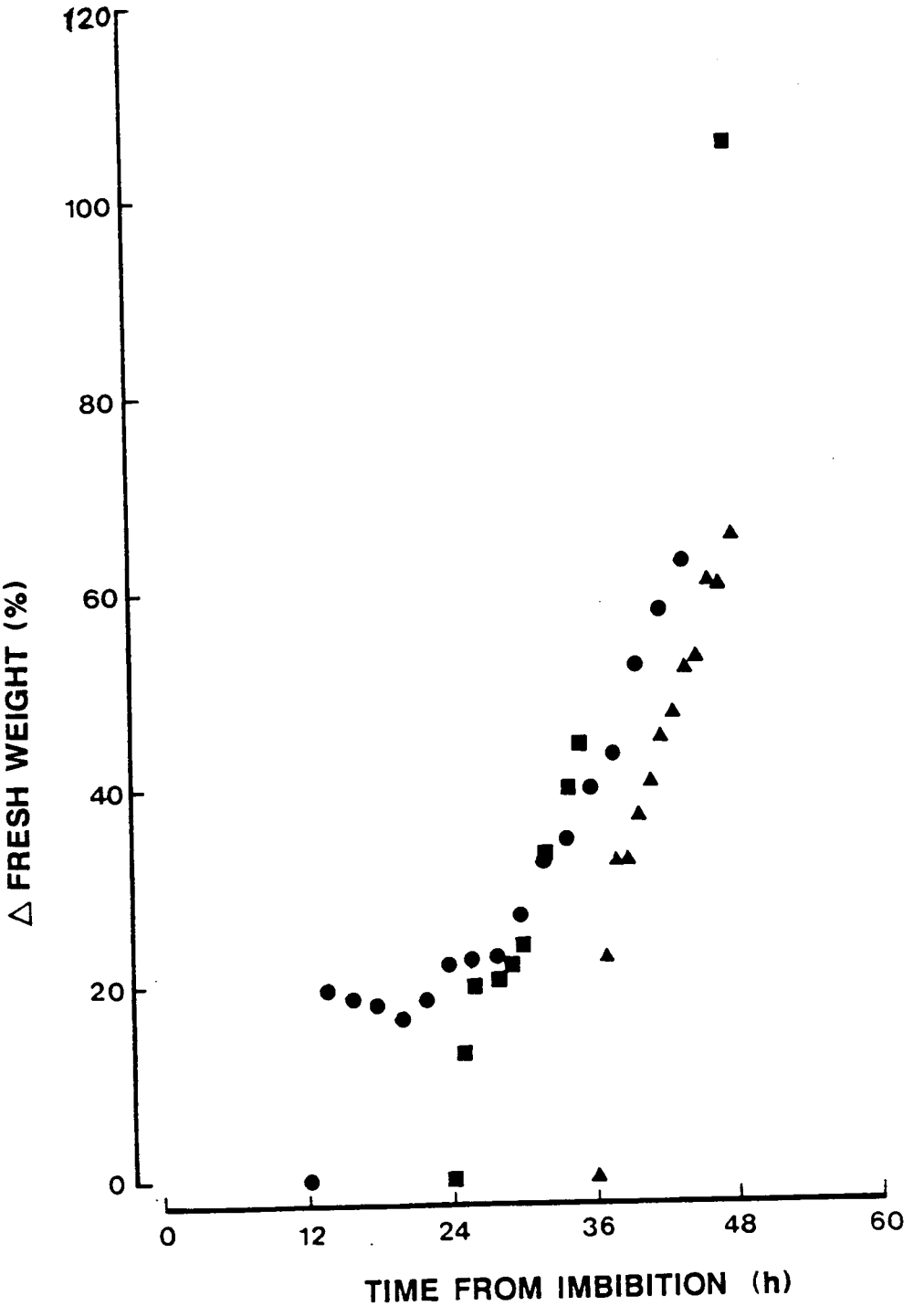

Fig. 2.6. Change in fresh weight of embryos excised from seeds 12 (●), 24 (■) or 36 h (▲) after the start of imbibition of the seed. Data shown are for individual embryos. Similar measurements were made on 46 embryos.

excised embryos began the linear phase of growth from 31 ± 0.67 h (mean ± s.e., n = 36) after the start of seed imbibition. Excision before this time did not advance the time at which growth began. The excised embryo Ψ rose to -0.3 MPa within 1 h. The Ψ of excised embryos remained unchanged thereafter (Fig. 2.7).

2.3.4 *Embryo Water-Release Curve*

The water-release curve for tomato embryos departed from linearity, indicating positive Ψ_p, at RWC >55% (water contents >63% DWt). Ψ values estimated from the water-release curve were in agreement with measured Ψ values of the embryos excised from imbibing seeds (Fig. 2.8). The water-release curve indicated that the Ψ_π at full turgor (RWC = 100%) was equal to -0.65 ± 0.03 MPa (mean ± s.e., n = 3), which was in good agreement with the value of -0.75 ± 0.04 MPa (n = 9) determined psychrometrically.

2.4 Discussion

Water uptake of UC 82B tomato seeds was triphasic (Fig. 2.1). During phase I the large water potential gradient between the seed and the solution resulted in rapid water uptake. During phase II the seed Ψ reached equilibrium with the water (Fig. 2.2). These observations are consistent with those of McDonough (1975) and Liptay and Schopfer (1983) and the suggestion of Bradford (1986), but contrary to the suggestion of Bewley and Black (1985) that seed Ψ was generally about -1.0 MPa during phase II. Water uptake

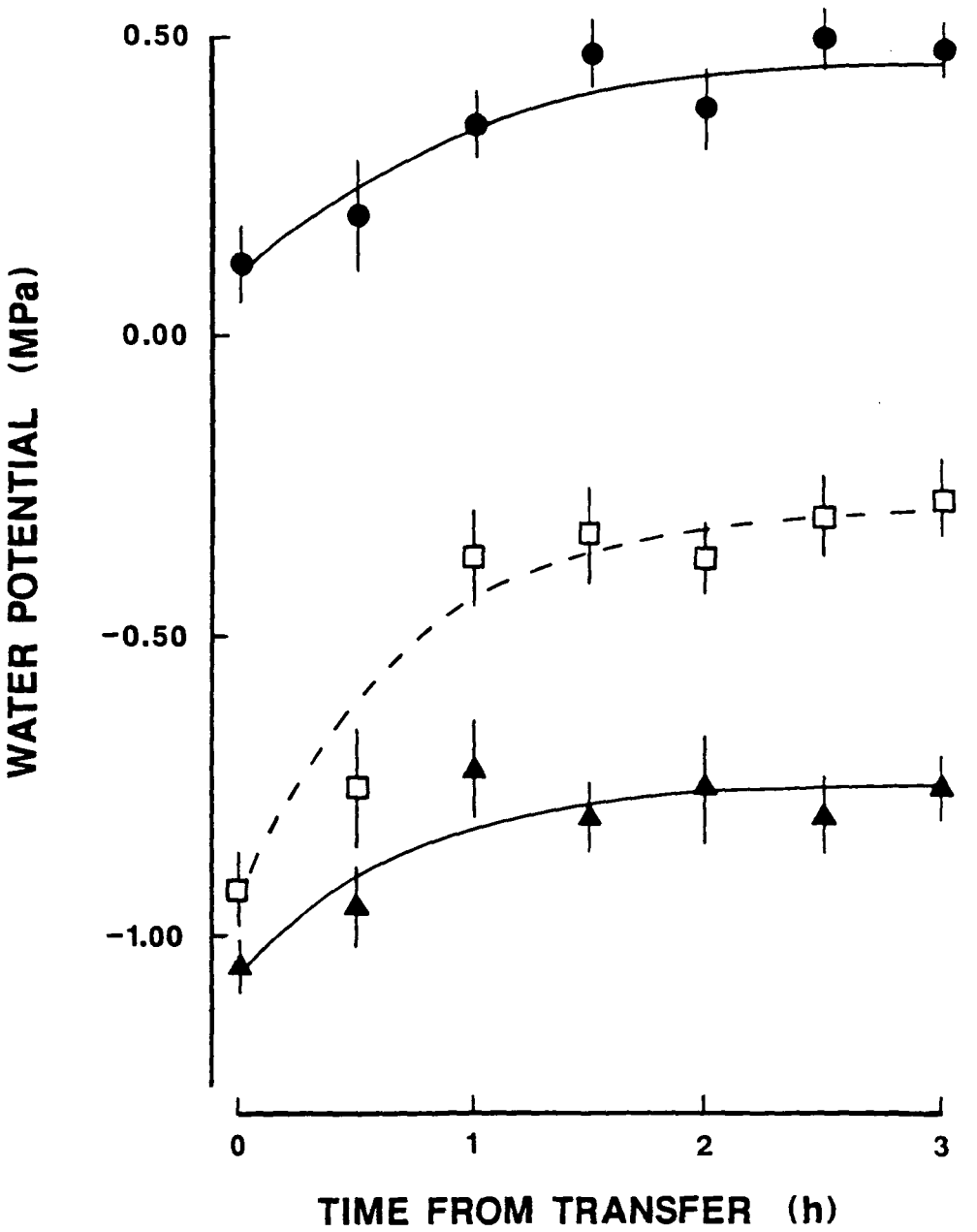

Fig. 2.7. Change in embryo Ψ (□), Ψ_π (▲), Ψ_p (●) after excision from the seed and transfer to water. Seeds were imbibed for 24 h before the embryos were excised. Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. Lines are drawn by eye.

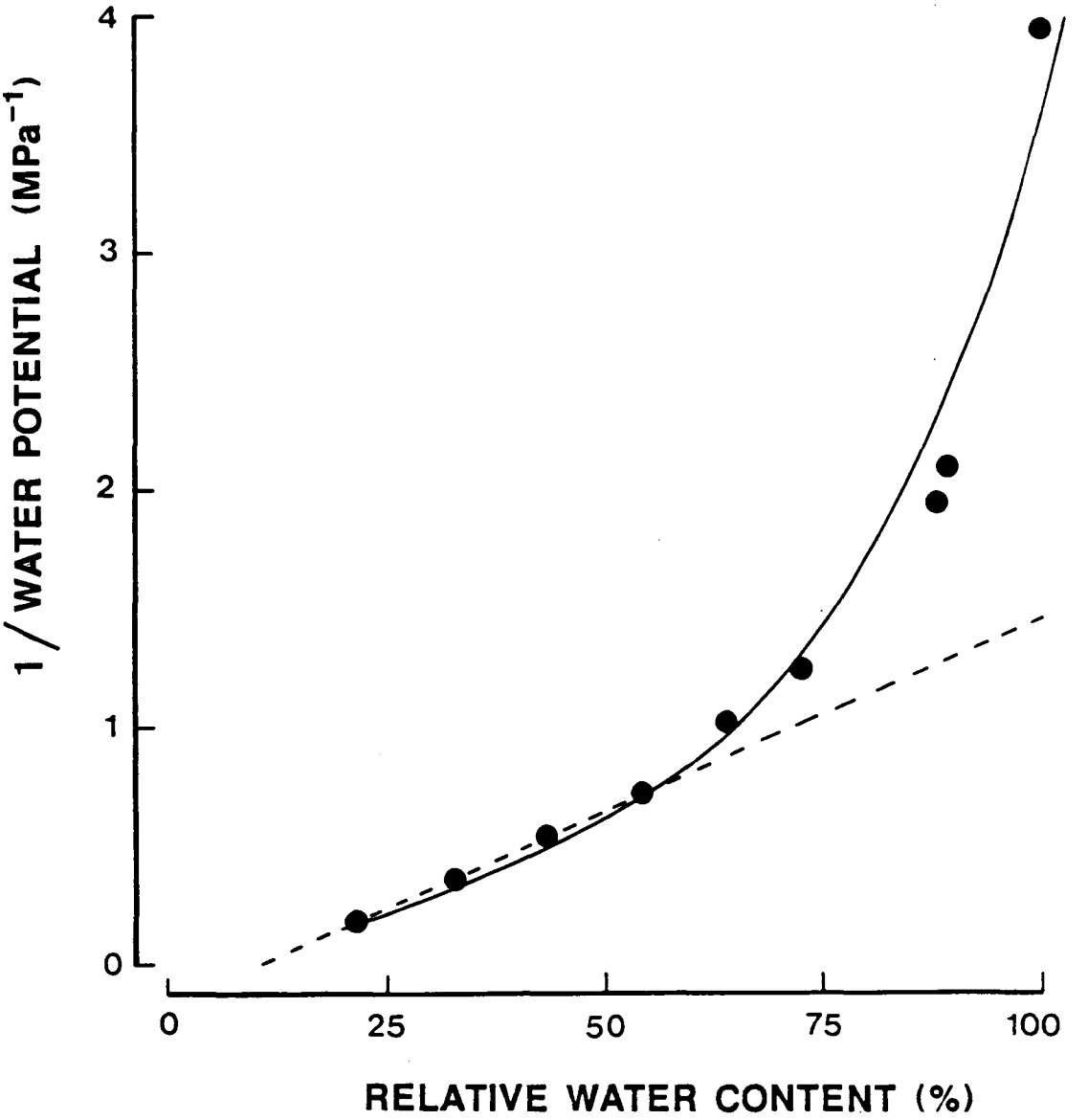

Fig. 2.8. Water-release isotherm of tomato embryos excised from seeds imbibed for 20 h then imbibed in water for 2 h. Data are from one experiment using five embryos. The experiment was repeated three times. Regression equations for the lines shown are: $\ln(1/\Psi) = -2.31 + 0.036$ RWC (r^2 = 98.4%) (———) and, for RWC <60%, $1/\Psi = -0.176 + 0.016$ RWC (r^2 = 99.9%) (– – –).

recommenced (phase III) with the onset of radicle emergence without a detectable change in seed water or osmotic potential (Fig. 2.2).

While the seed was in water potential equilibrium with the imbibitional solution during phase II, there appeared to be a large disequilibrium in Ψ within the seed because the embryo Ψ and Ψ_π remained well below that of the whole seed. There was no lowering of embryo Ψ_π nor buildup of Ψ_p during phase II (Fig. 2.4). This observation is in contrast to the suggestions of Bewley and Black (1985) and Bradford (1986). The maintenance of such a large $\Delta\Psi$ within the tomato seed raises some interesting questions about its water relations during germination.

Despite the large difference in Ψ between the embryo and the seed, two lines of evidence suggest that the reduced hydration level of the embryo was not caused by a permeability barrier within the embryo. Moreover, the existence of such a barrier to water uptake in the radicle would not be consistent with its role as the primary root of the developing seedling. Firstly, excised embryos immediately took up water upon transfer to moistened filter papers, indicating that there was no inherent barrier to water uptake (Fig. 2.6). Secondly, cutting of the seed coat and endosperm layers enclosing the embryo resulted in a rise in embryo Ψ (Fig. 2.5). Interestingly, the embryo Ψ of the cut seed rose only to -1.0 MPa, not to -0.3 MPa as happened when embryos were excised and placed in water. This indicated that some constraint to water uptake still existed

even though the embryo (cotyledons) had been exposed directly to water and some expansion (resulting in cotyledon protrusion) had occurred. In cut seeds from which cotyledons did not protrude there was no change in embryo Ψ (Fig. 2.5) indicating that embryo hydration was still fully constrained.

It can be concluded from this evidence that water uptake by the embryo was restricted by the endosperm layers enclosing it. Restriction of embryo water content could result from constraint on the expansion of the embryo by the endosperm. Such constraint would need to result in the development of a large Ψ_p of about 1.5 MPa if the embryo were to achieve water potential equilibrium with the rest of the seed.

Groot and Karssen (1987) have shown that the force necessary to puncture the endosperm layers enclosing the tomato embryo decreased during phase II and that this decrease was essential for radicle emergence. No change occurred in the small resistance shown by the seed coat. Control of endosperm weakening was mediated through the action of gibberellic acid produced by the embryo and could by substituted for by exogenous gibberellins. Control of production of gibberellic acid in the embryo and/or its action in the endosperm would control germination.

The embryo water relations measurements reported here were made on excised tissue and thus excluded the effects of constraint. Restraint of embryo expansion would increase

the *in situ* embryo Ψ_p to a higher value than that measured in the excised embryo. It is thus possible for a large embryo Ψ_p to exist during phase II without the requirement of solute accumulation to lower embryo Ψ_π. Thus radicle emergence would be dependent upon the reduction in endosperm constraint rather than upon the buildup of embryo Ψ_p.

Comparison of the results obtained in this study of tomato seeds with observations made on other species indicates that there may be a common mechanism controlling embryo water relations in morphologically different seeds. McIntyre and Hsiao (1985) reported that dormancy of *Avena fatua* was caused by factors which prevented water uptake by the embryo. Removal of or piercing the tissues enclosing the *A. fatua* embryo resulted in a rise in embryo water content followed by germination. Gibson and Bachelard (1986) reported that the Ψ of the *Eucalyptus sieberi* hypocotyl stabilized at -4.5 MPa prior to germination and concluded that the reduced hydration of the embryo resulted from the inhibition of water uptake by the inner integument.

The tomato embryos were capable of a linear rate of increase in FWt, *i.e.*, sustained growth, from 31 h after the start of imbibition although radicle emergence did not occur for at least another 5 h (Fig. 2.1). The timing of growth enablement was the same whether hydration of the embryo occurred in the seed or after excision, suggesting that the growth-enabling processes were gene regulated and not dependent on hydration level. The mechanism which controls growth enablement in tomato embryos requires investigation.

These results suggest that the water relations of the tomato embryo during germination are controlled by the endosperm and that lowering of embryo Ψ_π to buildup Ψ_p is not necessary for radicle emergence. The control of tomato seed germination thus appears to lie in the events which lead to weakening of the mechanical restraint of the endosperm tissue.

The sequence of events which are necessary for the germination of tomato seeds as identified by this study are as follows: (1) water is imbibed until a Ψ equilibrium is established between the seed and the imbibitional solution; (2) the embryo cell walls are loosened to enable their expansion when (3) the endosperm resistance is sufficiently reduced to permit radicle emergence. The changes to this sequence which prevent radicle emergence during priming are investigated in the next chapter.

Water Relations of Tomato Seeds during Priming

3.1 Introduction

In the previous chapter it was concluded that tomato seeds germinated as the result of weakening of the endosperm layers enclosing the radicle tip. It was also shown that the embryo of a germinating tomato seed was apparently not in equilibrium with the imbibitional solution during phase II of water uptake during germination. In this chapter I investigate the water relations of seeds during priming to ascertain possible mechanisms by which germination is prevented.

Seeds are primed by soaking in a solution of appropriate osmotic potential which permits some of the preliminary processes of germination, but which prevents radicle emergence (Heydecker and Coolbear 1975). The mechanism by which radicle emergence is prevented is not known.

Control of either water uptake or cell wall loosening may prevent radicle emergence during priming. For radicle emergence to occur there must be cell expansion within the embryo (Sect. 1.2). Cell expansion involves two interrelated physical processes: water absorption and cell wall yielding. Cell wall loosening reduces the cell water potential by the dissipation of turgor permitting water uptake which increases cell volume (Cosgrove 1986). Water uptake may be the controlling factor for the prevention of radicle emergence during priming.

Water uptake should cease during priming when a Ψ equilibrium is established between the seed and the priming solution. While water uptake by germinating seeds is a triphasic process (Bewley and Black 1985; Fig. 2.1) water uptake during priming should be restricted to the first two phases. As was shown for germinating seeds, phase I of water uptake during priming should occur because of the large $\Delta\Psi$ between the dry seed and the priming solution (*cf.* Figs 2.1 & 2.2).

A Ψ equilibrium between the seed and the solution may prevent germination by preventing the uptake of the water necessary for the expansion of the radicle. A similar mechanism of prevention of cell expansion has been proposed for the roots of salt affected plants. Cell expansion in these roots is initially limited by induced water deficits and reduced cell turgor (Munns and Termaat 1986). During priming cell expansion in the radicle could be prevented by either or both of two mechanisms. Firstly, the lack of an adequate $\Delta\Psi$ between the embryo and the priming solution which may restrict water uptake. Secondly, germination may be prevented by the embryo developing insufficient Ψ_p to overcome the resistance of the endosperm and seed coat layers enclosing the radicle.

A much reduced $\Delta\Psi$ between the embryo and the priming solution may prevent radicle emergence during priming if the embryo Ψ remained at the phase II level (-1.5 MPa) of seeds in water (Fig. 2.4). This is about 0.4 MPa below the

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Ψ_π of the priming solution used for these seeds, 0.090M K_2HPO_4 + 0.118M KNO_3 (Haigh and Barlow 1987). Measurement of seed and embryo water relations during priming would be necessary to clarify our understanding of these processes.

Prevention of germination during priming could also result from the prevention of the usual decrease in endosperm and seed coat mechanical resistance. The mechanical resistance offered by the endosperm tissue enclosing the radicle decreases prior to radicle emergence in tomato seeds. The decrease is mediated through gibberellic acid (GA) translocated from the embryo (Groot and Karssen 1987). Inhibited production of or action of GA during priming would prevent the decrease in endosperm resistance and thus prevent radicle emergence. This will not be directly tested in this study.

While endosperm resistance decreases prior to radicle emergence some mechanical restraint remains at the time of radicle protrusion (Groot and Karssen 1987). Although the restraining layers are weakened it is still necessary for the radicle to develop sufficient turgor to overcome this restraint. The embryo may be unable to overcome the endosperm restraint through insufficient development of turgor during priming. Turgor development would be associated with solute accumulation to lower embryo osmotic potential to permit water uptake. The water relations measurements will permit evaluation of this proposed mechanism.

An alternative mechanism for the prevention of

germination during priming may lie in embryo cell wall yielding. While it is necessary for a water potential gradient to exist for water uptake its existence is not sufficient for cell expansion to result, the embryo cells must be capable of expansion before radicle protrusion can occur. The embryos of tomato seeds were incapable of sustained growth during the first 30 h of imbibition in water at 25°C (Fig. 2.6). Should this incapacity not be relieved during priming then germination may be prevented. Measurement of embryo fresh weight gain following excision into water would enable evaluation of this hypothesis.

In this chapter I evaluate a number of alternative mechanisms which may prevent germination of tomato seeds during priming: (1) an insufficient $\Delta\Psi$ between the embryo and the priming solution; (2) lack of embryo cell wall yielding; (3) prevention of endosperm weakening; or (4) insufficient embryo turgor to overcome the endosperm and seed coat resistance. These mechanisms are evaluated through measurement of the water relations of priming tomato seeds.

3.2 Materials and Methods

3.2.1 *Water Uptake by Tomato Seeds during Priming*

Tomato seeds (UC 82B, Yates Seeds, Sydney, Australia) were primed in a solution of 0.090M K_2HPO_4 + 0.118M KNO_3 at 25°C for 6 days. Approximately 200 seeds were placed on filter papers (Ekwip U70, Industrial Equipment, Sydney,

Australia) moistened with approximately 16 ml of solution in 90 mm glass Petri dishes. At every measurement time 5 seeds were surface dried between layers of tissue paper before weighing on a CAHN 25 microbalance (Ventron Instruments Corporation, Cerritos, CA, U.S.A.) then dried at 80°C for 72 h before reweighing for dry weight.

3.2.2 *Water Relations of Tomato Seeds during Priming*

Approximately 200 seeds were primed at 25°C as above and seed Ψ and Ψ_π were measured in Peltier type thermocouple psychrometers at regular intervals during the 6 d priming treatment as described previously (Sect. 2.2.2).

3.2.3 *Water Uptake and Water Relations of the Embryo within the Priming Seed*

Seeds were primed at 25°C as above. At frequent intervals seeds were removed from the Petri dishes and the embryo excised, water content, Ψ and Ψ_π were measured as described previously (Sect. 2.2.3).

3.2.4 *Water Uptake by Embryos Excised from Priming Seeds*

Seeds were primed at 25°C as above. At a number of times embryos were excised from seeds, transferred to water-moistened papers and their water uptake was measured. Least squares regression equations were fitted individually to all data sets when they showed linear rates of increase in fresh weight.

3.3 **Results**

3.3.1 *Seed Water Uptake and Water Relations*

Water uptake during priming of tomato seeds was biphasic. Water content rose rapidly during the first day of priming then plateaued at about 72% DWt for the duration of the priming treatment (Fig. 3.1). Seed Ψ followed a similar pattern plateauing at -1.1 MPa during priming indicating that the seed was in equilibrium with the Ψ_π of the 0.090M K_2HPO_4 + 0.118M KNO_3 priming solution. Seed Ψ_π remained at about 0.17 MPa below the seed Ψ throughout priming indicating no pattern of solute accumulation. Seed Ψ_p was thus nearly constant at 0.17 MPa after the first day of priming (Fig. 3.2).

3.3.2 *Embryo Water Uptake and Water Relations within the Priming Seed*

Embryo water content increased rapidly during the first day of priming then plateaued at about 45% DWt (Fig. 3.3). The embryo Ψ during priming plateaued at -2.32 $\pm$ 0.03 MPa (mean $\pm$ s.e., n = 71). The embryo Ψ_π fell from -2.28 $\pm$ 0.22 MPa after 1 d to remain about -2.92 $\pm$ 0.06 MPa throughout priming, thus resulting in Ψ_p of 0.59 $\pm$ 0.05 MPa. There was a slight trend for of a lowering of embryo Ψ_π; however, this may not be significant as the measured values were not significantly different between 2 and 6 d of priming (Fig. 3.4).

3.3.3 *Water Uptake by Excised Embryos*

The change in fresh weight of excised embryos in water showed that they were capable of immediate expansion after

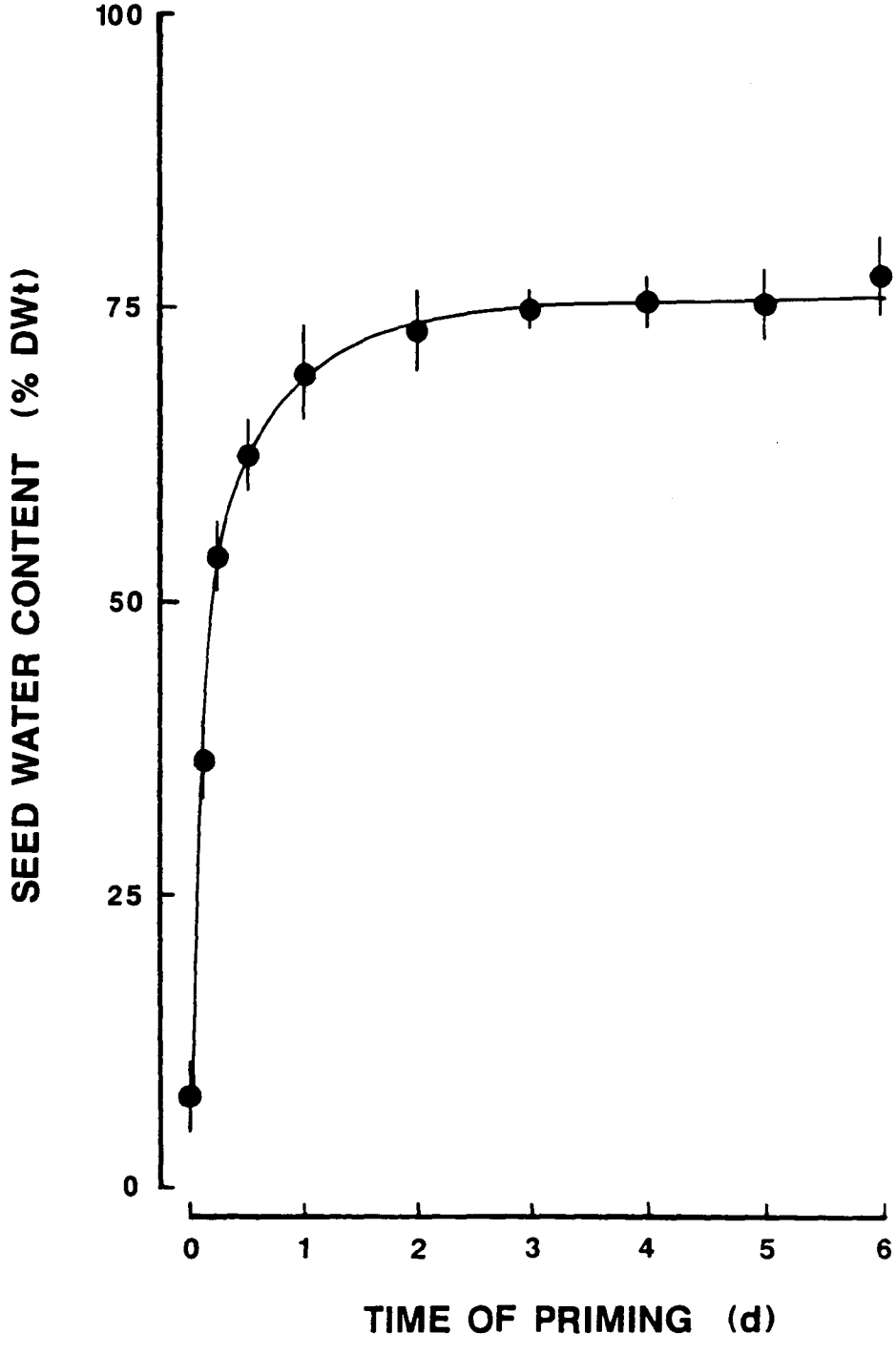


Fig. 3.1. Water uptake pattern of tomato seeds during priming. Vertical bars show s.e. of the means of 5 seeds. Lines are drawn by eye.

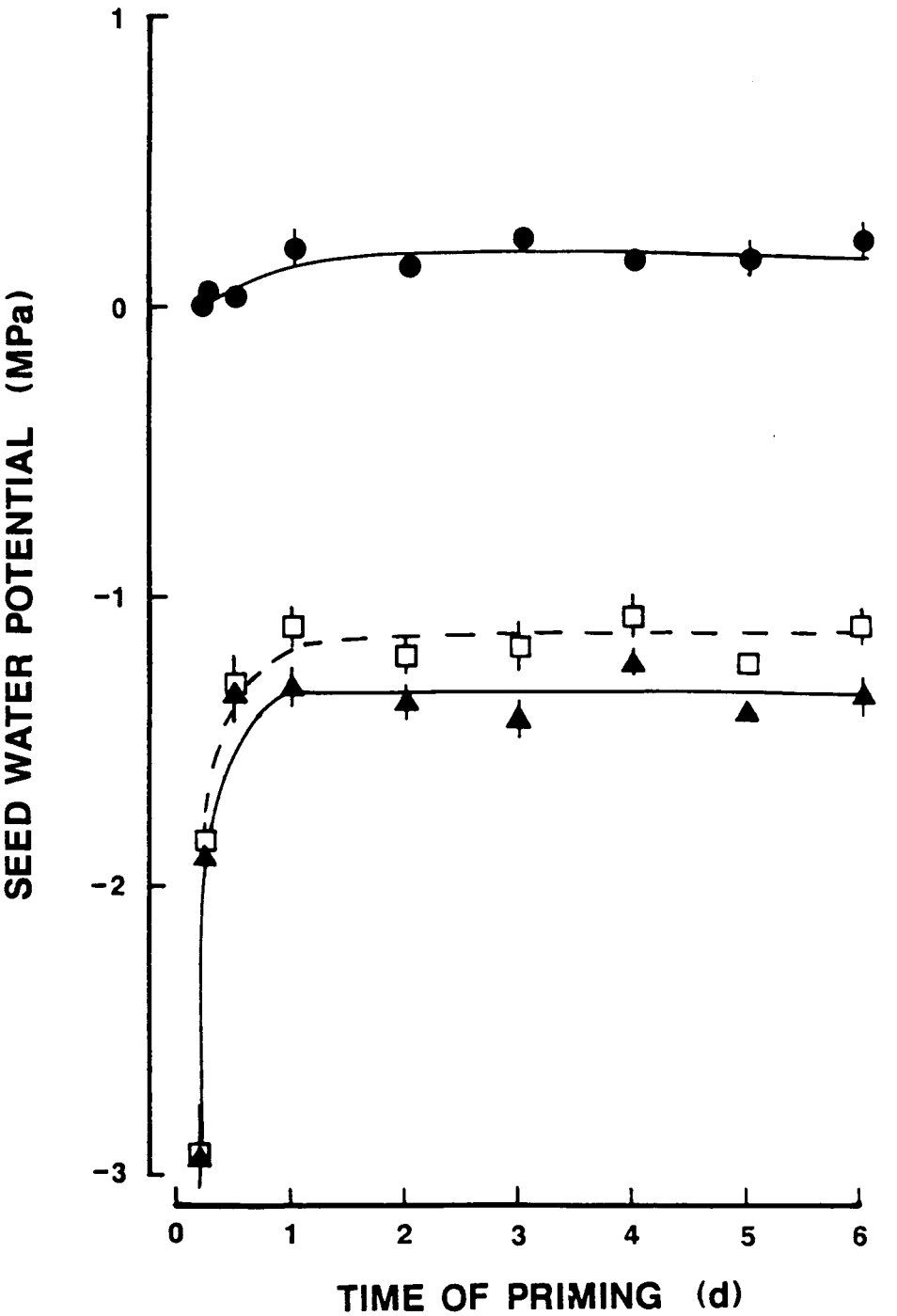


Fig. 3.2. Ψ (□), Ψ_π (▲), Ψ_p (●) of tomato seeds during priming. Vertical bars show s.e. of the means of 5 replicates of 15 seeds each. Lines are drawn by eye.

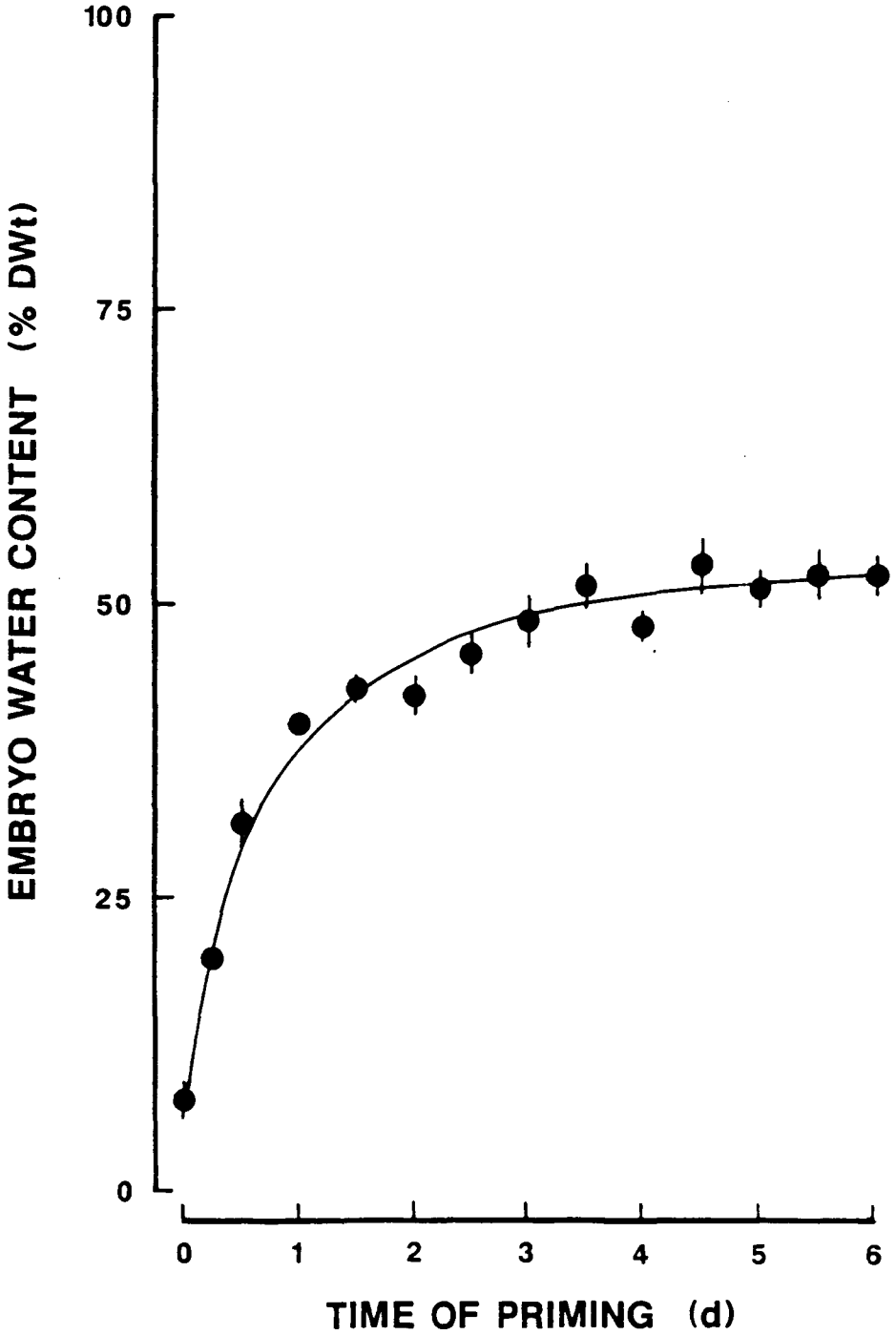


Fig. 3.3. Water uptake pattern of embryos within tomato seeds during priming. Vertical bars show s.e. of the means of 5 embryos. Lines are drawn by eye.

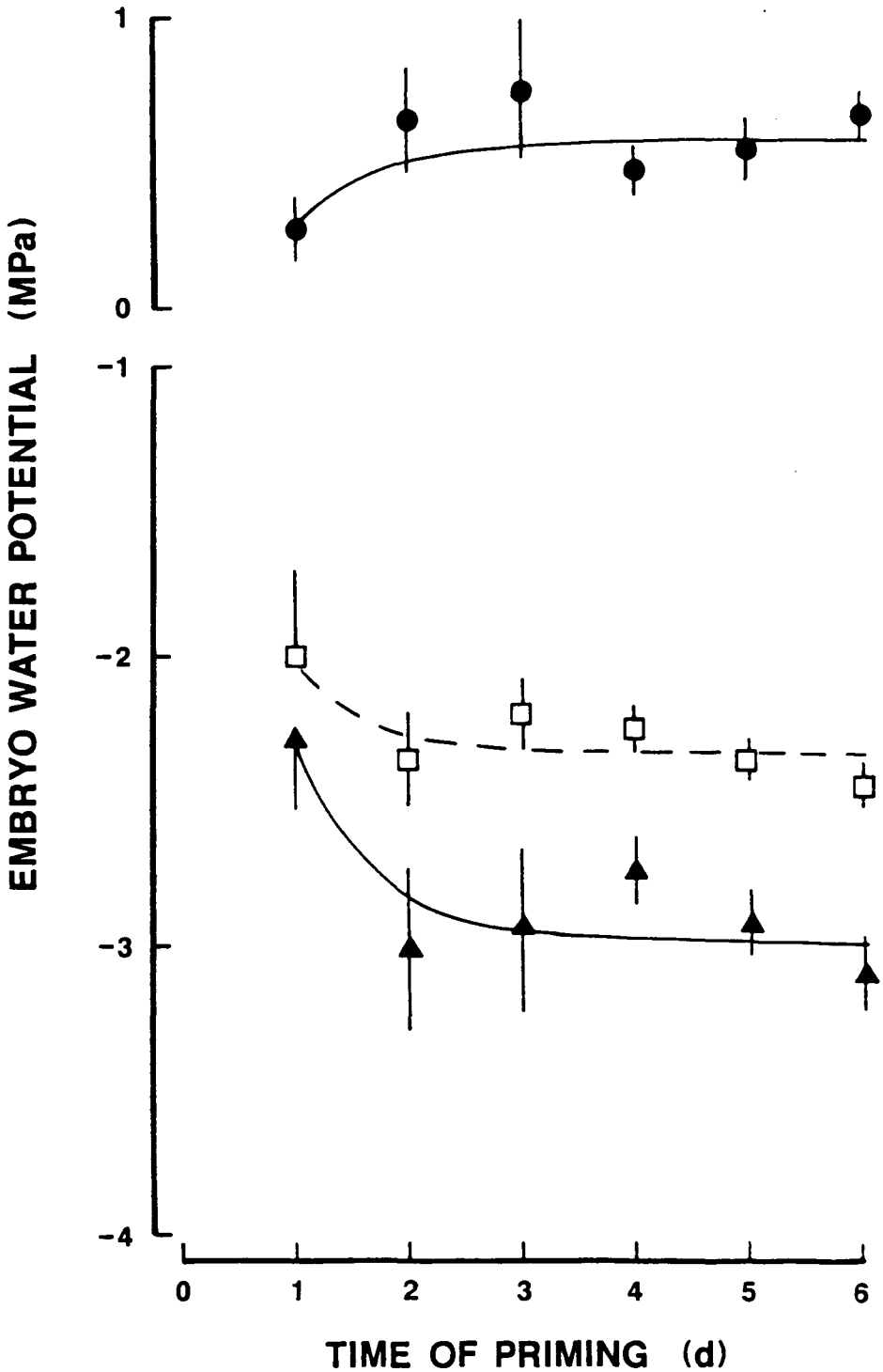


Fig. 3.4. Ψ (□), Ψ_π (▲), Ψ_p (●) of the embryo during priming of tomato seeds. Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. For simplicity of presentation only data at 24 h intervals are shown although more frequent measurements were made. Lines are drawn by eye.

2 d of priming. Embryos excised from seeds primed for 1 d were not capable of sustained water uptake until a further 12 h had elapsed (Fig. 3.5).

3.4 Discussion

Water uptake by tomato seeds during priming was biphasic (Fig. 3.1) because the Ψ of the seeds came into equilibrium with the Ψ_π of the priming solution (-1.1 MPa) after 1 d of priming (Fig. 3.2). Seed water content during priming was about 8% DWt lower than that of seeds in water during phase II of water uptake (Fig. 2.1). Similar reductions in water content have been reported for carrot, calabrese and lettuce seeds imbibed in a range of polyethylene glycol solutions (Hegarty 1977; Bradford 1986).

Prevention of radicle emergence during priming was not caused by a reduced $\Delta\Psi_\pi$ between the embryo and the priming solution. Imbibition in a -1.1 MPa solution directly lowered the embryo Ψ_π by this amount. At -2.9 MPa, embryo Ψ_π during priming was 1.1 MPa lower than during phase II of water uptake of seeds in water. The $\Delta\Psi_\pi$ between the embryo and the priming solution was thus exactly the same as that between the embryo and imbibing water during phase II of water uptake of non-primed seeds (Fig. 2.4). This large Ψ gradient between embryo and solution persisted throughout priming (Fig. 3.4).

Radicle emergence was not prevented by the lack of embryo cell wall loosening during priming. The water uptake data for embryos excised into water showed that the embryo

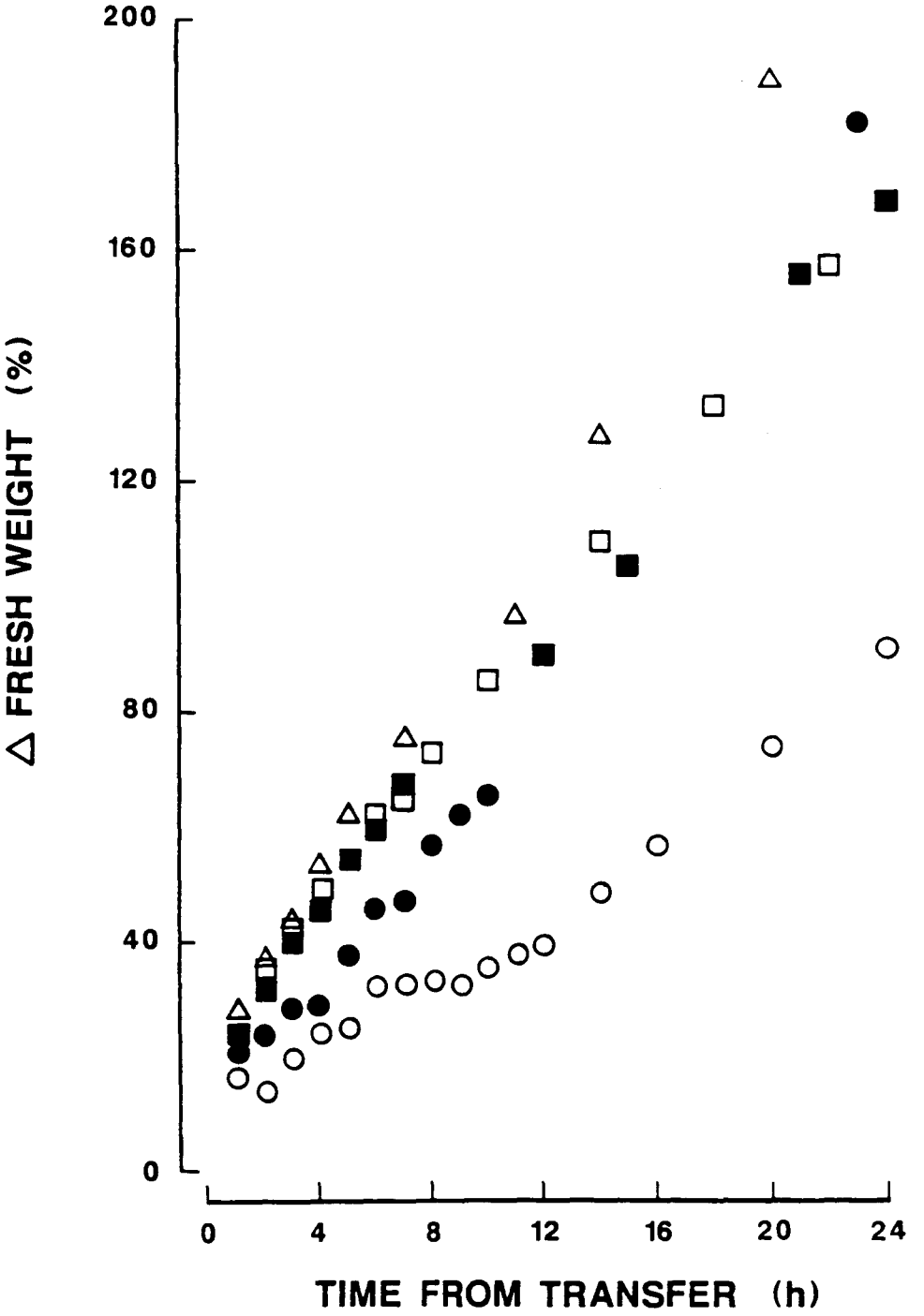


Fig. 3.5. Change in fresh weight of embryos excised from seeds 1 (○), 2 (●), 3 (□), 4 (■) or 6 d (△) after the start of priming of tomato seeds. Data shown are for individual embryos. Similar measurements were made on 70 embryos.

was readily capable of expansive growth from the second day of priming onwards. The timing of embryo cell wall loosening was the same in all situations in which it was determined. Embryos excised after 1 day of priming began to expand at the same time as those excised after 1 day in water (Figs 3.5 & 2.3). This may indicate that the process of growth enablement is developmentally regulated by a mechanism independent of fluctuations in water content.

Embryo Ψ_p during priming, at 0.6 MPa, was larger than that during phase II of water uptake of seeds in water (Fig. 2.4). As discussed above (Sect. 2.4) the reported embryo Ψ_p is based on the difference between measurements of Ψ and Ψ_π of embryos excised from their seeds. Therefore this may not be an accurate measure of actual *in situ* Ψ_p. It can be assumed to be somewhere between the calculated values and 1.8 MPa, the $\Delta\Psi_\pi$ between the embryo and the priming solution. Whatever the actual *in situ* Ψ_p value may be, it is insufficient to overcome the resistance offered by the enclosing endosperm and seed coat tissues during priming.

The measurements of embryo Ψ_π reported here reveal the difficulty associated with such tissue averaged measures. While there was some indication of a lowering in embryo Ψ_π during priming, it was of such a small order that the Ψ_π was not significantly different between 2 and 6 d of priming. A more direct measurement technique needs to be used to determine whether specific solutes accumulate in the embryo during priming. Bradford (1986) inferred from moisture-release isotherms of lettuce seeds that solute accumulation

had occurred during priming. Whole seed measurements reflect volume averaged changes in all tissues and therefore inferences concerning the radicle should be interpreted with caution.

While imbibition in a priming solution resulted in seed water contents below those of seeds in water, this reduction was unevenly distributed within the seed. There was 25% less water in the embryo during priming (reduced from 60% DWt to 45% DWt) (Figs 3.3 & 2.3), whereas the whole seed had 10% less water (from 80% DWt to 72% DWt) (Figs 3.1 & 2.1). This would indicate that the water potential/water content relationships of the embryo and the rest of the seed are quite different.

On a seed volume basis the embryo (as measured) occupies approximately one third, the cotyledons occupy another third with the endosperm and seed coat making up the balance. As the cotyledons are quite similar tissue to the rest of the embryo it is likely that the endosperm and seed coat cause the difference in water content. Solution uptake by the seed coat could be expected to be similar in a priming solution to that in water. The endosperm cells could be expected to have thickened walls by comparison with those of the embryo. These stiff walls would result in less volume change for an equivalent Ψ change in thin flexible walled tissue like that of the embryo.

In addition to the stiffer walls of endosperm cells their water content during priming may be maintained by some

degree of vacuolation of these cells. Vacuolation of endosperm cells during germination of tomato seeds has not been reported, but has been found prior to germination in celery (Jacobsen and Pressman 1979), lettuce (Psaras 1984) and sunflower seeds (Psaras 1985). No investigations of vacuolation of endosperm cells of priming seeds have been reported. However, the growth of carrot embryos during priming (Austin *et al.*, 1969; Weibe and Tiessen 1979) would have to be associated with endosperm cell degradation in these seeds.

The tomato embryo as measured here is composed of only hypocotyl and radicle tissue (Sect. 2.2.3) and thus changes in water content of the cotyledons were not directly measured. However, it is unlikely that the cotyledons would undergo considerable protein body breakdown and vacuolation during priming to permit higher water contents as protein body breakdown in the cotyledons has only been observed 3 days after germination commenced (Eggers and Geisman 1976). In my experience direct measurement of water content of endosperm or cotyledons was not possible without unacceptable damage during excision. However, the degree of vacuolation of endosperm cells and differences in cell wall thickness could be tested by a histological study.

It appears that prevention of radicle emergence during priming must result from the maintenance of sufficient resistance by the endosperm to prevent expansion of the radicle because neither cell wall loosening nor $\Delta\Psi_\pi$ between the embryo and the imbibing solution was any different

between seeds imbibing in priming solutions or in water.

Cell expansion is initiated by cell wall loosening in expanding cells of growing tissues (Cosgrove 1986). Cell Ψ_p can only have an effect on expansion once the walls have been loosened and water can enter the expanding cell. Thus a $\Delta\Psi_\pi$ can only have effect on expansion after restraint has been removed. Radicle expansion will be initiated by the removal of the restraint on expansion imposed by the endosperm.

The endosperm weakening which permits radicle emergence may be prevented by the inhibition of GA production or counteraction of its effects perhaps through the action of other growth regulators. The role of growth regulators in the control of germination and priming will not be investigated in this thesis; however, their possible involvement will be discussed.

Endosperm weakening is under the control of GA released by the embryo (Groot and Karssen 1987). GA synthesis or activity may be reduced during priming. Although GA activity was reduced in water-stressed lettuce leaves (Aharoni *et al.*, 1977) and GA synthesis occurs in roots (Torrey 1976), little is known about the synthesis and transport of GA during water or salt stress (Bradford and Hsiao 1982; Munns and Termaat 1986).

GA application increased growth of NaCl-effected red kidney beans (Nieman and Bernstein 1959). Munns and Termaat (1986) suggested that GA overcame the water deficit effects

of NaCl stress rather than the specific salt effect. Direct measurement of GA synthesis and transport during priming would be necessary to clarify this point.

Other growth regulators have been implicated in responses to water and salt stresses, but definitive proof of their involvement is lacking. Kinetin has been suggested as integrating shoot with root growth in water-stressed plants (Bradford and Hsiao 1982). However, application of kinetin had no effect on leaf expansion of NaCl-treated plants (Munns and Termaat 1986). Abscisic acid is also produced in the roots of water-stressed plants (Walton *et al.*, 1976), but the timing of its production appears to follow the diminution of growth (Lachno 1983). Considerable work is needed before our understanding of the roles of growth regulators in plant responses to osmotic stress is clear.

Priming appears to interrupt endosperm weakening which normally precedes phase III of water uptake during germination. The results presented here showed that prevention of germination during priming was caused by the maintenance of sufficient resistance by the endosperm and seed coat enclosing the embryo to prevent expansion of the radicle. It was shown that the prevention of radicle emergence during priming was not the result of an inadequate $\Delta\Psi$ between the embryo and the priming solution for water uptake, nor the result of lack of cell wall loosening in the embryo. The basis for the enhanced speed of germination of primed seeds will be investigated in the next chapter.

Water Relations of Germinating Primed Tomato Seeds

4.1 Introduction

In Chapter 2 I showed that the sequence of events during the germination of tomato seeds involved three distinct steps. Firstly, water was imbibed until a Ψ equilibrium was established between the seed and the imbibitional solution. This was followed by the loosening of the cell walls of the embryo and the weakening of the resistance offered by the endosperm to permit expansion of the radicle. In this chapter I address the question of why this sequence of events is completed by primed seeds in about half the time normally taken by non-primed seeds.

The time necessary for germination could be reduced by changes in any or all of the three main steps. In primed seeds, imbibition could be more rapid, embryo cell wall loosening could occur earlier and/or endosperm weakening could proceed more rapidly than during the germination of non-primed seeds.

Water uptake by seeds in water proceeded until Ψ equilibrium was established with the imbibitional solution (Figs 2.1 & 2.2). The dehydration of primed tomato seeds does not result in a return to the size of the seed prior to priming, primed seeds are about 25% larger in volume (Barlow and Haigh 1987). This increase in size may result from a change in conformation of the macromolecules and the associated swelling of the seed tissues during priming which is not reversed on drying. The increase in volume may

assist water uptake by increasing the hydraulic conductivity of the seed.

While cell wall loosening was shown to occur in the embryo of the tomato seed during priming (Fig. 3.5) it is not known what effect drying will have on the loosened walls. Wall loosening occurs to a limited extent in most tissues during periods of reduced water availability (Cleland 1986) and thus the relief of water stress is generally associated with a short period of rapid cell expansion. The effect of the level of dehydration associated with drying after priming has not been studied on cell wall properties. A complete reversal of embryo wall loosening may occur or perhaps no change may be induced by drying. Should cell wall loosening not be reversed by drying then a considerable enhancement in germination time could possibly result. As non-primed seeds in water germinated as a result of endosperm weakening, changes in the timing of embryo wall loosening may not in fact affect the timing of radicle emergence.

Radicle emergence was prevented during priming by the maintenance of sufficient resistance by the endosperm to prevent radicle expansion (Sect. 3.4); however, the possibility of some degree of endosperm weakening occurring during priming was not directly investigated. Should some endosperm weakening have occurred during priming then subsequent germination may be quicker.

After radicle emergence the rate of expansion of

radicle cells could be enhanced by changes in either the rates of water uptake or cell wall loosening. Increased rates of water uptake by radicle cells could result from solute accumulation which occurred during priming. A number of authors have suggested that the rapid germination of primed seeds may be due to solute accumulation in the embryo during priming (Hegarty 1978; Khan and Karssen 1981; Khan and Samimy 1982; Liptay and Schopfer 1983; Cantliffe *et al.*, 1984; Bradford 1986); however, direct evidence of solute accumulation has been lacking. Measurements of lowered embryo Ψ_π during priming, which would result from solute accumulation, did not provide clear cut evidence (Fig. 3.4).

Increased cell wall extensibility or a reduced cell wall yield threshold could also increase cell expansion following priming. Estimation of wall extensibility and yield threshold can be made through measurements of expansion of embryos imbibed in solutions with a range of Ψ_π (Cleland 1986).

4.2 Materials and Methods

4.2.1 *Water Uptake by Germinating Primed Tomato Seeds*

Tomato seeds (UC 82B, Yates Seeds, Sydney, Australia) were primed in a solution of 0.090M K_2HPO_4 + 0.118M KNO_3 for 6 days, air-dried to a water content of approximately 8% DWt then imbibed in water at 25^oC. Approximately 200 seeds were placed on filter papers (Ekwip U70, Industrial Equipment, Sydney, Australia) moistened with approximately 16 ml of water in 90 mm glass Petri dishes. At frequent intervals 5

seeds were removed, surface dried between layers of tissue paper, weighed on a CAHN 25 microbalance (Ventron Instruments Corporation, Cerritos, CA, U.S.A.) then dried at 80°C for 72 h before reweighing for dry weight.

4.2.2 *Water Relations of Germinating Primed Tomato Seeds*

Approximately 200 primed seeds were imbibed in water at 25°C as above (Sect. 4.2.1) and seed Ψ and Ψ_π were measured in Peltier type thermocouple psychrometers as described previously (Sect 2.2.2) at regular intervals until germination.

4.2.3 *Water Uptake and Water Relations of the Embryo within the Germinating Primed Tomato Seed*

Primed seeds were imbibed at 25°C as above (Sect. 4.2.1). At frequent intervals seeds were removed from the Petri dishes and the embryo excised by razor blade and its water content, Ψ and Ψ_π measured as described previously (Sect. 2.2.3).

4.2.4 *Water Uptake by Embryos Excised from Imbibing Primed Seeds*

Primed seeds were imbibed at 25°C as above (Sect. 4.2.1) for 3 h. Embryos were excised from seeds, transferred to water-moistened papers and their water uptake was measured. Least squares regression equations were fitted individually to all data sets when they showed linear rates of increase in fresh weight.

4.2.5 *Initial Seedling Growth of Primed Tomato Seeds*

Primed and non-primed seeds were imbibed in separate Petri dishes at 25°C as above. At germination 10 seeds from each dish were weighed and their fresh weight increase followed for 48 h on a CAHN 25 microbalance. From another 10 germinated seeds the embryos were excised, transferred to fresh water-moistened papers and their water uptake measured.

In another experiment, embryos from primed and non-primed seeds were excised at germination and allowed to imbibe water for a further hour. These embryos were transferred to Petri dishes containing filter papers moistened with water or solutions of polyethylene glycol (PEG) ranging in osmotic potential from -0.2 MPa to -1.2 MPa (Michel 1983). Fresh weight changes were followed for 10 h and water relations measured after 5 to 10 h in solution.

4.3 Results

4.3.1 *Water Uptake and Water Relations of Germinating Primed Tomato Seeds*

Water uptake by primed tomato seeds was more rapid than that of non-primed seeds. Primed tomato seeds displayed a triphasic pattern of water uptake. Water content increased very rapidly during phase I, then more slowly until radicle emergence which was associated with a rapid increase. Radicle emergence occurred after 24 h of imbibition at a seed water content of about 100% DWt. This was 16 h earlier than for non-primed seeds and at a water content which was

about 20% higher than that of non-primed seeds (Fig. 4.1). The Ψ of the imbibing primed seeds reached equilibrium with water by 12 h. The Ψ_π of imbibing primed seeds was slightly lower than that of non-primed seeds in water. This together with the higher water content of the seeds would suggest that more solutes were present in the imbibing primed seeds than in the non-primed seeds (Fig. 4.2).

4.3.2 *Water Uptake and Water Relations of the Embryo within the Germinating Primed Tomato Seed*

After the first 6 h embryo water content increased steadily until the large increase of radicle emergence. Just prior to radicle emergence water content was 65% DWt, not significantly higher than that of the embryo of non-primed seeds prior to radicle emergence (Fig. 4.3). Embryo Ψ rose rapidly to -1.8 MPa at 15 h, then remained about this value. Embryo Ψ_π was about -2.0 MPa after 15 h. These embryo water relations values were about 0.3 MPa below those of the embryo of non-primed seeds prior to germination. At radicle emergence embryo Ψ was -1.0 MPa and rose rapidly to -0.2 MPa as the radicle expanded (Fig. 4.4). The relationship of embryo Ψ to water content was thus different for primed embryos and non-primed embryos.

4.3.3 *Water uptake by Embryos Excised from Imbibing Primed Seeds*

Embryos excised from primed seeds which had been imbibing water for 3 h were immediately capable of

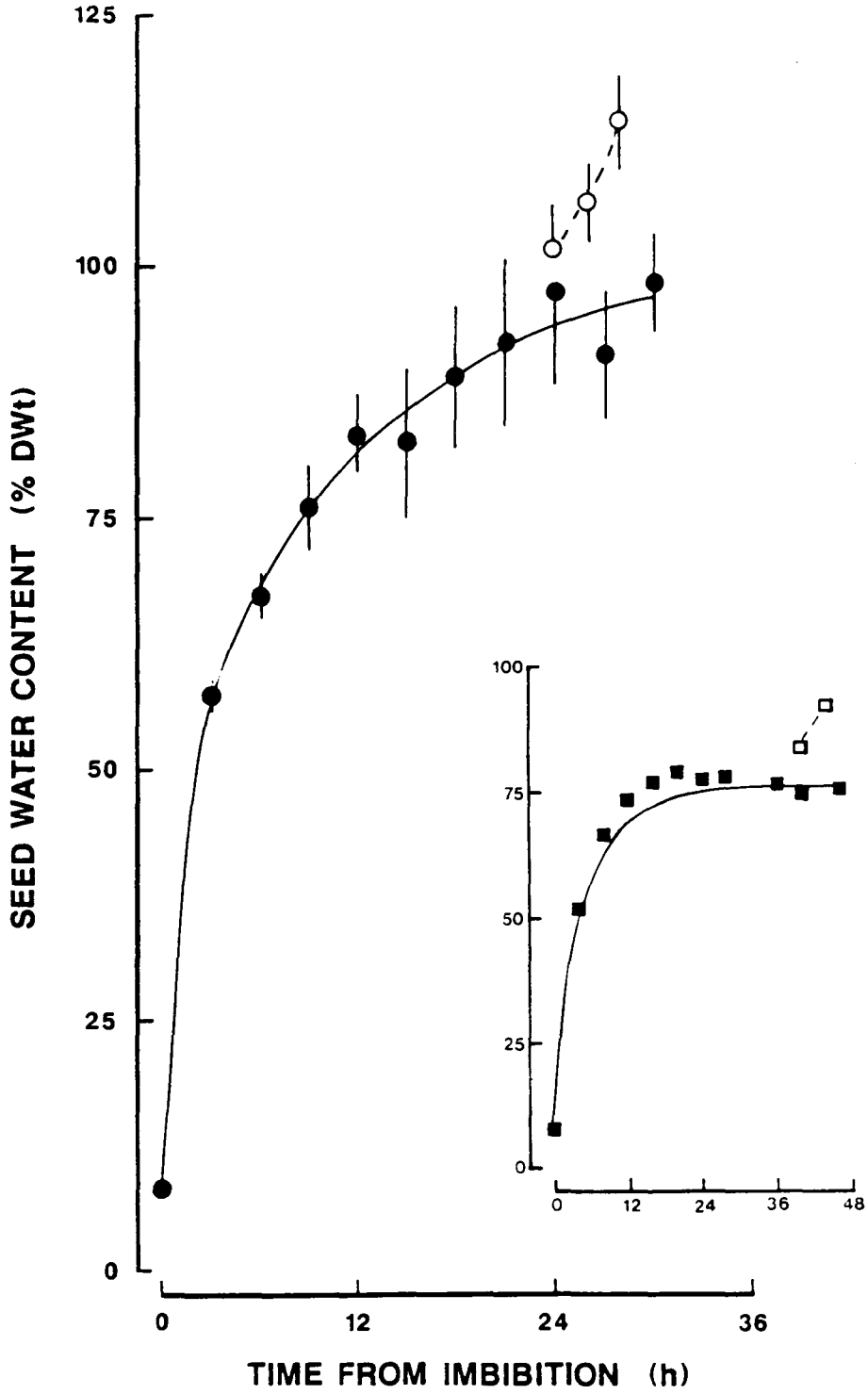


Fig. 4.1. Water uptake pattern of primed tomato seeds during germination. Vertical bars show s.e. of the means of 5 seeds. Inset shows water uptake pattern of non-primed seeds during germination (data from Fig. 2.1). Lines are drawn by eye.

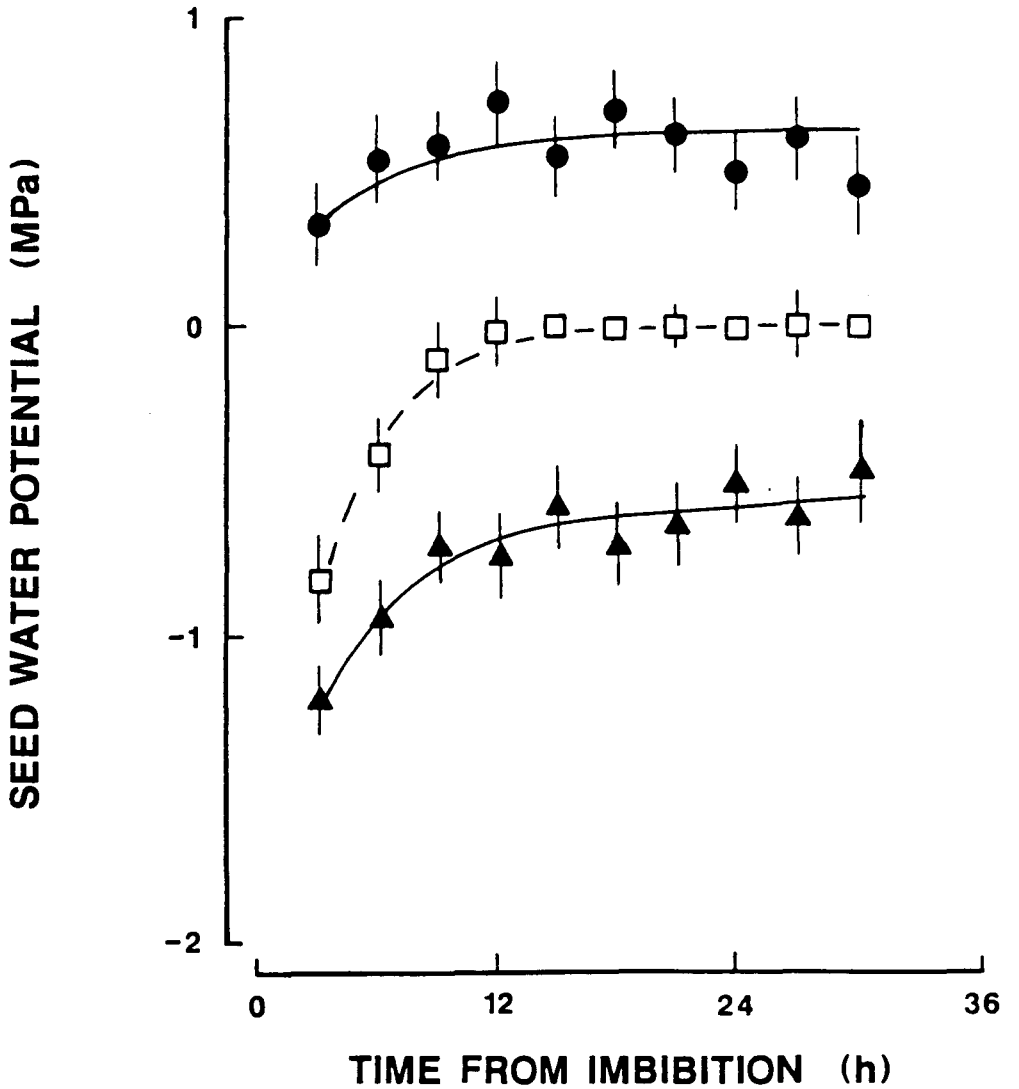


Fig. 4.2. Ψ (□), Ψ_π (▲), Ψ_p (●) of primed tomato seeds during germination. Vertical bars show s.e. of the means of 5 replicates of 15 seeds each. Lines are drawn by eye.

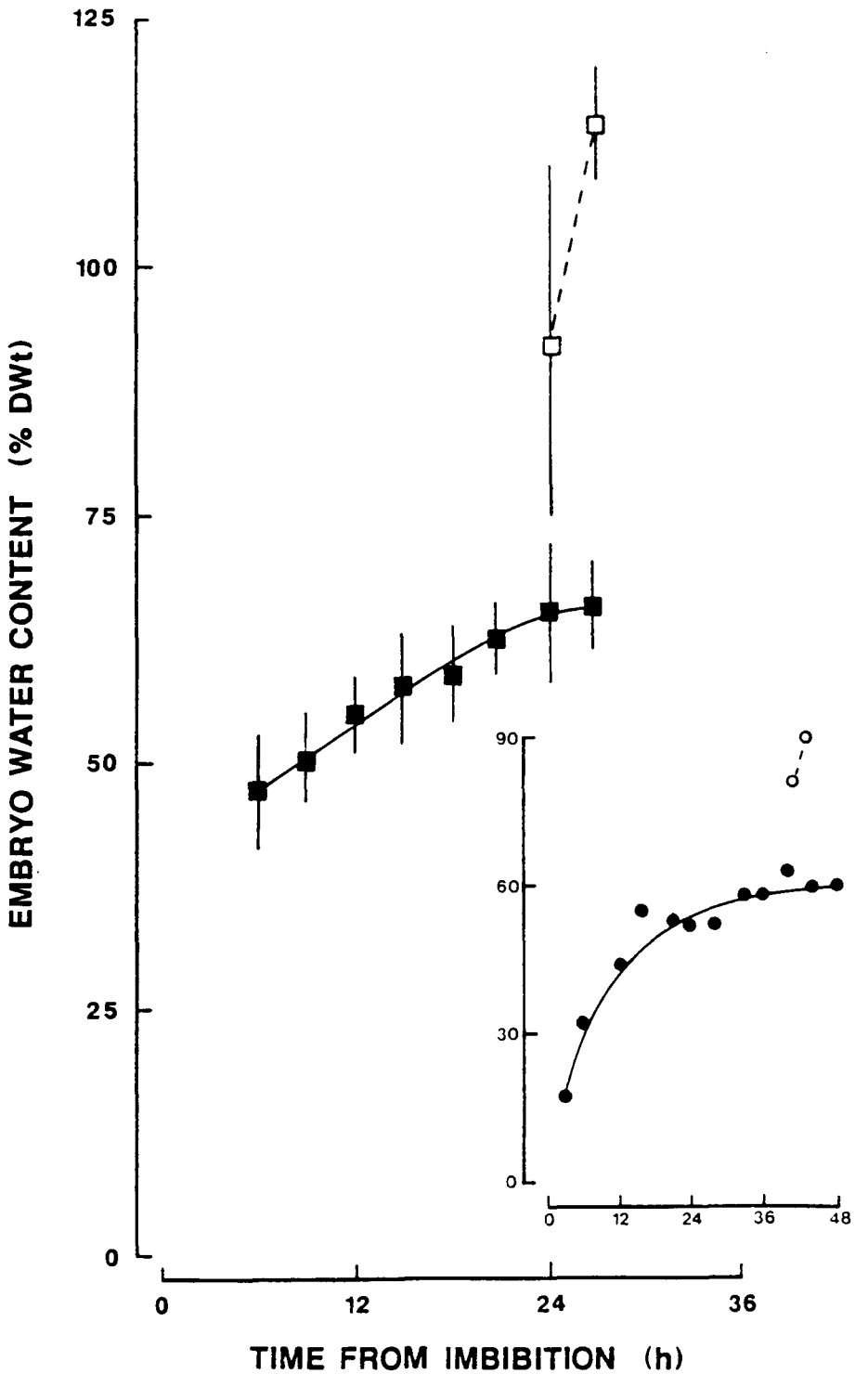


Fig. 4.3. Water uptake pattern of embryos within primed tomato seeds during germination. Vertical bars show s.e. of the means of 5 embryos. Inset shows water uptake pattern of embryos from non-primed seeds during germination (data from Fig. 2.3). Lines are drawn by eye.

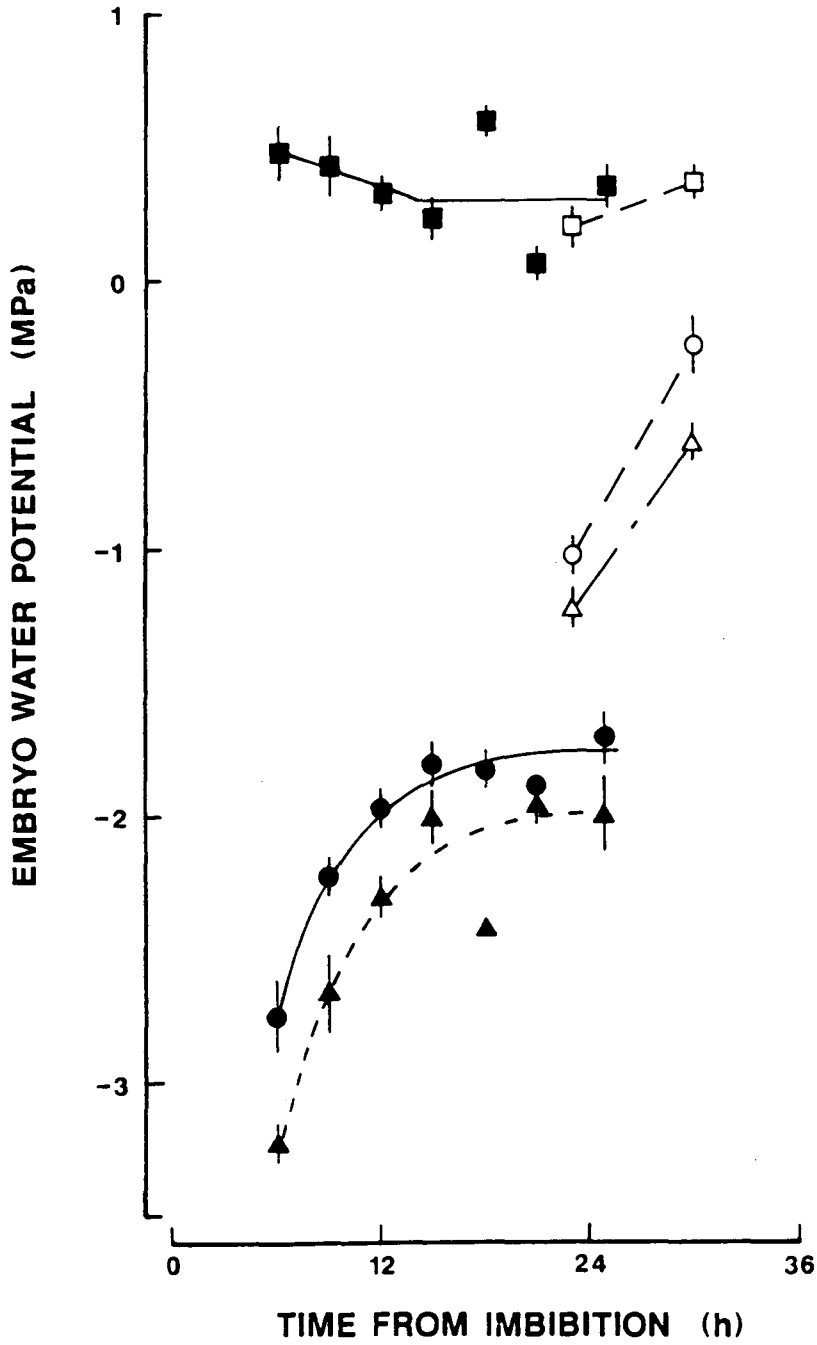


Fig. 4.4. Ψ (○, ●), Ψ_π (△, ▲), Ψ_p (□, ■) of the embryo of primed tomato seeds during germination. Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. Open symbols are for embryos post radicle emergence. Lines are drawn by eye.

expansion. As it was not possible to excise embryos before this time it indicated that the drying which followed priming did not reverse the cell wall loosening which had occurred during priming (Fig. 4.5). The Ψ of excised embryos rose to -2.5 MPa within 1 h of transfer to water (Fig. 4.6).

4.3.4 *Initial Seedling Growth of Primed Tomato Seeds*

The fresh weight of primed seeds increased rapidly from germination and throughout the following 48 h period of observation. The fresh weight of primed seedlings was higher than that of non-primed seedlings at all times (Fig. 4.7). Similar increases in fresh weight were observed when isolated embryos were measured (Fig. 4.8). The absolute increase in fresh weight of both seedlings and embryos were similar throughout the first 12 h of growth. The relative growth rates of primed seedlings and embryos were higher than those of non-primed seeds and embryos during the first 12 h of growth. After this time relative growth rates were similar (Fig. 4.9).

The expansion rates of primed embryos were greater than those of non-primed embryos expanding in a range of external Ψ_π. Both primed and control embryos ceased to expand in the -0.8 MPa PEG solution (Fig. 4.10). There was no difference in the water relations of the expanding embryos; however, primed embryos had higher Ψ in solutions which caused the tissue to shrink and the $\Delta\Psi_\pi$ between embryo and solution only differed in -1.2 MPa solutions (Fig. 4.11). There

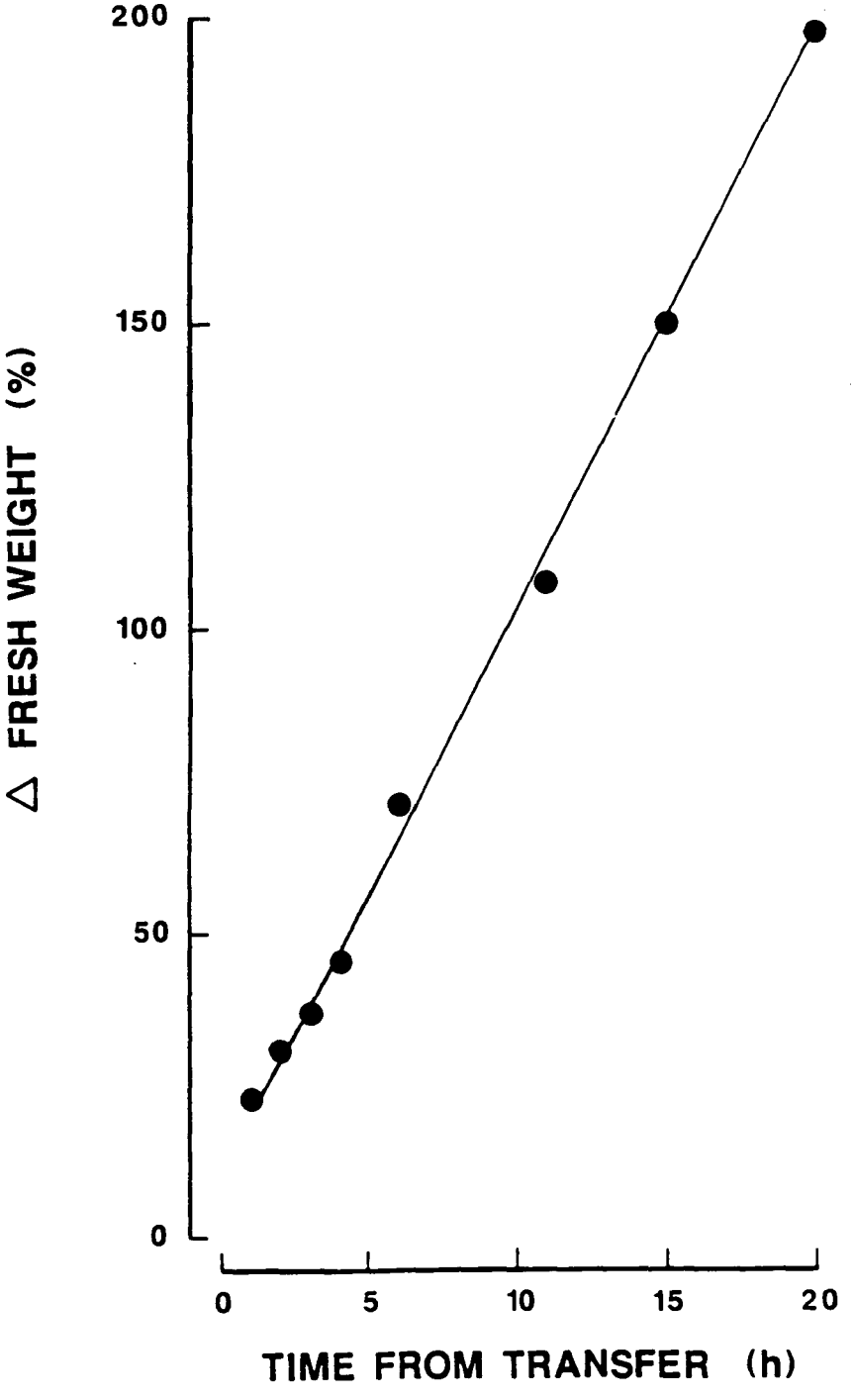


Fig. 4.5. Change in fresh weight of embryos excised from primed seeds imbibed in water for 3 h. Data shown are for individual embryos. Similar measurements were made on 10 embryos.

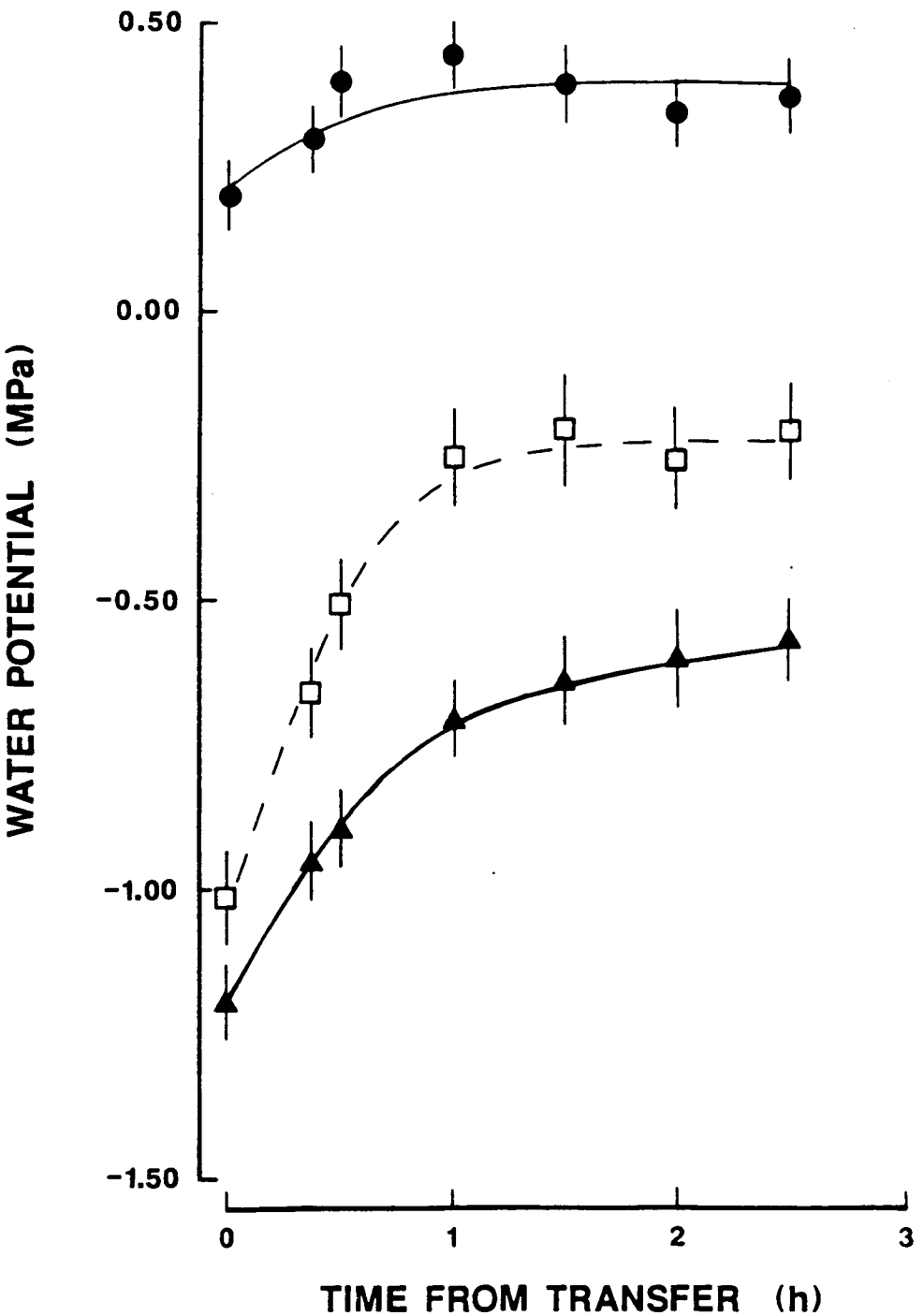


Fig. 4.6. Change in embryo Ψ (□), Ψ_π (▲), Ψ_p (●) after excision from the seed and transfer to water. Seeds were imbibed for 3 h before the embryos were excised. Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. Lines are drawn by eye.

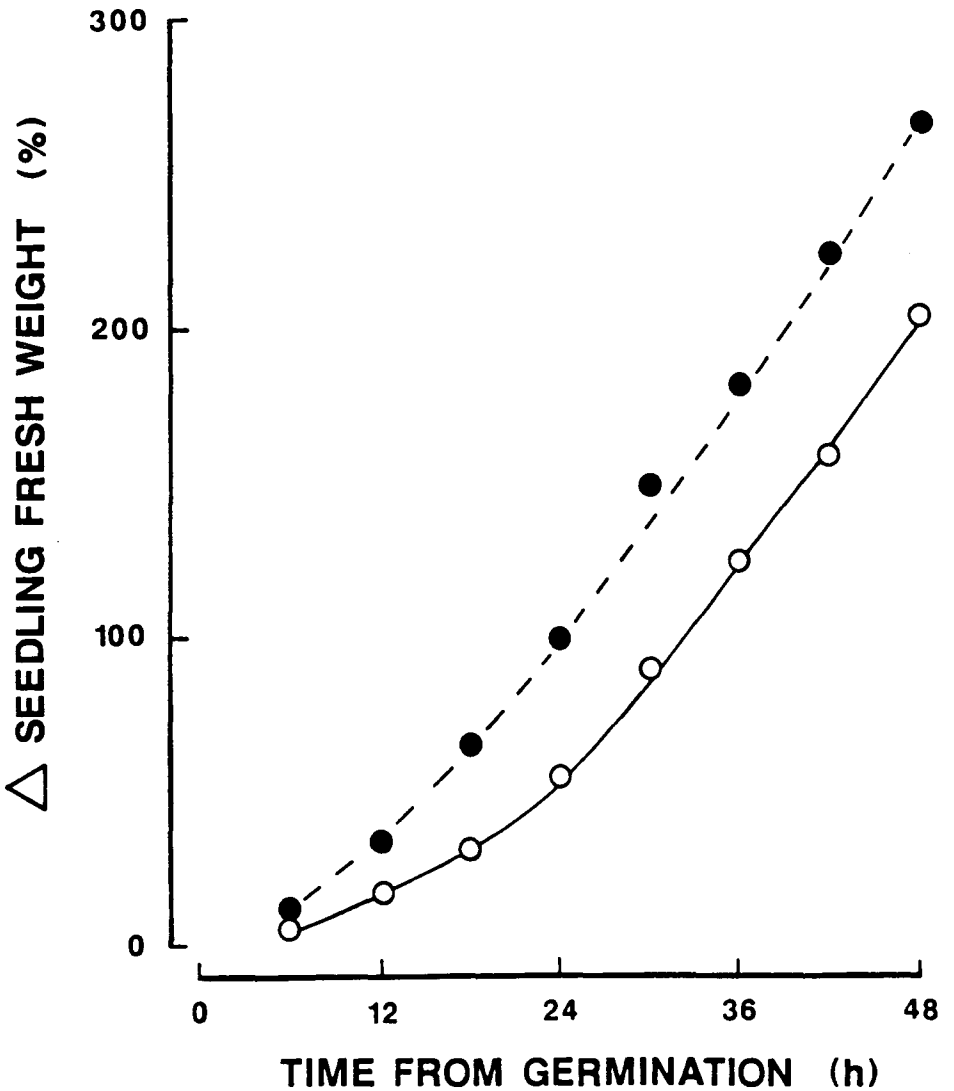


Fig. 4.7. Change in fresh weight of primed (●) and non-primed (○) seedlings following germination. Vertical bars show s.e. of the means of 10 replicates. Lines are drawn by eye.

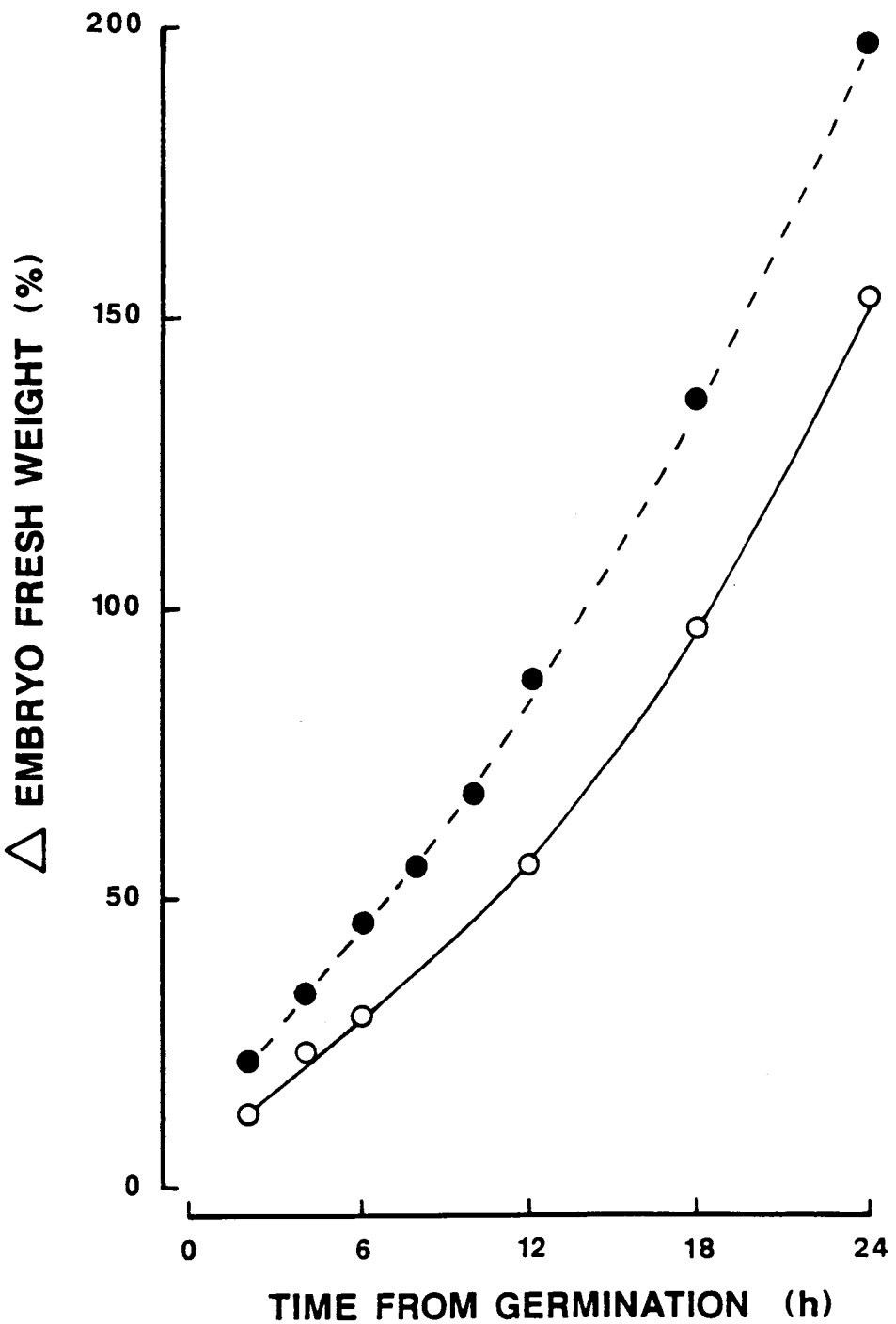


Fig. 4.8. Change in fresh weight of isolated embryos from primed (●) and non-primed (○) seeds following germination. Vertical bars show s.e. of the means of 10 replicates. Lines are drawn by eye.

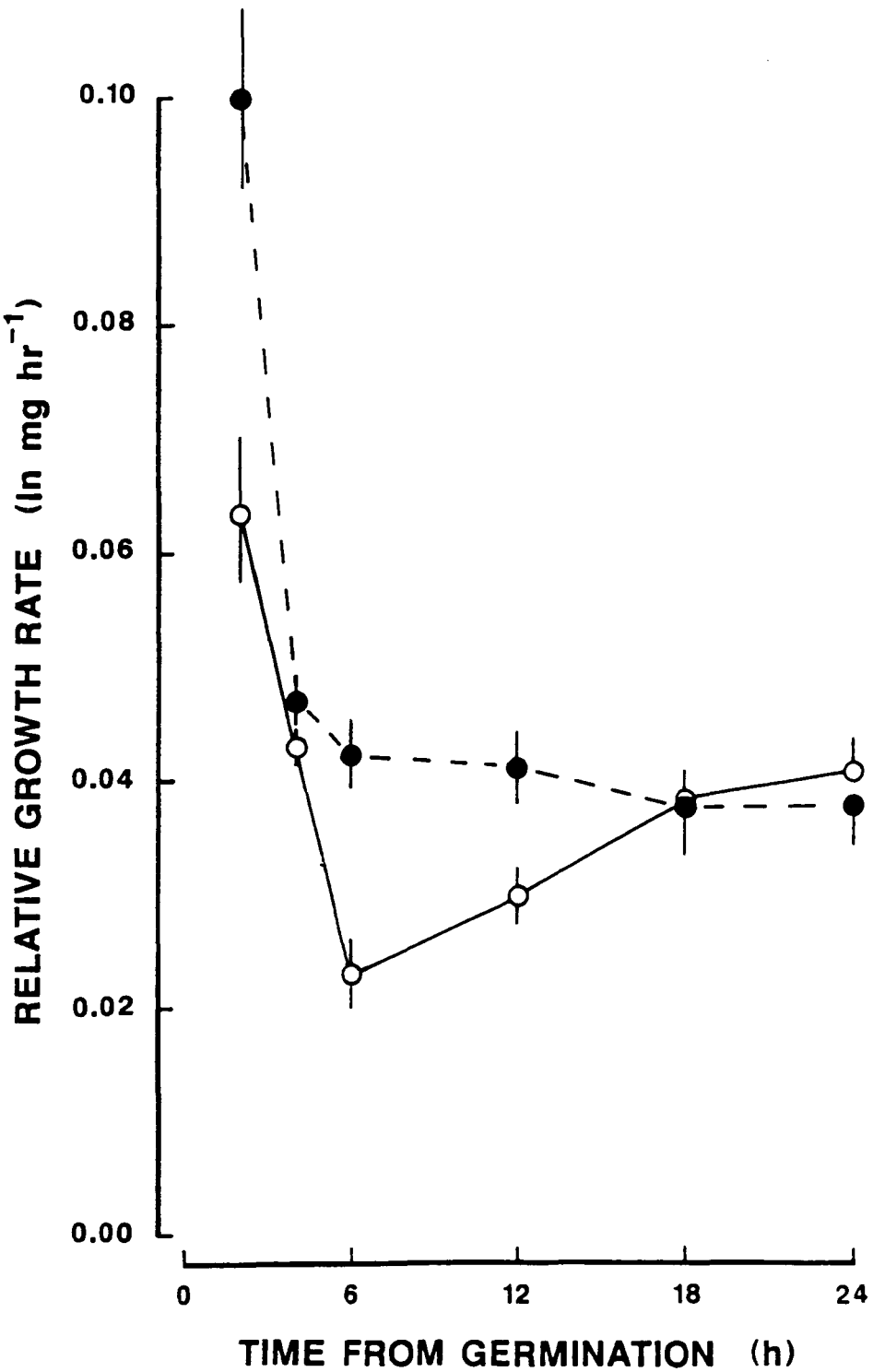


Fig. 4.9. Relative growth rates of isolated embryos from primed (●) and non-primed (○) seeds following germination. Vertical bars show s.e. of the means of 10 replicates. Lines are drawn by eye.

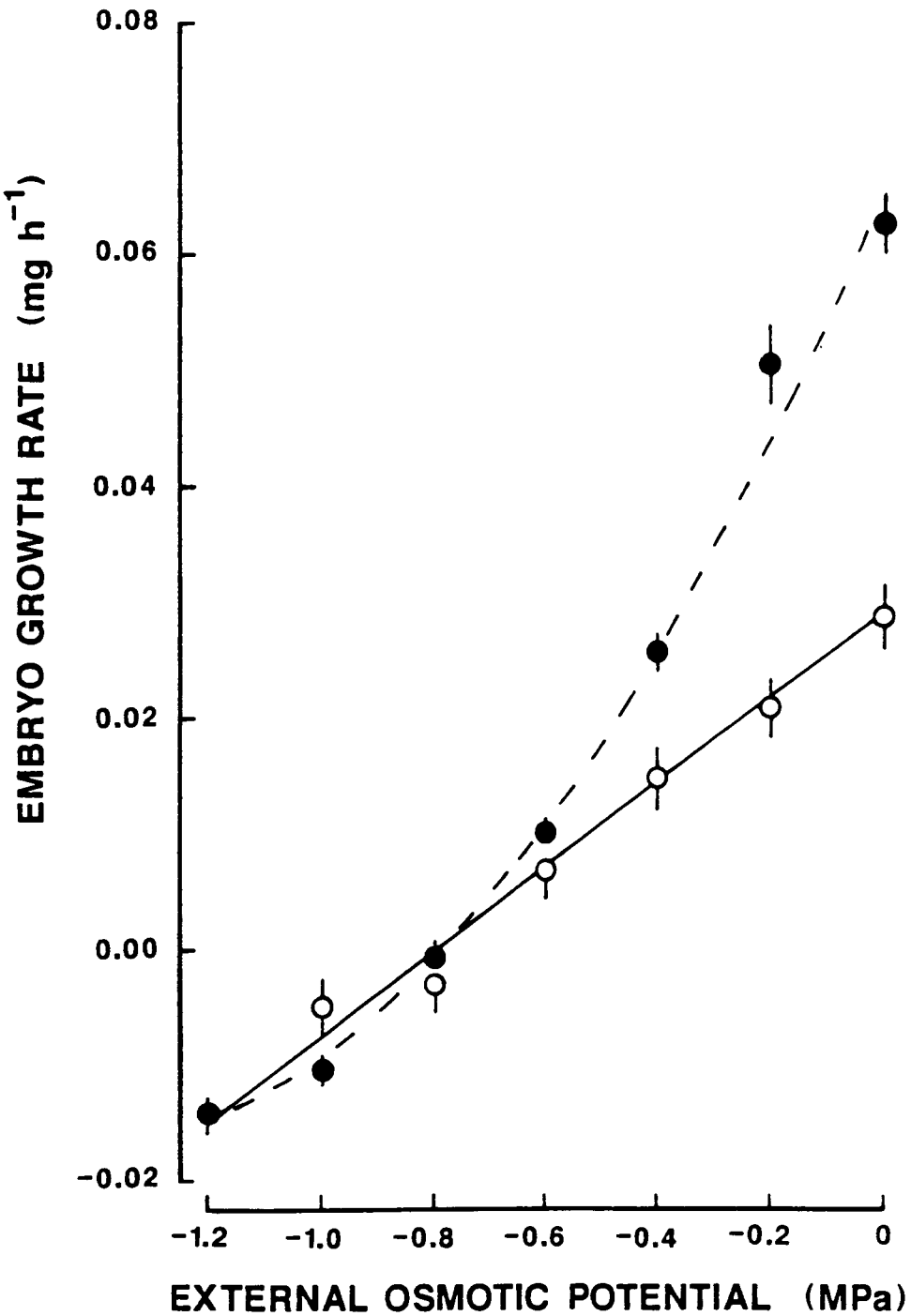


Fig. 4.10. Growth rates (water uptake rates) of isolated embryos from primed (●) and non-primed (○) tomato seeds in a range of PEG solutions. Vertical bars show s.e. of the means of 10 replicates. Lines are drawn by eye.

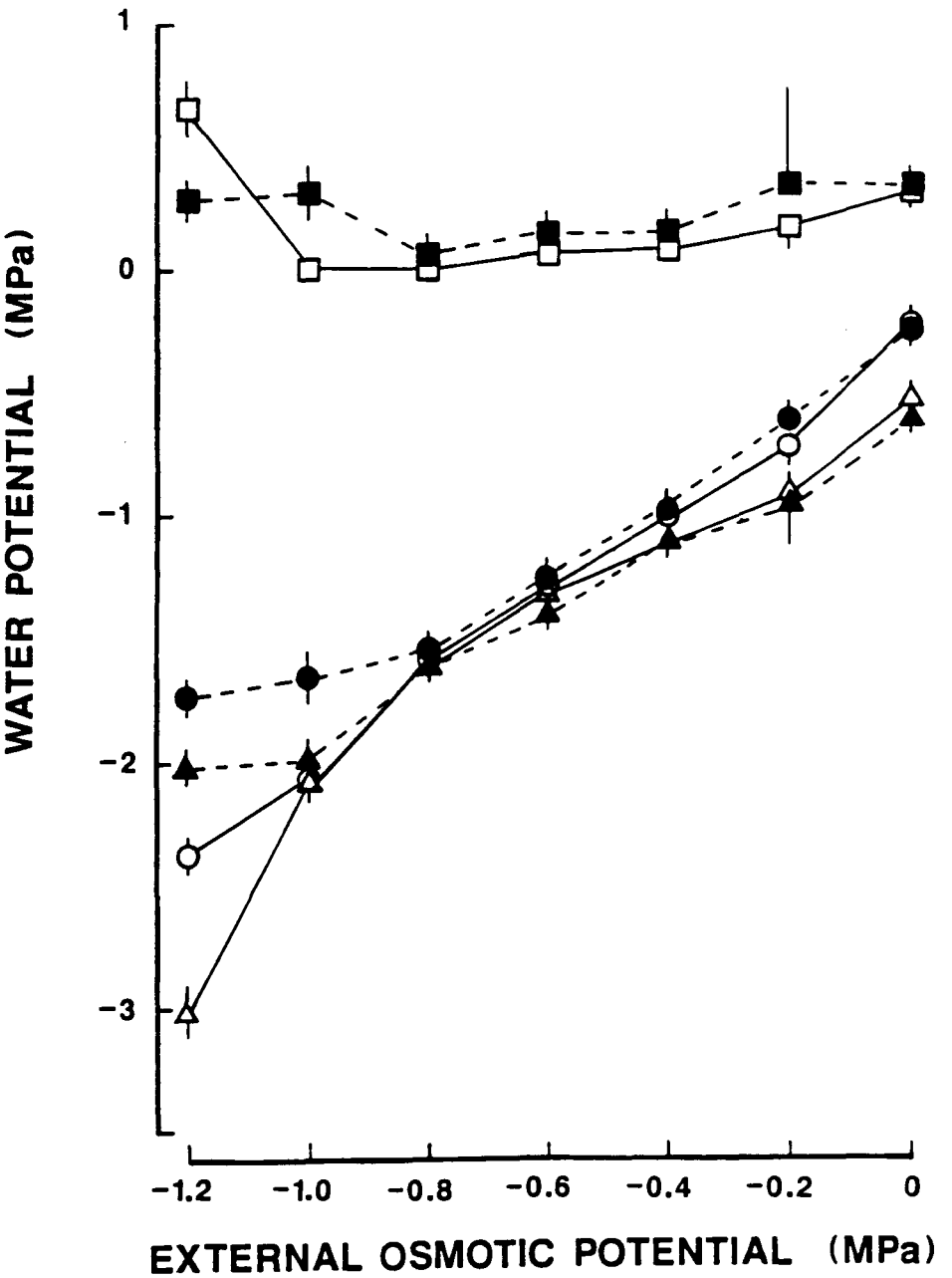


Fig. 4.11. Ψ (○, ●), Ψ_π (△, ▲), Ψ_p (□, ■) of isolated embryos from primed (●, ▲, ■) and non-primed (○, △, □) tomato seeds in a range of PEG solutions. Vertical bars show s.e. of the means of 3 replicates of 6 - 10 embryos. Lines are drawn by eye.

difference between the slopes of the growth rates v. embryo Ψ_p curves. This may have reflected some of the difficulties involved in determining embryo Ψ_p (Fig. 4.12).

4.4 Discussion

Primed tomato seeds germinated more rapidly than non-primed seeds because all three major steps in germination occurred more rapidly.

Water uptake by primed seeds was more rapid than that by non-primed seeds (Fig. 4.1). Water potential equilibrium with the imbibing water was reached about 3 to 6 h earlier in primed seeds (9 to 12 h) than in non-primed seeds (12 to 15 h) (Figs 4.2 & 2.2). The faster uptake of water of primed seeds may have been due to the lower Ψ_π of primed seeds during imbibition or to changes in the hydraulic conductivity of the seed.

The primed seeds reached higher water contents prior to radicle emergence than did non-primed seeds (Fig. 4.3). These were accompanied by somewhat lower seed Ψ_π values indicating that the primed seeds had higher concentrations of solutes than did the non-primed seeds (Figs 4.2 & 2.2). Similarly, the embryos of germinating primed seeds had lower Ψ and Ψ_π values with unchanged water contents compared to those of non-primed embryos (Figs 4.4 & 2.4). It can be inferred from these results that some accumulation of solutes occurred. These results are consistent with Bradford's (1986) data on primed lettuce seeds where primed seeds had higher RWC when imbibed at the same Ψ_π. Bradford

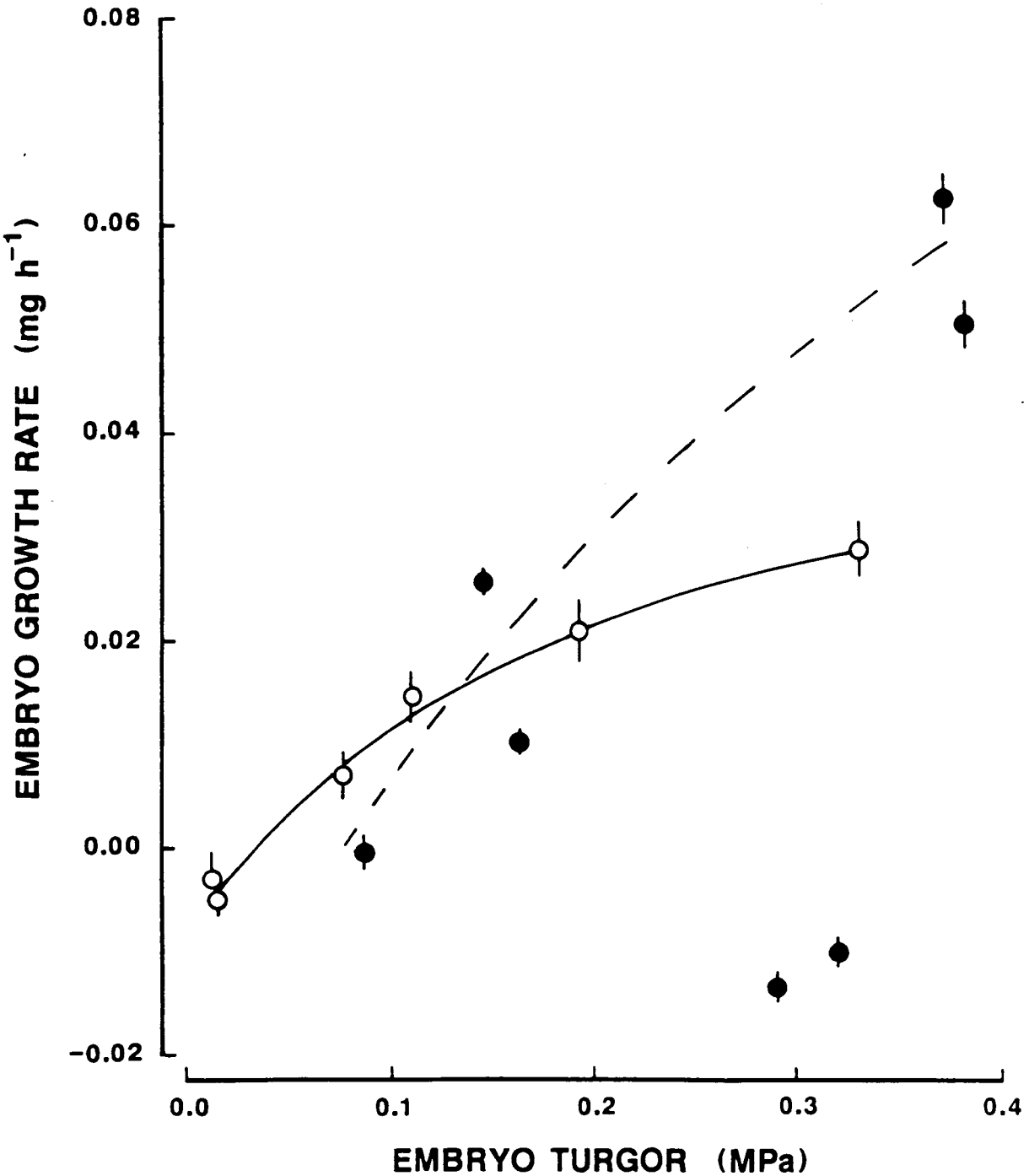


Fig. 4.12. Growth rates (water uptake rates) of isolated embryos from primed (●) and non-primed (○) tomato seeds in a range of PEG solutions expressed as a function of embryo turgor. Vertical bars show s.e. of the means of 10 replicates. Lines are drawn by eye.

(1986) interpreted his results as indicating that the lettuce seeds had accumulated solutes during priming. As this was not apparent during priming it must have occurred during drying of primed seeds.

The embryos from primed seeds were capable of expansion at the earliest time at which they could be excised from the seeds (Fig. 4.5). This indicated that the cell wall loosening which had occurred during priming was not reversed by drying. Thus the 31 h normally necessary to initiate cell wall loosening during germination of non-primed seeds had been completely eliminated by priming.

The pattern of water uptake by primed seeds and embryos did not show a distinct plateau as was found for non-primed seeds (Figs 4.1 & 4.3). This plateau phase of non-primed seeds resulted from the restriction on embryo cell expansion caused by the endosperm. Its absence from the water uptake pattern of primed seeds may indicate that endosperm weakening in primed seeds occurs more rapidly or that considerable weakening had occurred during priming. Direct measurement of endosperm resistance will be necessary to clarify this. This will be examined in Chapter 6.

Upon radicle emergence expansion of primed radicles was more rapid than that of non-primed radicles (Fig. 4.8). In an attempt to identify changes in radicle cell wall extensibility, embryo growth (water uptake) rates were measured in a range of PEG solutions. The large differences in growth rates in PEG solutions indicated that the cell wall extensibility of primed embryos was higher than that of

non-primed embryos (Fig. 4.10). However, some difficulties were experienced with this analysis.

Both primed and non-primed embryos ceased to expand when placed in a -0.8 MPa PEG solution (Fig. 4.10). By definition the $\Delta\Psi_\pi$ between the embryo and the solution at this point should equal the cell wall yield threshold (Y) (Cleland 1986). In this solution, both primed and non-primed embryos had Ψ_π of -1.6 MPa, thus Y equalled 0.8 MPa. This analysis assumes that Ψ equilibrium existed between the embryo and the solution, but the Ψ measurements did not show this. The measured Ψ values most probably underestimated the actual Ψ due to cell wall relaxation during measurement resulting in underestimates of Ψ and Ψ_p (Cosgrove *et al.*, 1984). However, this relaxation was extremely rapid when compared to reported measurements, occurring in minutes rather than hours (Hsiao *et al.*, 1985; Van Volkenburgh and Cleland 1986).

The $\Delta\Psi_\pi$ between the embryos and the PEG solutions which permitted expansion showed that the wall yield threshold changed as a consequence of the Ψ_π of the solution in which the embryos were expanding. In water, the Ψ_π of the embryos were about -0.6 MPa thus Y must have been less than the 0.8 MPa which was determined from the water uptake rate *v.* external Ψ_π curve. The wall yield threshold of expanding tissues can also be determined from a water uptake rate *v.* embryo Ψ_p curve.

The attempted analysis of cell wall extensibility

through the embryo water uptake rate v. Ψ_p curve (Fig. 4.12) was not successful due to wall relaxation during Ψ measurements. This is unfortunate as the slope of the growth rate v. external Ψ_π curve gives $(mL_p)/(m + L_p)$ not m which would have been derived from the growth rate v. Ψ_p curve. It was not possible to exclude the effects of hydraulic conductance on embryo growth by this approach. Based on the rates of water loss for shrinking embryos in -1.0 MPa and -1.2 MPa PEG solutions there was no difference in L_p between primed and non-primed embryos. The establishment of Ψ equilibrium after excision and transfer to water also occurred in similar time for both primed and non-primed embryos (Figs 4.6 & 2.7).

From the analyses of the modification of embryo growth rate by external Ψ_π it can be concluded that there was no significant difference between the wall yield thresholds nor between the hydraulic conductances of primed and non-primed embryos. Therefore, the differences in the growth rates caused by priming must have been due to changes in embryo cell wall extensibility.

That the relative growth rates were only significantly different up to 12 h after radicle emergence indicated that only a limited number of cells had had their wall properties modified during priming. The similarity of relative growth rates after 12 h post radicle emergence may indicate that cell division began at about this time and that the new cells were no different between primed and non-primed roots after this time. A histological study of germination and

radicle expansion would confirm or refute these suggestions.

This study of the water relations and growth rates of germinating primed seeds has shown that priming advanced the timing of radicle emergence by improving the rate of water uptake by the seeds; by eliminating the time necessary for the loosening of embryo cell walls and by reducing the time necessary for endosperm weakening. Embryos from primed seeds were found to have improved cell wall extensibilities which permitted higher relative growth rates during the first 12 h post radicle emergence. The morphological changes during priming and germination will be examined in the next chapter. This will be followed by an examination of the changes in the resistance of the endosperm during priming and germination.

Histological Study of Tomato Seed Germination and Priming

5.1 Introduction

It was concluded from the water relations studies that the control of tomato seed germination lay in the events which led to the weakening of the endosperm tissue enclosing the embryo (Chapter 2). The nature of these events are not known. Comparison with seeds of similar structures may indicate the possible events.

The tomato seed is up to 4 mm in length, flattened and ovoid with a curved linear embryo embedded in a non-starchy endosperm (Martin 1946; Vaughan 1970). The seeds take about 50 days to develop from pollination to maturity. After about 35 days the embryo and endosperm are filled with reserve material in the form of oleosomes and protein (aleurone) bodies (Smith 1935). The mature seeds contain about 22% protein and 15% oil (Vaughan 1970).

The morphological changes occurring during germination may resemble those which have been identified in lettuce seeds (Jones 1974; Psaras 1984) which have less endosperm and celery seeds (Jacobsen and Pressman 1979) which have more endosperm than tomato seeds (Martin 1946).

Lettuce endosperm is composed of two cell layers enclosing the entire seed with an additional two or three layers at the radicle tip. The endosperm cell walls were broken down during germination (Jones 1974), but specific observations of degradation of the cells enclosing the radicle tip were not reported. The lettuce endosperm cell

walls were composed of galactomannan (Halmer *et al.*, 1975). These walls were rapidly broken down following radicle emergence in association with the appearance of endo-B-mannanase activity (Halmer *et al.*, 1978; Halmer and Bewley 1979). However, this work was conducted with whole endosperms and did not identify any wall hydrolysis at the micropylar end before radicle emergence as would be consistent with the appearance of the cracks and pits found in this region by Pavlista and Valdovinos (1978). Protein body breakdown and vacuole formation occurred in the micropylar endosperm cells prior to radicle emergence in lettuce (Psaras 1984) and sunflower (Psaras 1985).

Celery endosperm cells were extensively degraded prior to germination, apparently under the control of gibberellic acid. Degradation resulted in protein body breakdown, vacuole formation and eventual cell separation associated with embryo growth before radicle emergence (Jacobsen and Pressman 1979). In castor bean (Vigil 1970; Gifford *et al.*, 1983), fenugreek (Reid and Meier 1972) and carob (Seiler 1977) protein body breakdown also led to vacuole formation after germination.

The water content data for priming seeds suggested that vacuolation of endosperm cells may occur during priming (Sect. 3.4). The growth rates of primed seeds suggested that priming changed the wall extensibilities of a limited number of radicle cells. These possible change will be investigated in a histological study of priming and

germinating tomato seeds.

5.2 Materials and Methods

Tomato seeds were imbibed in water at 25°C until germination. At specific times seeds were removed and fixed in a solution of 3% Gluteraldehyde in K_2HPO_4 pH 6.8 for 24 h then dehydrated in a range of ethanol solutions before embedding in LR White plastic resin (London Resins, Basingstoke, U.K.). To improve fixation seeds were cut in half transversely or a longitudinal section of the seed coat was removed.

Sections (0.5 μm) were cut on an ultramicrotome (Reichert-Jung Universal Microtime 1140/Autocut, Nusslach bei Heidelberg, F.R.G.) and stained with 0.65% Toluidine Blue in 1% $NaHCO_3$ from at least three seeds at each sampling time. To identify protein material some sections were stained with 0.5% Napthalene Black in 10% CH_3COOH.

5.3 Results

5.3.1 *The Dry Seed*

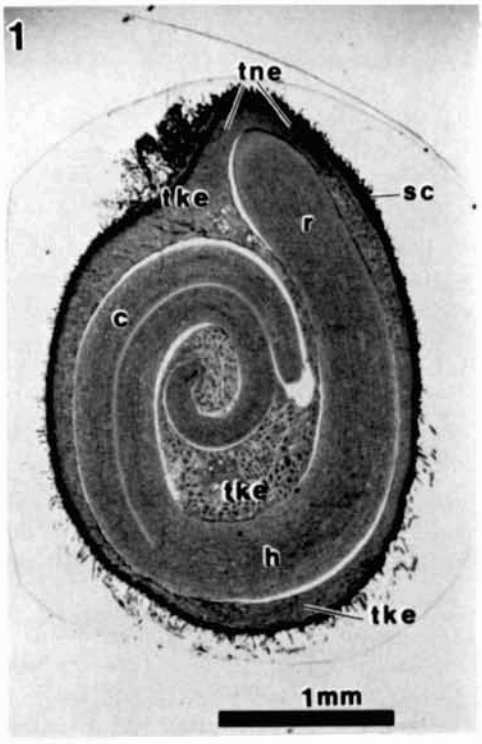
The tomato seed coat was composed mainly of two epidermal layers. Both layers were massively lignified. The outer epidermis of the testa gave rise to the seed hairs which were triangular in longitudinal section. There were from 4 to 10 layers of endosperm cells between the seed coat and the embryo. The embryo was composed of about 150 000 cells, but no difference could be identified between radicle and hypocotyl tissue (Fig. 5.1).

Fig. 5.1. A longitudinal section through a dry tomato seed. *r* radicle, *h* hypocotyl, *c* cotyledon, *sc* seed coat, *tke* thick walled endosperm, *tne* thin walled endosperm.

Fig. 5.2. The thick walled endosperm cells of a dry tomato seed. *ow* outer wall, *p* protein body, *o* oleosome, *c* protein crystalloid.

Fig. 5.3. A longitudinal section of the embryo of a dry tomato seed. The section was stained with Napthalene Black to highlight protein bodies. *rc* root cap, *pv* provascular tissue, *p* protein body.

Fig. 5.4. A longitudinal section through the cotyledon of a dry tomato seed. *pm* palisade mesophyll, *sm* spongy mesophyll.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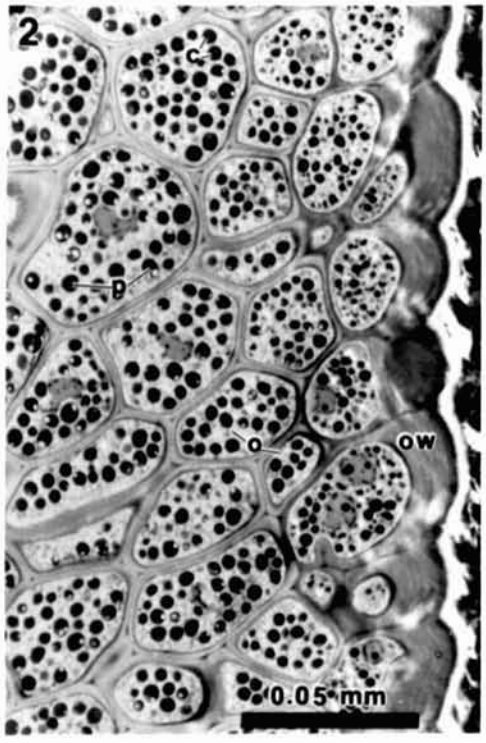
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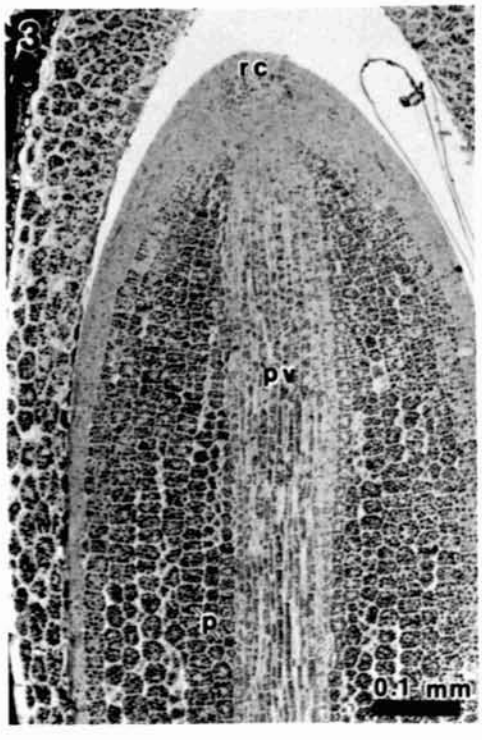
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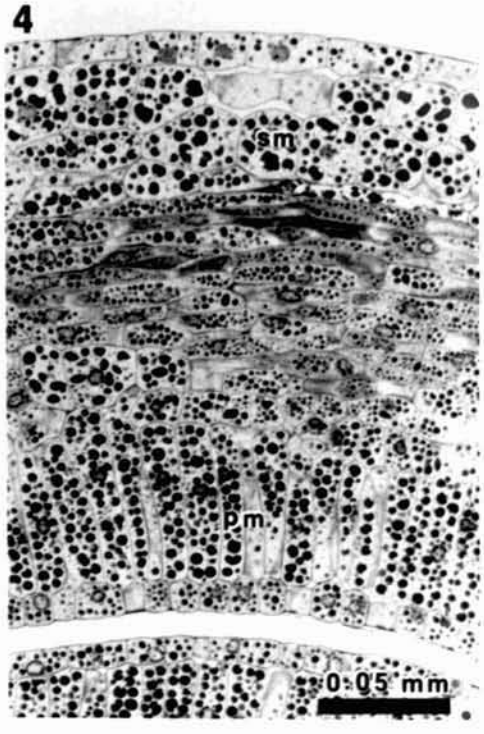
4
sm
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The endosperm was composed of two cell types which were distinguished on the basis of wall thickness. Most of the endosperm cells were thick walled (Fig. 5.2), but those which enclosed the radicle tip had much thinner walls (Fig. 5.5). (Figures are presented in an order which facilitates comparison of identified changes rather than in the order in which they are mentioned in this section.) Endosperm cells with massively thickened outer walls were found immediately inside the testa (Fig. 5.2), except near the micropyle. Most cells contained many protein bodies which stained intensely blue in Toluidine Blue (Fig. 5.2) and black in Napthalene Black (Fig. 5.3). Protein crystalloids were evident in some of the protein bodies. Oleosomes (lipid bodies) were evident in all cells (Fig. 5.2).

The embryo was completely enclosed by endosperm tissue. The embryo showed considerable cellular differentiation (Fig. 5.3). Provascular tissue was evident throughout and the cotyledons showed both palisade and spongy mesophyll cell layers (Fig. 5.4). The radicle epidermis and root cap contained few protein bodies, whereas the ground parenchyma and provascular tissue contained many. Few small vacuoles were found prior to imbibition (Fig. 5.3).

5.3.2 *Protein Body Breakdown*

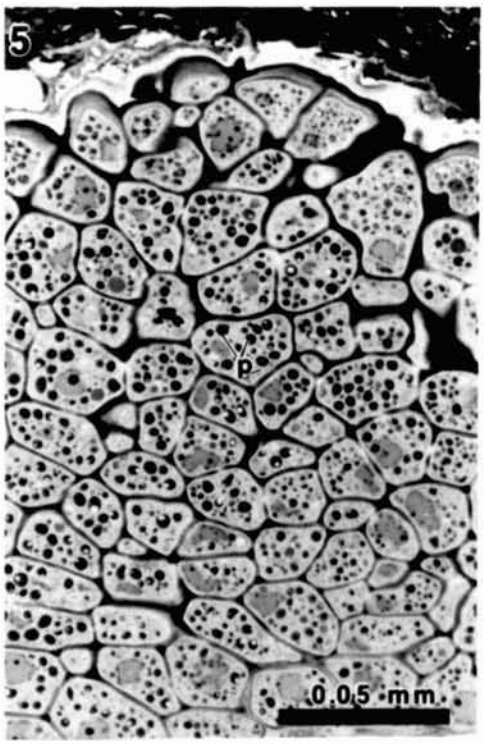
Following imbibition the protein bodies in the thin walled endosperm began to breakdown and vacuoles appear. After 24 h imbibition protein bodies were similar to those found in dry seeds (Figs 5.5 & 5.6). By 48 h protein body

Fig. 5.5. The thin walled endosperm cells from the micropylar region of a dry tomato seed. *p* protein body.

Fig. 5.6. The thin walled endosperm cells from the micropylar region of a tomato seed imbibed in water at 25°C for 24 h. *p* protein body.

Fig. 5.7. The thin walled endosperm cells from the micropylar region of a tomato seed imbibed in water at 25°C for 48 h. *p* protein body, *v* vacuole.

Fig. 5.8. The thin walled endosperm cells from the micropylar region of a tomato seed after 6 d in a priming solution, 0.090 M K_2HPO_4 + 0.118 M KNO_3, at 25°C. *v* vacuole.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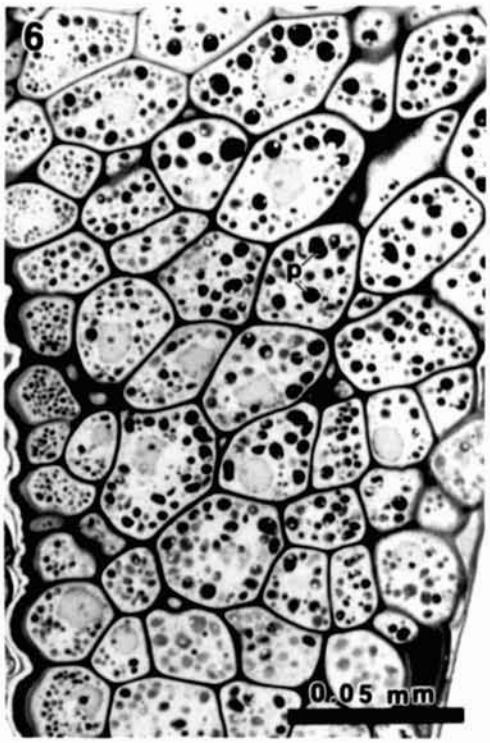
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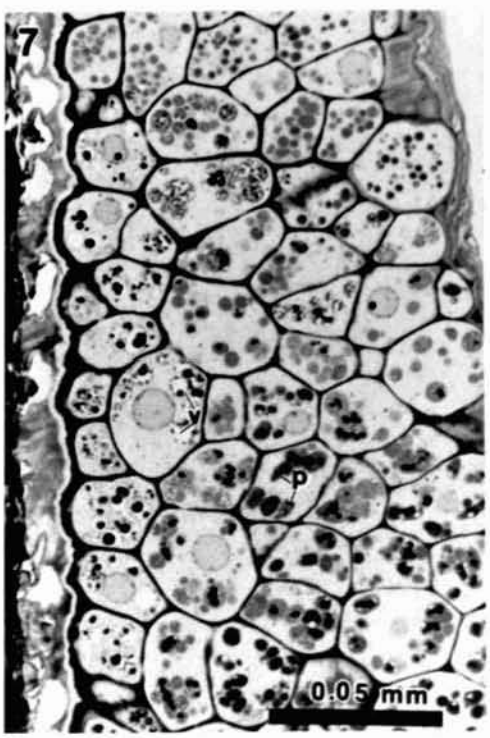
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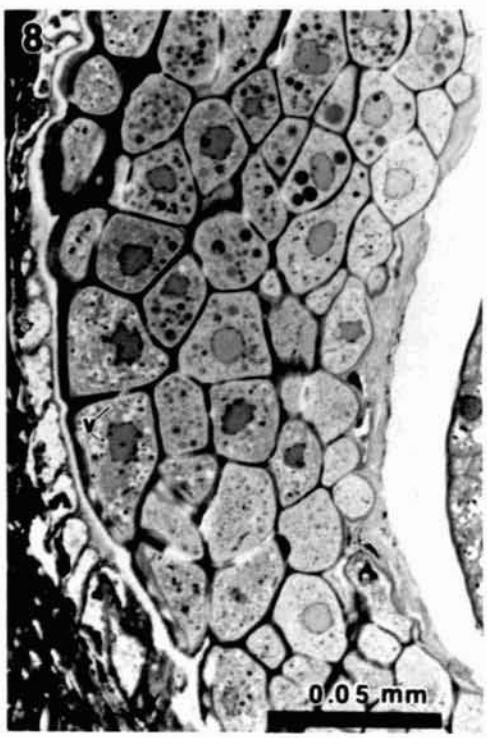
8
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numbers had been reduced and vacuoles were more extensive (Fig. 5.7). Few protein bodies in the radicle appeared to change before radicle emergence (Fig. 5.9).

5.3.3 *Radicle Emergence*

The radicle emerged through the thin walled endosperm cells and the inner integument. The endosperm cell layers parted at a point consistent with the location of the suspensor cells although these were not distinguished after radicle emergence.

Radicle emergence was accompanied by a limited amount of crushing of endosperm cells. Longitudinal sections showed 4 - 6 intact cells either side of the embryo. A few crushed endosperm cells were found adjacent to the embryo (Fig. 5.11). The extent of crushing varied between samples (Fig. 5.12), but tended to occur more on the cotyledonary side of the embryo (*i.e.*, the endosperm cells on the left hand side of the radicle tip in Fig. 5.1).

5.3.4 *Priming Seeds*

Priming of seeds led to greater protein body breakdown and vacuolation of the thin walled endosperm cells than occurred in untreated seeds prior to radicle emergence (Fig. 5.8). The primed embryo showed extensive vacuole development and protein body degradation (Fig. 5.10).

5.3.5 *Cell Expansion after Radicle Emergence*

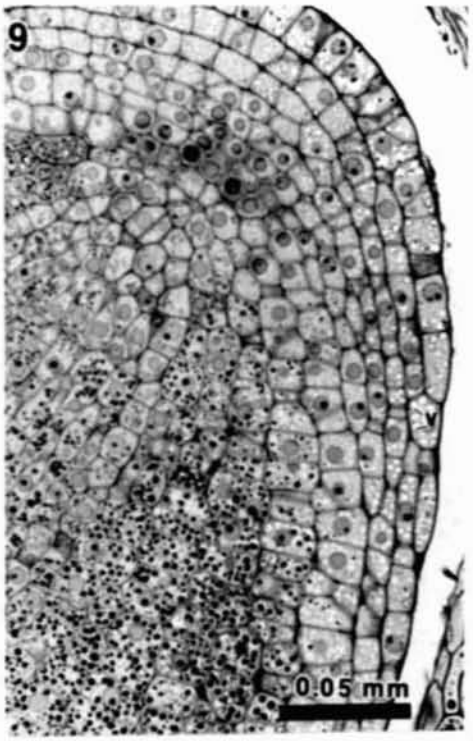
After radicle emergence, the cells of the radicle of primed seeds were about 50% larger than those from non-

Fig. 5.9. A longitudinal section of the radicle tip of a tomato seed imbibed in water for 48 h at 25°C. v vacuole.

Fig. 5.10. A longitudinal section of the radicle tip of a tomato seed after 6 d in a priming solution, 0.090 M K_2HPO_4 + 0.118 M KNO_3, at 25°C. v vacuole.

Fig. 5.11. A longitudinal section of the thin walled endosperm cells at germination 48 h in water at 25°C. r radicle.

Fig. 5.12. A longitudinal section of the thin walled endosperm cells at germination 48 h in water at 25°C showing the region of crushed cells on the cotyledonary side of the radicle. r radicle.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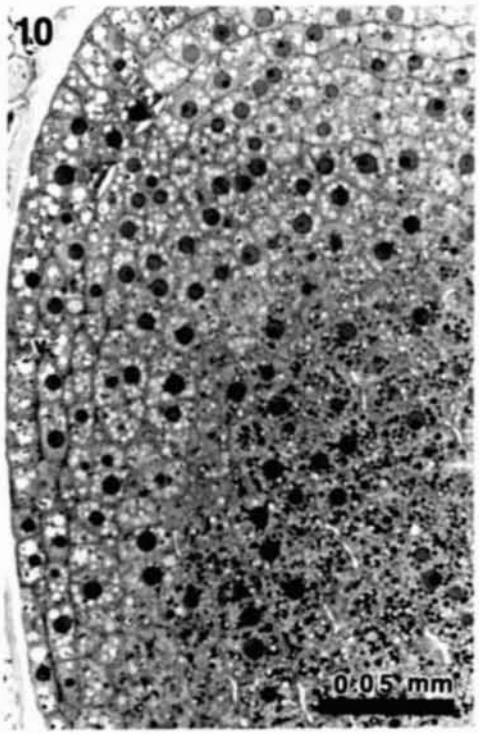
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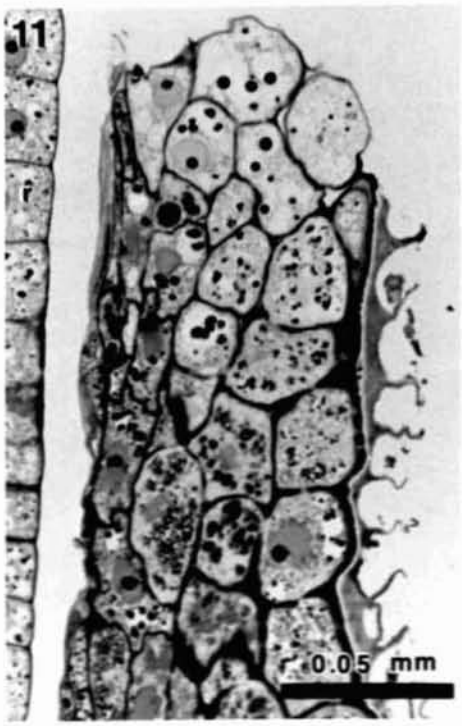
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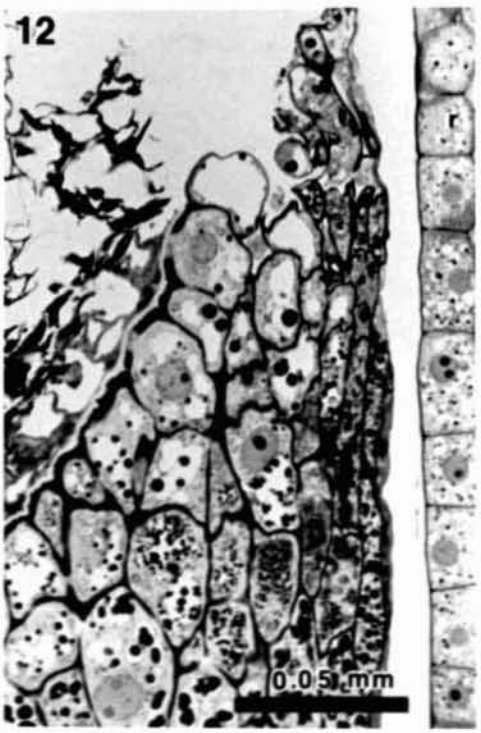
12
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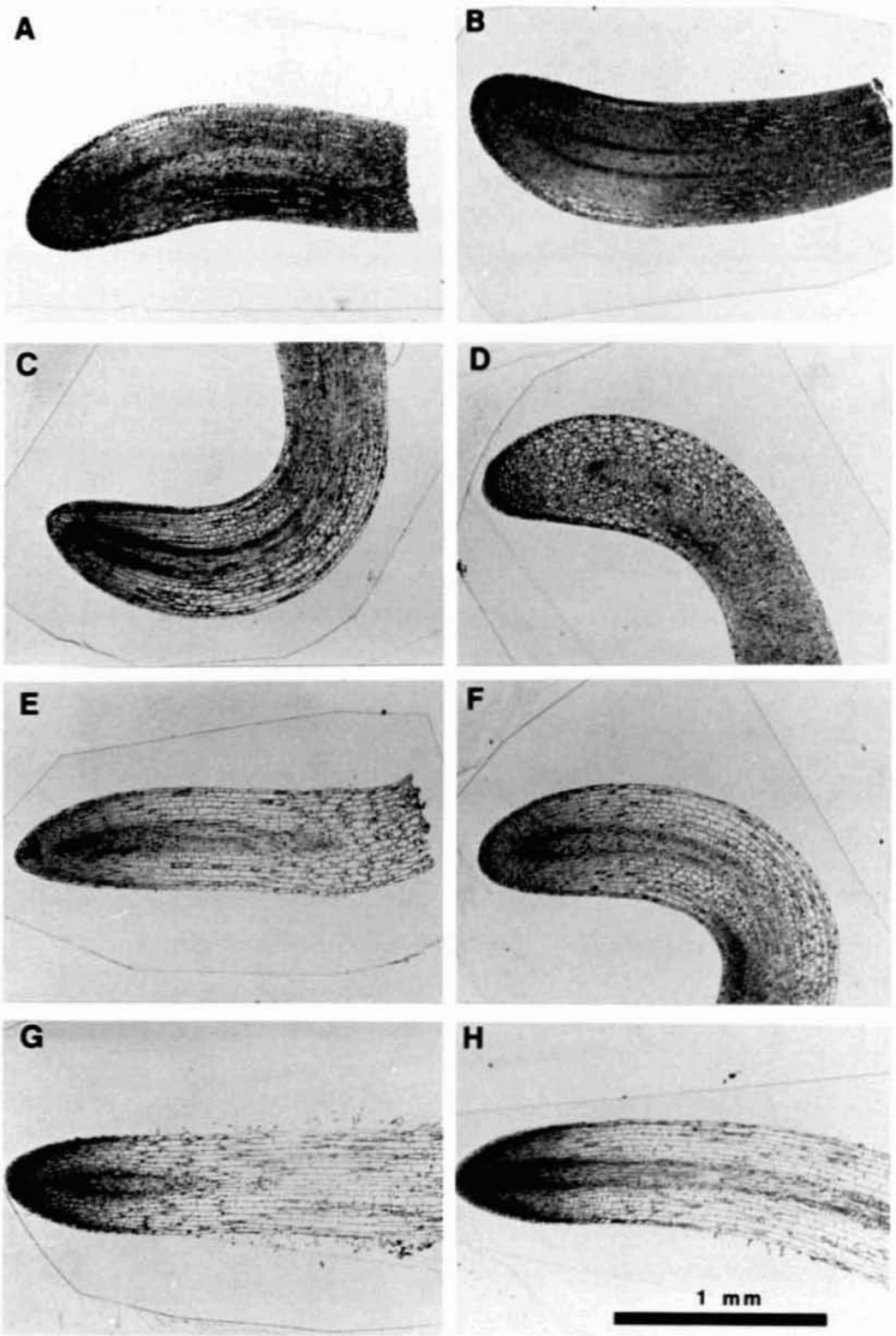
primed seeds. This size difference was overcome after about 4 h, thus the size of cells of primed radicles 8 h post radicle emergence was reached by non-primed radicles by 12 h. Although not distinguishable in these figures, both primed and non-primed radicles commenced cell division about 8 h after radicle emergence (Fig. 5.13).

5.4 Discussion

In tomato seeds protein bodies were found in great abundance in endosperm cells and to a lesser extent throughout the embryo. Such protein bodies are common storage organelles in many seeds (Lott 1980). The breakdown of these protein bodies led to the formation of vacuoles in the endosperm cells of the micropylar region before germination. A similar process of protein body breakdown and vacuole formation has been reported in castor bean (Gifford *et al.*, 1983), celery (Jacobsen and Pressman 1979), lettuce (Psaras 1984) and sunflower (Psaras 1985).

In castor bean and celery the changes do not occur simultaneously throughout the endosperm, but first appear adjacent to the embryo and spread outwards (Vigil 1970; Jacobsen and Pressman 1979). Similar to tomato seeds the changes began at the micropylar end of lettuce and sunflower seeds (Psaras 1984 1985). In celery, castor bean and lettuce seeds, endosperm degradation may be controlled by the embryo through GA induction of proteolytic enzymes in the endosperm, but definitive proof of endogenous GA involvement is lacking (Gifford *et al.*, 1984; Jacobsen *et*

Fig. 5.13 Longitudinal sections through expanding radicles after germination of non-primed (*A,C,E,G*) and primed (*B,D,F,H*) seeds. *A, B* at radicle emergence, *C* 4 h after radicle emergence, *D* 2 h after radicle emergence, *E* 8 h after radicle emergence, *F* 4 h after radicle emergence, *G* 12 h after radicle emergence, *H* 8 h after radicle emergence.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al., 1976; Psaras and Georghiou 1983).

GA action is essential for the germination of tomato seeds (Groot and Karssen 1987). Although not tested here it would appear feasible that the changes identified in the endosperm cells of the micropylar region of tomato seeds could also be GA induced. That similar changes occurred during priming would indicate that GA would be active during this treatment. That both priming and control seeds underwent similar endosperm changes would indicate that these changes must be preliminary to radicle emergence not controlling it. The similarity of the changes in priming and germinating seeds may indicate that some degree of endosperm weakening may occur during priming.

The radicle emerged through the cell layers of the endosperm. There was little evidence of cell crushing as would be consistent with a radicle push to puncture the endosperm and seed coat. The small layer of "crushed" endosperm cells adjacent to the emerged radicle indicated that little destruction occurred during radicle emergence. Separation of endosperm cell layers along a single fissure would permit radicle emergence and result in the observed pattern.

No evidence of cell division was found in the embryo prior to radicle emergence nor during priming, although only a small number of seeds were examined. Cell division was evident in embryos at 8 h after radicle emergence in both primed and non-primed seeds. Embryo growth during priming

has been claimed to be the cause of the observed effects of seed priming in carrot seeds (Austin *et al.*, 1969; Weibe and Tiessen 1979). This does not seem to be the case with tomato seeds. However, Kurth *et al.*, (1986) have suggested that cell division increased the number of cells in the tomato embryo by about 50% (from 200 to 300 cells) prior to radicle emergence and also during salinity treatments which prevented germination. These treatments are analogous to priming. My results differ greatly from those of Kurth *et al.*, (1986) and support Coolbear and Grierson's (1979) report of no change in DNA content during priming of tomato seeds. My own estimates of cell numbers per embryo suggest that there are about 150 000 cells. It is not clear how the embryos measured by Kurth *et al.*, (1986) could have so few cells. Smith's (1935) observations of embryo development would suggest that it passed the 300 cell stage before 18 days after pollination, and continued to grow to maturity for a further 30 to 35 days.

This study has shown that priming had beneficial effects to at least two processes of germination. Endosperm cell degradation was more advanced in a primed seed than in a control seed at radicle emergence. Considerable vacuole development occurred in the radicle during priming. The cells of the emerged radicle of primed seeds expanded more rapidly than in control radicles. During the first 12 h after radicle emergence the cells of the radicles from primed seeds were about 50% larger than those from non-primed seeds. A similar fresh weight difference between

growing primed and control embryos for the first 12 h after radicle emergence. This is also consistent with the changes in cell wall extensibility measured in Chapter 5.

Endosperm Weakening during the Germination and Priming of Tomato Seeds

6.1 Introduction

Seeds which do not have impermeable seed coats may have tissues enclosing the embryo which may influence germination by mechanically restraining the expansion of the embryo (Bewley and Black 1985). In lettuce seeds two mechanisms for overcoming endosperm restraint have been proposed: the mechanical force of the embryo pushing against the endosperm, and an enzymatically induced weakening of the endosperm (Ikuma and Thimann 1963).

Prevention of endosperm weakening by Cl-releasing compounds led to lettuce embryo expansion without radicle emergence or in a small proportion of seeds (<5%) germination occurred by protrusion of the cotyledons or splitting of the seed coat about midway between the micropyle and the cotyledonary end (Pavlista and Haber 1970). The force necessary to puncture the micropylar endosperm tissue decreased prior to germination in both lettuce (Tao and Khan 1979), capsicum (Watkins and Cantliffe 1983) and tomato (Groot and Karssen 1987). Endosperm weakening was under the control of GA released by the embryo of tomato (Groot and Karssen 1987) and was promoted by GA application in capsicum (Watkins and Cantliffe 1983). The testa did not weaken during germination but offered only low resistance to radicle emergence (Groot and Karssen 1987).

Guedes *et al.*, (1981) suggested that priming permitted

lettuce seeds to overcome thermodormancy by removing the restriction on radicle emergence imposed by the endosperm. However, they provided no explanation for the prevention of radicle emergence during priming.

The water relations studies indicated that endosperm weakening controlled the timing of germination of tomato seeds (Chap. 2). It was also concluded that endosperm restraint prevented germination during priming (Chap. 3). The histological study of germination and priming revealed that considerable degradation of endosperm cells occurred both prior to radicle emergence and during priming (Chap. 5). In this chapter I have used direct measurements of endosperm resistance to investigate the role of endosperm weakening during the germination and priming of tomato seeds.

6.2 Materials and Methods

Tomato seeds were imbibed in water, primed in 0.090M K_2HPO_4 + 0.118M KNO_3, or reimbibed in water following priming and air drying in Petri dishes containing Ekwip U70 filter papers at 25°C. At various times seeds were removed and the mechanical restraint of the tissues enclosing the radicle were measured. Mechanical restraint was measured by cutting seeds in half and removing the radicle tip from the micropylar end by applying gentle pressure and using forceps. The seed halves were placed in an aluminium holding block over a hole of 0.8 mm diameter. A chrome-plated needle (0.46 mm diam.), with its tip rounded to

resemble that of a radicle, was inserted into the space vacated by the radicle. The alignment of the needle was maintained constant by the upper portion of the aluminium block (Fig. 6.1). The block was placed onto the centre of a 20 Newton load cell of an Instron 1122 universal testing instrument (Instron Ltd., High Wycombe, Bucks., U.K.). The crosshead of the Instron was lowered onto the needle at a speed of 5 mm min^{-1} until after the needle had punctured the tissues opposite the radicle tip. At each sampling time 30 seeds were measured.

6.3 Results

The mechanical restraint of the endosperm and testa opposite the radicle tip decreased during germination of tomato seeds. The restraint fell prior to the time at which radicle emergence began in the population of seeds (42 h) with little change after that time. When germination was near complete (85% at 60 h) the restraint of the enclosing tissues of the ungerminated seeds was higher than that measured earlier (Fig. 6.2).

During priming mechanical restraint fell gradually throughout the first 4 d of treatment. The mechanical restraint of the priming seed was lower than that measured for control seeds when germination had begun in their population (Fig. 6.3). During reimbibition of primed seeds the mechanical restraint of the enclosing tissues was initially low and fell a little further to values slightly lower than those measured during priming (Fig. 6.4).

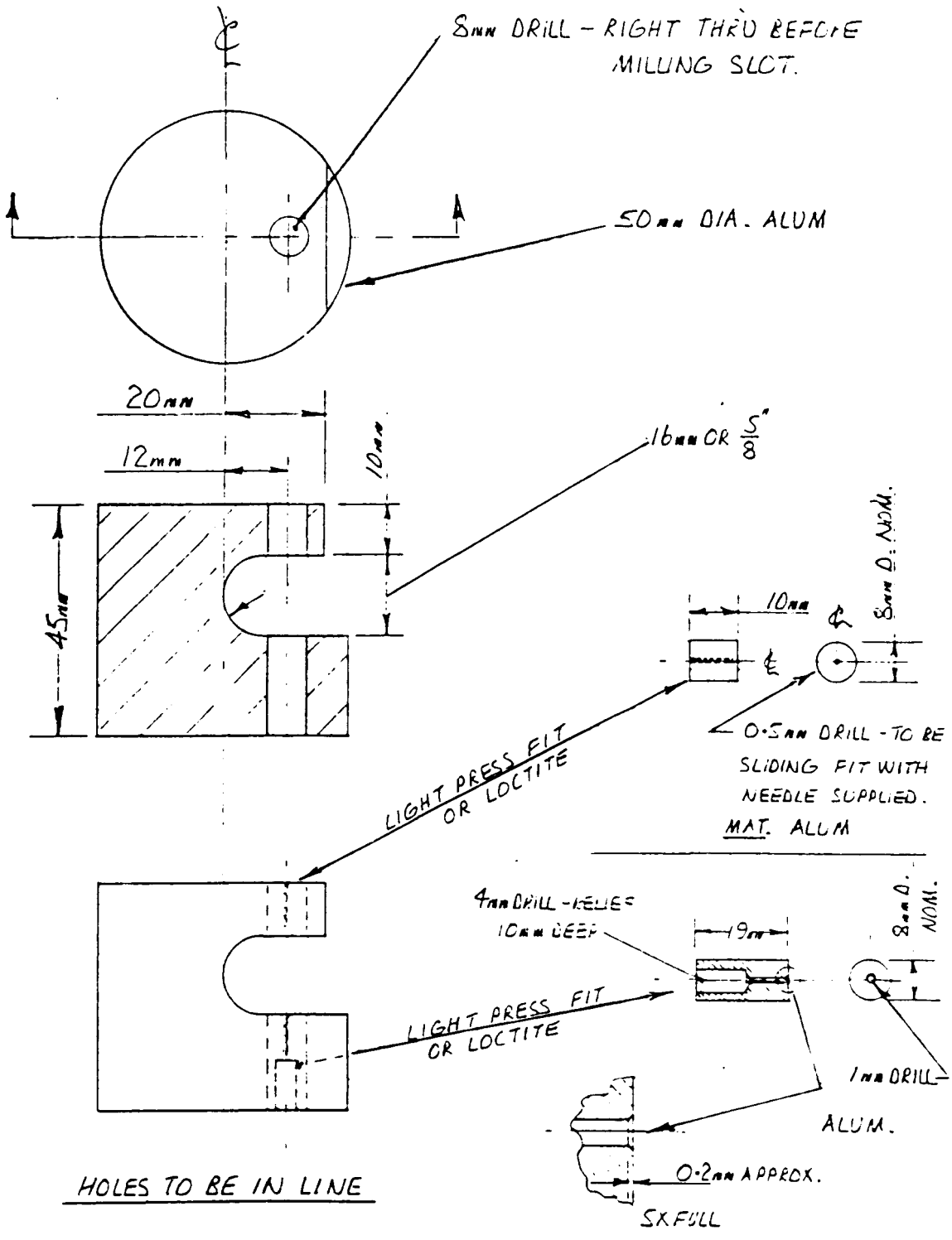

Fig. 6.1. Workshop construction diagram of the apparatus used to test the force necessary to puncture the endosperm and seed coat tissue enclosing the radicle tip of a tomato seed. The aluminium block was used to hold half seeds and needle for puncture force measurements with an Instron 1122 universal testing instrument.

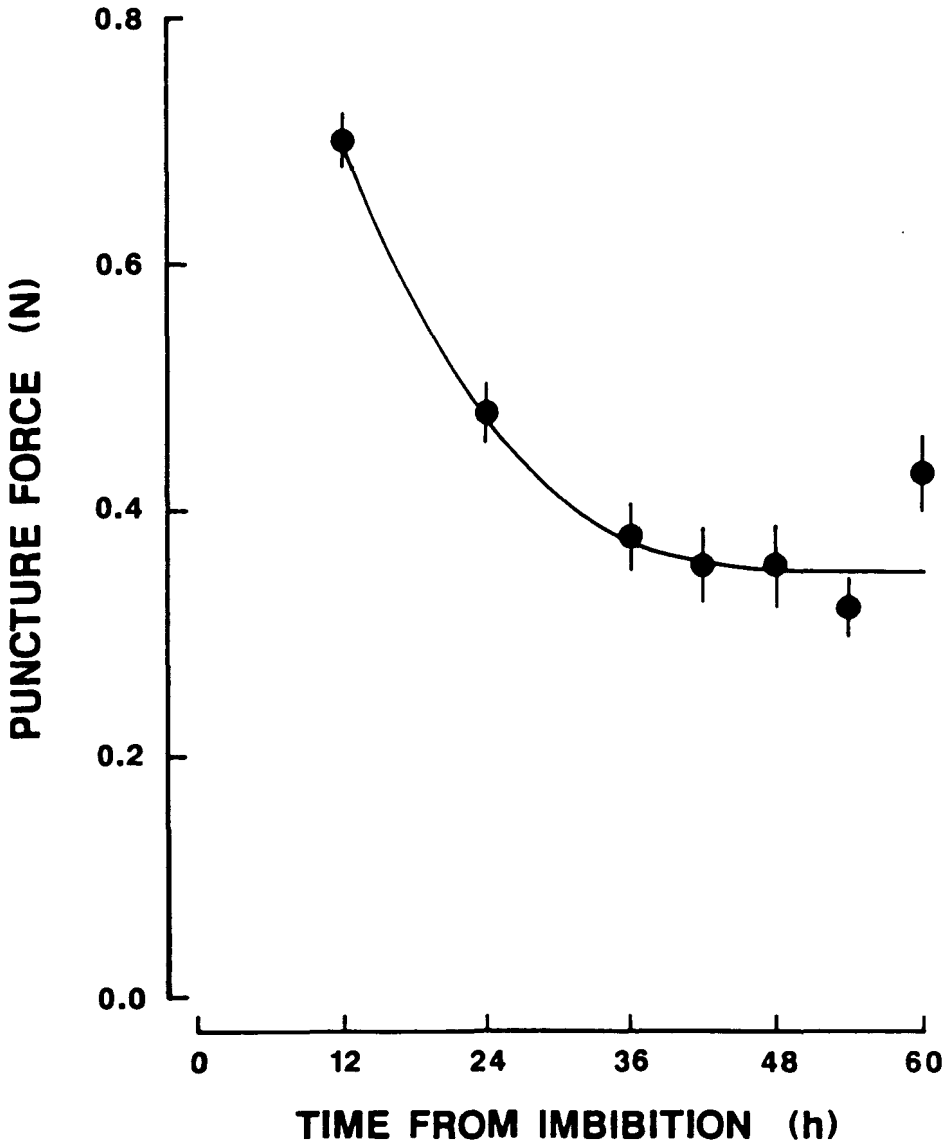

Fig. 6.2. The force required to puncture the endosperm and seed coat tissues of tomato seeds imbibed in water at 25°C.

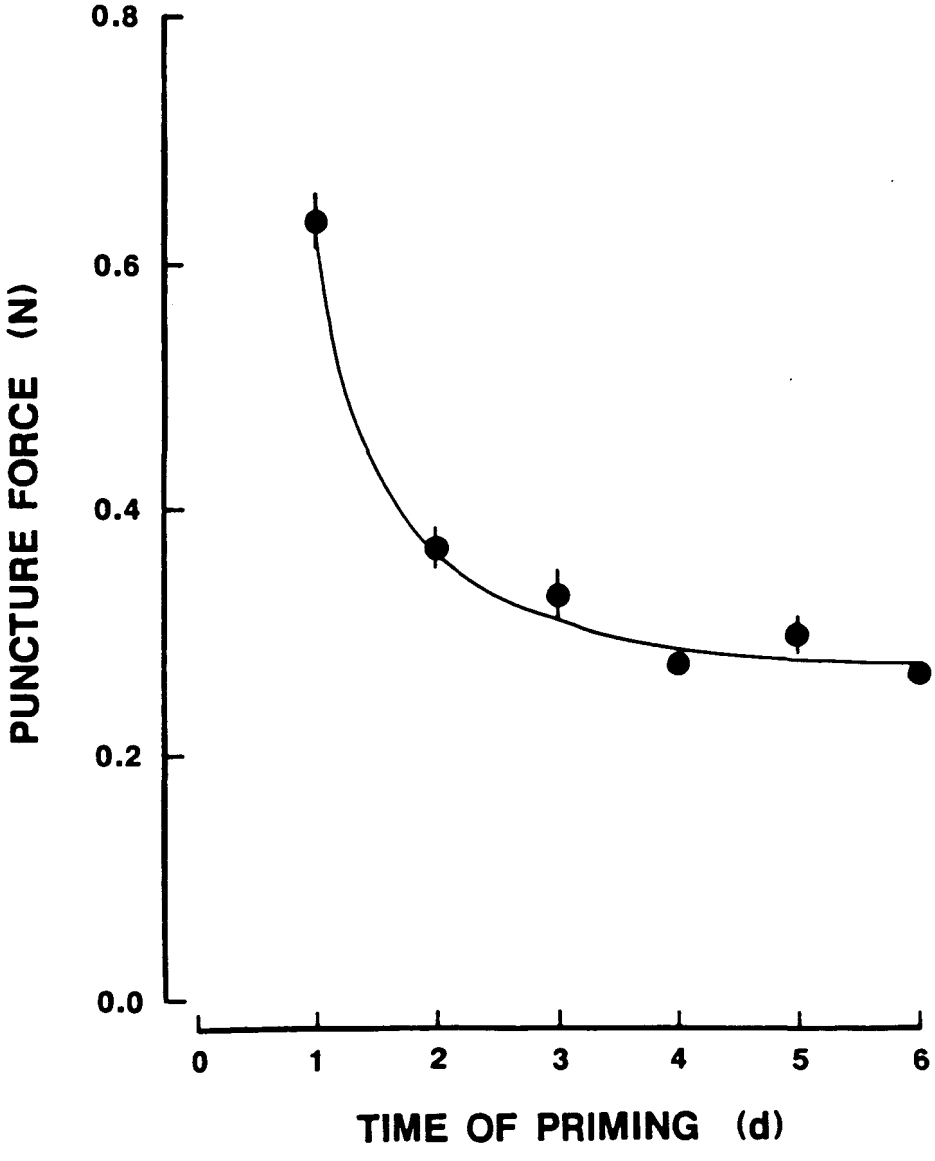

Fig. 6.3. The force required to puncture the endosperm and seed coat tissues of tomato seeds during priming in 0.090M K_2HPO_4 + 0.118M KNO_3 at 25°C.

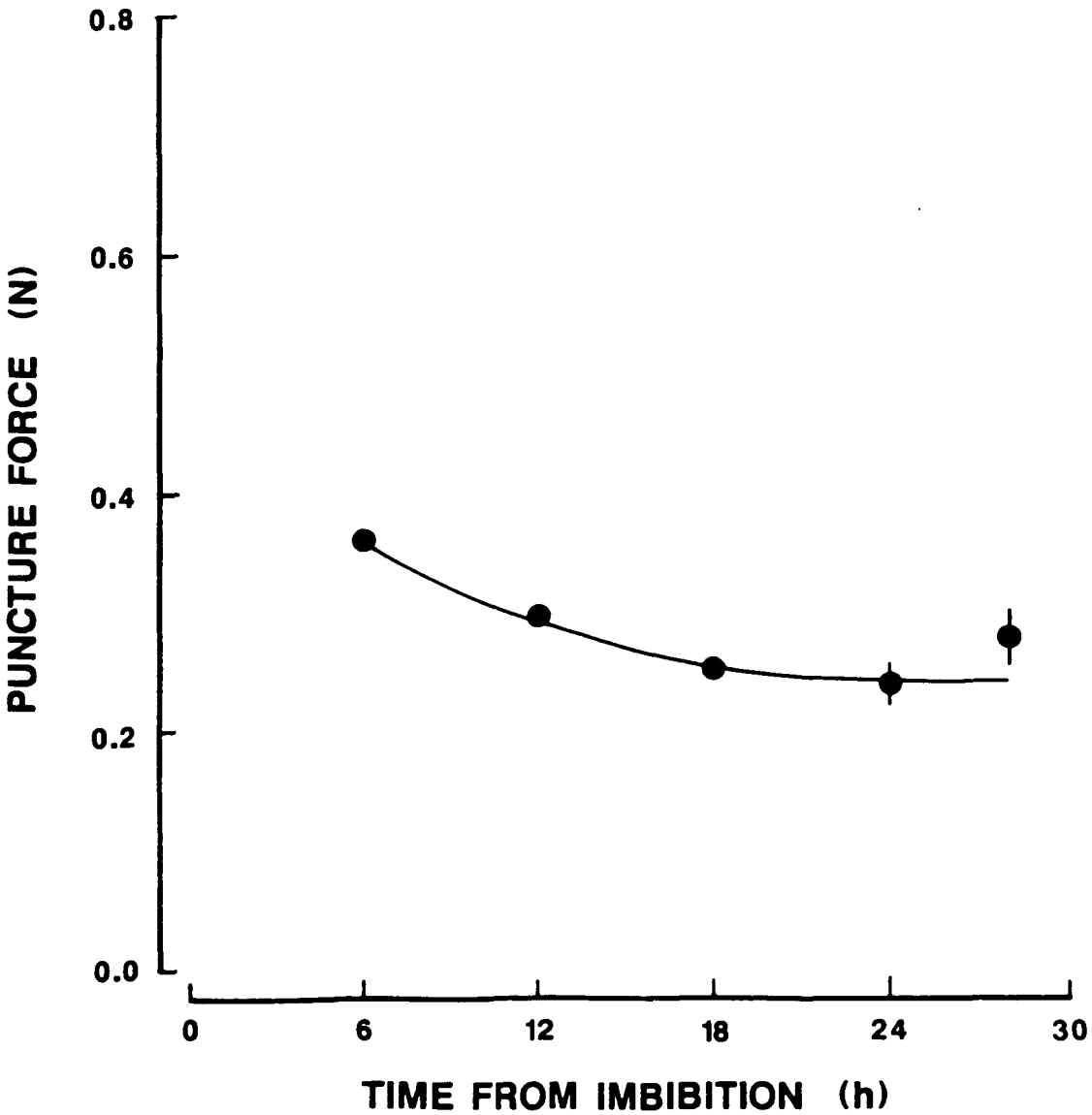

Fig. 6.4. The force required to puncture the endosperm and seed coat tissues of primed tomato seeds imbibed in water at 25°C.

6.4 Discussion

The force necessary to puncture the endosperm and testa tissues opposite the radicle tip decreased during imbibition prior to radicle emergence (Fig. 6.2). These results are similar to those reported in lettuce (Tao and Khan 1979), capsicum (Watkins and Cantliffe 1983) and in tomato (Groot and Karssen 1987) although the timing of endosperm weakening and germination differed between the tomato variety used by Groot and Karssen and that used in this investigation.

The results were consistent with the hypothesis that germination of tomato seeds is controlled by the weakening of the endosperm tissue opposite the radicle tip. The higher puncture force values measured for slow germinating seeds add weight to this evidence. The values measured for priming and reimbibed primed seeds were lower than those measured for non-primed seeds indicating that endosperm weakening during priming may have been more extensive than that occurring during normal germination. These puncture force measurements parallel the extensive changes during priming observed in the histological study (Fig. 5.8).

Ikuma and Thimann (1963) proposed that there were two processes involved in the germination of lettuce seeds: the embryo push and endosperm resistance. It was argued in Chapter 3 that only the removal of the endosperm resistance could result in radicle emergence, *i.e.*, that the embryo can have no expansive push until the restraint is removed. This argument can be supported by the additional data presented in this Chapter.

The $\Delta\Psi_\pi$ from the embryo to the external environment was 1.8 MPa for both primed and non-primed seeds (Figs 2.4 & 3.4), while that for reimbibed primed seeds was about 2.0 MPa (Fig. 4.4). The puncture force measurements can be converted into pressure measurements by division by the cross-sectional area of the needle used. From this calculation non-primed seed endosperm and testa layers would require a pressure of 2 MPa to puncture them. Thus these layers would be capable of restricting embryo expansion. However, the endosperm and testa of priming and germinating primed seeds require less pressure to puncture them (about 1.5 MPa) than would be appear to be generated by the measured $\Delta\Psi_\pi$ between the embryo and the environment.

For radicle emergence to occur some cell expansion must be necessary and cell expansion requires water uptake. This water uptake can only result from the removal of restraint. The maintenance of restraint results in Ψ equilibrium between the restrained tissue and the environment and thus prevents water flow. Therefore, for radicle emergence to occur a further weakening of the endosperm would be necessary. Thus the process of endosperm weakening would appear to occur in two steps.

The values of puncture force were constant within the latter parts of each experiment also suggesting that endosperm weakening for radicle emergence may be a two step process. The first step being composed of a slow weakening of cell wall material resulting in softening to the

resistances measured by the Instron technique. A second endosperm weakening would be necessary to release the embryo during radicle emergence. This second weakening process would be short and immediately precede radicle emergence. It would probably be a cell separation process perhaps similar to abscission rather than an extensive breakdown of wall material.

The first step in tomato endosperm weakening was accompanied by the induction of endo-β-mannanase activity and the release of mannan from the endosperm cell walls (Chapter 3 of Groot 1987). The second step would require the rapid synthesis of a cell separating enzyme to remove the restraint on radicle expansion. Such a separation would be consistent with the observed histological changes associated with radicle emergence (Figs 5.11 & 5.12).

In abscission zones cell separation occurs in a discrete 1 to 3 cell wide separation layer. Separation results from the hydrolysis of the cell wall and the development of mechanical stresses (Sexton *et al.*, 1985). Two wall hydrolases have been found in many abscission zones: B1:4 glucan 4 glucan hydrolase (cellulase) (Abeles 1969) and polygalacturonase (Riov 1974).

In an attempt to test whether polygalacturonase may have been involved in the weakening of endosperm of tomato seeds, studies were undertaken with an anti-polygalacturonase polyclonal antibody. These antibody studies were inconclusive, but showed that the antibody reacted with a molecule found in the endosperm of tomato

seeds. This molecule had a much greater molecular weight than that of polygalacturonase. Considerably more research effort would be required to identify this molecule and its action during endosperm weakening than was possible for me to undertake as part of this study.

The low values necessary to puncture the enclosing tissues of priming seeds indicated that part of the time reduction for germination of primed seeds may result from the weakening of the endosperm which occurred during priming as was suggested by the water uptake data for germinating primed seeds (Sect. 4.4). Whether priming also facilitates the second step in endosperm weakening, namely at radicle emergence, cannot be determined from this data or by using this technique.

General Discussion

It was the aim of this thesis to investigate the mechanisms involved in priming of tomato seeds. These investigations were framed around attempts to answer two questions: why do tomato seeds prime and why do tomato seeds germinate more rapidly after priming?

7.1 Why do Tomato Seeds Prime?

My approach to answering this question has involved a water relations study. This has followed from regarding germination as a growth phenomenon. Expansive growth of plant cells results from cell wall yielding and water absorption. Expansion is initiated with a yielding of the cell wall and continues under the effects of Ψ_p. Wall relaxation reduces the cell Ψ by dissipation of Ψ_p and gives rise to water influx, which in turn increases cell volume (Cosgrove 1986).

As seed priming involves the soaking of seeds in a solution of appropriate osmotic potential to permit the preliminary processes of germination but prevent radicle emergence, it was hypothesised that this prevention may result from interference with the normal water relations of germinating seeds. Before it was possible to ask relevant questions about the mechanisms involved in prevention of germination during priming it was first necessary to study the water relations of normally germinating tomato seeds.

The water relations study of germinating tomato seeds

revealed that the seeds came into Ψ equilibrium with the imbibitional solution (Fig. 2.2), whereas, the embryo was measured at a much lower Ψ. There was no evidence for a lowering of embryo Ψ_π during phase II prior to radicle emergence (Fig. 2.4), as was suggested by Bradford (1986).

The embryo Ψ measurements should be interpreted with caution for they are *ex situ* measurements and thus do not directly measure these properties in the seed. It is thermodynamically improbable that a large $\Delta\Psi$ could be maintained within the seed during imbibition. Thus the existence of a large endosperm resistance to embryo expansion can be inferred from these measurements.

The endosperm tissue enclosing the embryo was found to restrict the hydration level of the embryo prior to its emergence. As it was shown that the embryo was capable of expansive growth prior to radicle emergence (Fig. 2.6), it was concluded that the weakening of the endosperm was the controlling process in germination of tomato seeds.

During priming radicle emergence is prevented. The water relations study of tomato seed germination revealed that radicle emergence could be prevented by a number of mechanisms. Firstly, the $\Delta\Psi_\pi$ from the embryo to the priming solution may be insufficient for water uptake to occur for radicle expansion. Secondly, the radicle cells may not be capable of expansion. Thirdly, endosperm restraint may prevent radicle expansion.

Water relations studies of priming tomato seeds showed that the seed was in Ψ equilibrium with the priming solution

(Fig. 3.2), but the embryo was not. The $\Delta\Psi_\pi$ from embryo to solution during priming was the same as that for embryos of germinating seeds in water (Figs 3.4 & 2.4). As the embryo was capable of growth after 2 days of the 6 day priming treatment (Fig. 3.5) it was concluded that germination was prevented by the maintenance of the endosperm restraint at a level which prevented radicle expansion.

The force necessary to puncture the endosperm and seed coat decreased prior to radicle emergence in tomato seeds in water (Fig. 6.2). Consistent with a hypothesis of control of radicle expansion by the weakening endosperm, the slower germinating seeds within the population had higher values for endosperm resistance. Priming led to endosperm weakening which resulted in values for endosperm resistance which were lower than those measured from a population of germinating non-primed seeds (Fig. 6.3).

Gibberellic acid (GA) produced by the tomato embryo caused the mechanical restraint of the endosperm layers enclosing the radicle tip to decrease prior to radicle emergence (Groot and Karssen 1987). Endosperm weakening is associated with the induction of endo-B-mannanase activity and the degradation of the mannan-rich endosperm cell walls (Chapter 3 of Groot 1987). However, as Instron-measurable endosperm weakening occurred to a greater extent during priming than during germination of non-primed seeds, an additional weakening process would appear to be necessary for radicle emergence to occur. This second step would be

immediately precede radicle emergence and would probably resemble a cell separation process similar to abscission rather than be an extensive breakdown of wall material. As it is impossible to predict the exact time of radicle emergence in individual seeds, this second stage would be very difficult to measure.

These studies have shown that tomato seeds prime because the endosperm does not weaken sufficiently to permit expansion of the radicle. The mechanism by which some endosperm weakening was permitted but the final weakening for radicle emergence was prevented was not identifiable from these studies. There are two main areas for investigation. Firstly, what enzymes are involved in the second step of endosperm weakening. Secondly, how does the seed sense the suitability of the external environment.

7.2 Why do Primed Tomato Seeds Germinate Rapidly?

The second fundamental question asked about seed priming was why do primed seeds germinate more rapidly?

It was hypothesised that the time necessary for radicle emergence could be reduced by changes in any or all of the three main steps of germination. In primed seeds, imbibition could be more rapid, embryo cell wall loosening could occur earlier and/or endosperm weakening could proceed more rapidly than during the germination of non-primed seeds.

Primed tomato seeds germinated more rapidly than non-primed seeds because all three major steps in germination

occurred more rapidly.

Water uptake by primed seeds was more rapid than that by non-primed seeds (Fig. 4.1) and may have resulted from the lower Ψ_π of primed seeds during imbibition or from improved hydraulic conductivity (Fig. 4.2). It was concluded from the water relations studies of germinating primed seeds that some solute accumulation occurred as was suggested by a number of authors (Hegarty 1978; Khan and Karssen 1981; Khan and Samimy 1982; Liptay and Schopfer 1983; Cantliffe *et al.*, 1984; Bradford 1986). These results are consistent with Bradford's (1986) data on primed lettuce seeds where primed seeds had higher RWC when imbibed at the same Ψ_π. However, it appeared that the solute accumulation occurred during drying following priming. The effects of this solute accumulation appeared to be short lived during embryo expansion after germination.

Primed embryos were capable of expansion at the earliest time at which they could be excised from the seeds. This indicated that the cell wall loosening which occurred during priming was not reversed by drying. Thus the 31 h normally necessary to initiate cell wall loosening during germination of non-primed seeds had been completely eliminated by priming. However, as the timing of radicle emergence was controlled by endosperm weakening, the changed embryo cell wall properties may not affect the timing of germination, but merely the rate of subsequent expansion.

During imbibition in water of primed and dried seeds the mechanical resistance of the enclosing tissues was

initially low and fell a little further to values slightly lower than those measured during priming (Fig. 6.4). This indicated that a large part of the time reduction for germination of primed seeds resulted from the weakening of the endosperm which occurred during priming as was suggested by the water uptake data for germinating primed seeds (Sect. 4.4).

The endosperm of tomato seeds was found to consist of two distinct cell types found in separate locations within the seed (Fig. 5.1). At the micropylar end of the seed the endosperm cells had thin walls (Fig. 5.3), whereas those in the rest of the seed had thickened walls (Fig. 5.2). All cells, except those of the root cap, contained protein bodies (Fig. 5.4). During priming protein body breakdown was more extensive in the micropylar region endosperm cells (Fig. 5.13) than was observed prior to germination in non-primed seeds (Fig. 5.9). These changes during priming appear to associated with the measured endosperm weakening as measured by Instron-puncture force (Fig. 6.3). The changes in endosperm morphology are part of the enhancement of germination caused by priming.

After radicle emergence expansion of primed radicles was more rapid than that of non-primed radicles (Fig. 4.7). The large differences in embryo growth rates in PEG solutions indicated that the cell wall extensibility of primed embryos was higher than that of non-primed embryos (Fig. 4.9). There was no identifiable change caused by

priming in either the cell wall yield threshold nor in the hydraulic conductance. Therefore, the changes in embryo Ψ_π caused by priming were of little consequence to radicle emergence.

The relative growth rates of primed embryos were significantly different from those of non-primed embryos for 12 h after radicle emergence. This indicated that only those radicle cells which had had their wall properties modified during priming affected subsequent radicle growth. The histological study of radicle expansion after germination showed that cell division began by 8 h after germination in both primed and non-primed embryos.

This study of germinating primed tomato seeds has shown that priming advanced the timing of radicle emergence by improving the rate of water uptake by the seeds; by eliminating the time necessary for the loosening of embryo cell walls and completing the first step of the endosperm weakening process. Embryos from primed seeds were found to have improved cell wall extensibilities which permitted higher relative growth rates during the first 12 h post radicle emergence.

7.3 A Theory of Seed Priming

A number of general points arise from this study of the mechanisms involved in the control of germination and the changes induced by priming of tomato seeds.

Essentially, tomato seeds prime because of the presence of endosperm tissue enclosing the embryo. Endosperm tissue

prevents radicle emergence during priming by maintaining sufficient resistance to prevent radicle expansion. Because the radicle is prevented from expanding it is possible for changes to occur to the cell wall extensibility of the radicle.

Most of the seeds which have been successfully primed have endosperm which restricts the expansion of the radicle. Many of these seeds have extensive endosperm tissue and small embryos. In some of these species, the development of the embryo is arrested when still immature. Germination of these seeds follows a period of embryo development after the seed is shed from the mother plant (Bewley and Black 1978).

Reinterpretation of the work of Hegarty and Ross (1978 1981; Ross and Hegarty 1979) provides additional evidence for the crucial role of the endosperm during priming. Hegarty and Ross (1981) determined that radicle emergence of a range of species was prevented at higher solution osmotic potentials than that which inhibited radicle growth subsequent to germination. Hegarty and Ross (1981) interpreted their results as indicating that the initiation process for cell elongation was inhibited. Rather these results should be interpreted as indicating that endosperm weakening for radicle emergence was inhibited as was found to be the case for tomato seeds.

Embryo growth during priming has been reported for carrot seeds (Austin *et al.*, 1969). Weibe and Tiessen (1979) showed that embryo size correlated with the time to radicle emergence in both primed and non-primed carrot

seeds. This embryo growth during priming would follow the degradation of the endosperm tissue. As radicle emergence is prevented during priming of carrot seeds, the radicle must still be constrained by some endosperm. Control of endosperm degradation must be independent of the processes which weaken the endosperm layers enclosing the radicle tip to permit radicle expansion.

The breakdown of endosperm cell walls and the mobilization of mannans has been shown to occur during the germination of some dicot seeds. The induction and activity of endo-B-mannanases in the mobilization of carbohydrates from lettuce endosperm cell walls has been demonstrated (Halmer and Bewley 1979). Endo-B-mannanase activity was also evident in the endosperm of tomato seeds prior to radicle emergence and could be induced in GA-deficient seeds by GA application (Chapter 3 of Groot 1987). However, as was demonstrated by measurement of endosperm weakening during priming (Fig. 6.3), additional processes must be necessary for the endosperm weakening which permits radicle emergence.

Most work on the endosperm of germinating seeds has concentrated on the mobilization of the reserves after radicle emergence, particularly in cereals. The similarity between the changes occurring in the cereal aleurone layer and the dicot endosperm cells has been recognised (Jacobsen 1984), but little attention has been paid to the control of endosperm weakening during the germination of dicot seeds or

to the role of the aleurone layer enclosing the cereal embryo.

It is probable that the weakening processes occurring in dicot endosperm also occur in the aleurone layer enclosing the embryo of cereals. Similar to tomato seeds, in wheat this aleurone layer is thin-walled (Bradbury *et al.*, 1956). This layer has been shown to control the dormancy of *Avena fatua* seeds by controlling the uptake of water by the embryo (Raju *et al.*, 1986). Therefore, priming should be able to be used to enhance the germination of all seeds that have endosperm which constrains the expansion of the radicle, should the manipulation of the weakening of this endosperm layer be achieved.

Priming has not been particularly successful for seeds without extensive endosperm tissue, particularly when the primed seeds have been dried before subsequent germination. Drying of primed soybean and sorghum seeds reduced the benefits considerably (Bodsworth and Bewley 1981). However, the disadvantageous effects of drying may be independent of the small endosperm layer. When radicle emergence is constrained by the enclosing endosperm tissue priming should be of benefit to germination.

The control of radicle emergence during germination and priming may benefit from studies of the mechanisms involved in other processes of tissue weakening such as fruit ripening and abscission. These studies may provide means for manipulating endosperm weakening to permit priming. In addition to studies of endosperm weakening, the most

fundamental process needing investigation is the mechanism by which the seed can determine the suitability of the external environment for germination.

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