THE GERMINATION AND ESTABLISHMENT OF 
E. sieberi L. Johnson IN SEEDBEDS 
SUBJECT TO WATER DEFICIT

by

Ann Gibson

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Doctor of Philosophy
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AN ABORIGINAL SIMILE

There was no stir among the trees,
No pulse in the earth,
No movement in the void;
The grass was a dry white fire.
Then in the distance rose a cloud,
And a swift rain came:
Like a woman running,
The wind in her hair.

Mary Gilmore
STATEMENT OF ORIGINALITY

Except where acknowledged, this thesis is my own work.

[Signature]

Ann Gibson
ACKNOWLEDGEMENTS

I wish to thank my supervisors Professor D.M. Griffin, Professor E.P. Bachelard and Mr K.W. Groves for introducing me to the biology of the forest, and for their interest in the project as it developed. I thank Professor Bachelard for teaching me to write more scientifically than I normally do.

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Many members of the Department of Forestry have supported me during this project: I thank them for their care. I thank my husband Alan Gibson for his unqualified respect for my wish to practice this discipline.
Observations of seed germination and seedling establishment of *Eucalyptus sieberi* L. Johnson, silvertop ash, from seed broadcast on seedbeds typical of severely, moderately and lightly disturbed field sites suggested that these seeds and seedlings are specifically adapted for survival on cohesive seedbeds receiving an intermittent water supply. Penetration of the soil surface occurred on all sites, although, following severe disturbance the soil was sufficiently compacted to restrict development of the root system to 30% of that on lightly disturbed sites.

The seed coat is broken by the hypocotyl which curves towards the soil surface and pushes the tip of the radicle into the soil. The hypocotyl exerts more force, by virtue of its relatively broad, rigid structure, than the radicle. The 'clinging disc' around the collet anchors the seedling against growth pressures which tend to force it out of the soil, and stabilizes the seedling while the cotyledons are raised, preventing it from toppling and pulling the radicle from the soil.

Placement of the radicle vertically downwards in or against the surface facilitates establishment by increasing the pressure it can exert and by increasing the rate at which it extends. Radicles growing downwards (1 x gravity) extend at twice the rate of those growing on a 45° slope (0.7 x gravity). The hypocotyl compensates for changes in orientation during germination, ensuring that the radicle
reaches the surface as rapidly as possible, in a vertical direction.

The peripheral hairs on the 'clinging disc' provide water to raise the cotyledons before the radicle is established. Desiccation of the seedling between germination and placement of the radicle in the soil does not prevent establishment. The hypocotyl and the collet end of the radicle tolerate several cycles of wetting and drying, and if they are lost or debilitated, establishment can still occur from adventitious roots which emerge from the undamaged apical end of the hypocotyl. The production of adventitious roots depends on a supply of sucrose and auxin from the cotyledons and these resources are channelled into two roots which both extend rapidly.

Early expansion of the cotyledons relative to the establishment of the radicle allows photosynthesis to begin soon after germination but exposes the seedling to damage from desiccation. Expansion of the cotyledons is delayed by intense light, and anthocyanin, which absorbs UV light, accumulates in exposed tissue. The upper surface of the cotyledons has xeromorphic features but is wettable. Water droplets adhering to it are absorbed directly through the cuticle and may supplement the seedlings' water supply.

Germination occurs in wet weather, after the seed has been 'primed' by successive wet periods. Progress towards germination is cumulative and may be interrupted for up to 8 months with little loss of viability or of 'priming'. The water relations of the germinating seed are controlled
by an interaction between the two separate integuments, the position of the seed on the surface, and the atmospheric relative humidity. This interaction ensures that the seed does not germinate, however wet the seedbed, if the atmospheric conditions are unfavourable.

The seed coat is broken by pressure from a small group of rapidly expanding cells in the collet. Should germination not succeed, food reserves further up the hypocotyl are mobilized and several attempts to germinate can be made. The apical meristem of the radicle remains inactive during germination; it is resistant to desiccation and uses no food reserves.

This specialized germination strategy in *E. sieberi* may be related to selection pressures against vulnerable apices which delay the onset of radicle growth and emphasize the role of the hypocotyl for germination and establishment.
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CHAPTER I
GENERAL INTRODUCTION

Logged eucalypt forest may regenerate from seed shed from capsules on standing seed trees or on branches left as débris. Seed beds in which this seed germinates differ from natural seed beds because logging disturbs the soil and a post-logging burn may cover the beds unevenly with ash.

Disturbance varies from compression or removal of the litter layer of the soil where a tree has fallen or a log skidded, to heavy compaction and destruction of the A horizon of the soil where logging traffic has been constant, on skid trails and log landings. After clear felling, representative figures suggest that about 35% of the site is undisturbed, 15% is heavily compacted and 50% has suffered some degree of disturbance (Jacobsen 1983, Bridges 1983). Disturbance associated with logging activity degrades the structure of the soil, increasing the bulk density and decreasing the pore space (Greacen and Sands, 1980). These changes may affect root growth directly by making the soil difficult to penetrate or indirectly by affecting the supply of air, water or nutrients to roots.

Two recent studies compared the amount of root growth under skid trails and in adjacent undisturbed soil. Schuster (1979), working in karri forest on podsolic soils in Western Australia found 0.4 and 0.2 kg m$^{-3}$ root dry matter in compacted soil near the surface and at 40 cm
depth, compared with 3.0 and 2.0 kg m\(^{-3}\) respectively in undisturbed soil. He predicted a serious loss of productivity, since 20-30% of the soils in the forest are compacted to the level sampled. Jacobsen (1983), working in alpine ash forest on a kraznozem in the Australian Capital Territory, logged over 40 years ago, found 0.4 and 0.2 kg m\(^{-3}\) of root dry matter 2-10 and 30-50 cm below a skid track surface, compared with 2.2 and 1.3 kg m\(^{-3}\) below undisturbed soil at the same depths. His crude estimate was that productivity on skid tracks is about 20% of that on undisturbed soil. This agrees with results of Wert and Thomas (1981) who found that production from 32 year old Douglas firs on old skid tracks was only 26% of that on undisturbed areas.

The Australian studies compared the amount of root tissue in undisturbed and heavily compacted soil, but did not sample disturbed soil which occurs on 50% of a logged site. The aim of this study is to measure the establishment and growth of seedlings in slightly disturbed, disturbed and severely disturbed seed beds derived from granite and sedimentary parent materials in the coastal dry sclerophyll eucalypt forest at Eden, New South Wales.

Disturbance due to logging alters the surface on which seed must germinate, as well as the structure of the mineral soil in which the seedling becomes established. Therefore an assessment of factors affecting germination on disturbed sites was an integral part of the project.
Bridges (1983) remarked that "it is not known whether the mosaic of soil disturbance, débris and ash accumulation [after integrated logging at Eden] contributes any beneficial or detrimental effects to germination and establishment". Christensen and Schuster (1979) found that ash beds inhibited germination of karri, perhaps by resisting initial wetting of the seed bed, but enhanced the survival of the seedlings that did germinate. Annels (1980) found only 5 to 20% of plots considered unfavourable for germination (compacted and unburned) were stocked with karri, while 20-33% of disturbed plots were stocked. Fifty to 70% of ash bed plots were stocked in this case, presumably because germination was not inhibited, the rain causing germination lasting long enough to overcome any difficulty with initial wetting. These studies indicate that changes in the surface due to logging and post-logging activities do influence germination and establishment of eucalypts.

Eucalyptus sieberi L. Johnson, silvertop ash, was chosen to test the effect of logging disturbance on germination and establishment at Eden. It accounts for 30% of eucalypt seedlings regenerating after logging there (Bridges, 1983), and individuals occur on heavily compacted skid tracks and log landings. Moreover, Ellis (1971) found that E. sieberi competed well against other eucalypt seedlings in pot trials in soils of increasing bulk density, suggesting that it could be established in the more severely disturbed soils in the field at Eden.
E. sieberi seed is non-dormant and germinates quickly and vigorously (Grose and Zimmer, 1958). A large supply of Certified Seed was available from the CSIRO Division of Forest Research - Seedlot 12129, from 10 parent trees growing at Timbillica, NSW, Latitude 37°22', Longitude 149°37', Altitude 244 m.

Ten week old seedlings of E. sieberi grown in seed flats have a relatively shallow fibrous root system and large seedling leaves with a Root/Shoot ratio of 2.7 (Zimmer and Grose, 1958). This suggested that seedlings in the field would be easy to measure, easy to dig out for assessment of root growth, and less likely to escape the compacted surface soil than seedlings of species which develop a deep tap root.

Thus vigorous germination, balanced growth habit, ability to grow in high bulk density soils, and commercial value for timber, led to the use of E. sieberi to measure the effect of logging disturbance and soil compaction on germination and establishment. Germination occurs on exposed seedbed surfaces and establishment requires that the radicle penetrate the compact surface. The morphology and physiology of the germinating seed and the newly germinated seedling are described in this thesis and related to the successful establishment of seedlings from seeds lying on compact seedbeds.
CHAPTER 2

THE EFFECT OF LOGGING DISTURBANCE ON GERMINATION AND ESTABLISHMENT OF *E. sieberi* REGENERATING FROM SEED

INTRODUCTION

The nature and degree of soil disturbance and compaction in logged forests is highly variable because the harvesting operations are not evenly spread over the area and because the soil varies in its response to the load placed on it (Graacen and Sands, 1980). A boulder or branch on the surface may, for example, protect the soil beside it or move in the soil under pressure, opening a crack and thus providing a favourable microsite for growth in otherwise severely disturbed soil. Accordingly, the trial designed to test the effect of logging disturbance sampled seedbed surfaces representative of disturbed sites and at the same time ensured that seedlings used to assess the effect of compaction were not in favourable microsites.

To sample both surface conditions and soil compaction, three scales were included in the experimental design.

Scale 1: seed broadcast evenly over the surface of 25 m$^2$ plots representative of low, intermediate and high levels of disturbance and left unprotected.

Scale 2: seed broadcast evenly over 1 m$^2$ plots selected for homogeneity and absence of favourable microsites, positioned within 25 m$^2$ plots and protected by Sarlon shade cloth fences.
Scale 3: 1000 cm³ clods of compacted soil taken from beside each 1 m² plot and set in gypsum casts to retain the structure: seed sown on clod and conditions for germination and growth controlled.

Thus the 25 m² plots sampled establishment after germination in the range of seedbed conditions characteristic of a particular level of disturbance and the 'clods' gave a measure of seedling growth in compacted soil without climatic extremes, for comparison with growth in the field.

The trial was set out in August 1982. However, the Scale 1 plots were not sown because the 'drought breaking' rain in March was not followed up in April and May and if the drought had continued the subsoil would have been too dry to support growth through summer. Scale 2 plots were sown as it would have been possible to water them through summer, but insufficient seedlings became established to justify watering. The 'clod' trial was carried out.

2.1 FIELD TRIAL

Design

Objective data on the effect of logging on the soil and on regeneration of the eucalypts in the State Forest at Eden was not available, so the Field Trial was designed to give as much information as possible, within the constraints of cost and time, to obtain a general data base for more precise experiments. The interaction of 4 soils, 2 on granite and 2 on sedimentary parent material, with 3 levels of logging disturbance was tested.
Logging disturbance was judged by eye as **Low**, litter layer crushed and mineral soil exposed, **Intermediate**, litter layer removed and mineral soil compressed, and **High**, surface structure of mineral soil destroyed. Undisturbed sites were not included since a heavy litter layer prevents establishment (Jacobs, 1955) and clearing would have made an artificial seedbed, unrepresentative of the logged sites.

The two granite sites were on opposite sides of a ridge running east-west 380 m above sea level in Compartment 344 of the Yambulla State Forest (Fig. 2.1) site A in coupe 7 and site B in coupe 3. Logging 12 months earlier had been followed by a post logging burn.

The two sedimentary sites were on the northern side of a ridge in Compartment 224 of the Timbillica State Forest, approximately 15 km east of the granite sites and at an elevation of 130 m. Coupe 13, site C, and coupe 15, site D, had been burned in a wild fire after regeneration had started following logging 18 months earlier.

Climatic conditions were standardized by selecting sites with an exposed northerly aspect, close enough together to receive similar amounts of rain. Plots were sited on flat areas to minimize run off. Each square metre plot was fenced with 0.5 m wide Sarlon shade cloth, one side running due E-W to standardize the amount of shade provided to the plot. The fences protected the plots from wind and animals as well as providing shade. No precautions were taken against foraging ants as they are
Fig. 2.1 Site of Field Trial at Eden N.S.W.
Top: Location of sites in State Forests
Bottom: Location of plots in logged sites
not sufficiently active in the forest in early spring to remove a significant amount of seed (Bridges, pers. comm.).

There were 3 plots at each disturbance level on granite site A and sedimentary site C and 2 at each level on granite site B and sedimentary site D. The design was unbalanced to limit the trial to 30 plots, enabling all data to be recorded on the one day of each monthly inspection. Seed was broadcast evenly on the plots at a rate of 300 viable seeds m$^{-2}$.

Over 70 mm of rain fell in September, after the August sowing, and the first assessment of establishment of the seedlings was made in mid October. The percentage establishment was lower than expected, providing too few seedlings for systematic sampling of the root system through the summer. Therefore the top growth only of the seedlings was measured, month by month.

**Result and Discussion**

Fig. 2.2 shows the rainfall at the Timbillica Weather Station during the trial period. Rainfall in September caused germination and allowed establishment of some seedlings, but very low rainfall in October and November, January and February subjected them to drought conditions.

Germination and establishment occurred during September and October on the granite sites as shown by the number of seedlings in the plots in October and November (Table 2.1) but in September, only, on most sedimentary sites.
Fig. 2.2 Annual Rainfall at Timbillica Station
Table 2.1  Establishment of *E. sieberi* on seedbeds disturbed by logging

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<th>SITE</th>
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<td>High disturbance</td>
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<td>16</td>
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<td>2</td>
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<tr>
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Variation between the replicate plots of a treatment was as great as that between the three levels of logging disturbance on any particular site. Thus some factor or factors other than level of disturbance determined whether *E. sieberi* seedlings became established in the spring of 1982.

On both granite sites at least half the seedlings on low and high disturbance plots survived the summer but fewer than 10% survived on intermediate level plots (Fig. 2.3). On sedimentary site C few seedlings survived into December and on site D seedlings on highly disturbed plots survived but all other seedlings died by November.
Fig. 2.3 Survival of *E. sieberi* seedlings, summer 1982-3
The growth of the surviving seedlings was reduced on sites on which few seedlings survived (Fig. 2.4). Intermediate disturbance on granite supported less growth than high or low disturbance, although the effect was less severe on site B than on site A. Site C did not support growth past the cotyledon stage and seedlings established on the high disturbance plots of site D grew through the driest months.

Representative seedlings taken from the plots in March 1983 (Plate 2.1) varied considerably in size. Seedlings from low disturbance sites were consistently larger than those on high disturbance sites. Thus it appeared that the compacted soil of highly disturbed sites reduced growth rate although it did not affect survival or production of leaf pairs.

The length of time a seedling survived depended on its position in the plot. All seedlings established on plots with dark surfaces were within the shade cast by the Sarlon cloth fence (Plate 2.2) and those seedlings established on the unshaded side of plots with light coloured surfaces were the first to die in that plot. An analysis of survival in relation to surface colour of the plot (Table 2.2) shows that seedlings survived on plots with light coloured surfaces on both granite sites at low and intermediate disturbance, but not on plots with dark coloured surfaces where grey ash and charcoal absorbed more heat than did the light surfaces (Lilley, 1980). Low and intermediate disturbance plots of site D, on which no seedlings survived, were very dark with ash and charcoal from wildfire that burned regrowth after logging.
Plate 2.1 Representative seedlings from disturbed sites.

Granite Soil B
(L. to R. - Low, Intermediate and High, 3 times)

Sedimentary Soil D
(All high disturbance)

Plate 2.2 Cotyledonary seedlings established in shade, October 1982.

(a) Sarlon Shade cloth  (b) Shaded micro-site
Fig. 2.4 Growth of *E. sieberi* seedlings, Summer 1982-3
Table 2.2 The effect of colour of surface on seedling survival

<table>
<thead>
<tr>
<th>Soil and site</th>
<th>% Survival of seedlings Oct '82-Mar '83</th>
<th>Low disturbance</th>
<th>Intermediate</th>
<th>High disturbance</th>
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<tbody>
<tr>
<td></td>
<td>Light</td>
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<tr>
<td>Granite A</td>
<td>66</td>
<td>7</td>
<td>10</td>
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<td>75</td>
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<td>50</td>
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<td>Granite B</td>
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<td>100</td>
<td>27</td>
<td>56</td>
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<td>Sedimen. C</td>
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<td>Sedimen. D</td>
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All plots at the high level of disturbance were light since the light subsoil was exposed and any ash had been washed off the compact surface. Moreover, these plots had stones on the surface and cracks formed by shrinkage as the plot dried, making the surface sufficiently rough to provide shaded microsites for germination and establishment. The sedimentary site C was uniformly light grey in colour but the surface of all plots was smooth, high level disturbance having compacted it severely, without bringing stones to the surface or causing cracks to form.

Thus, establishment of seedlings from seed broadcast on the surface of sites disturbed by logging depended on the roughness and colour of the surface. Rough surfaces provided shaded microsites and light surfaces reflected insolation, keeping the surface cool relative to a dark surface (Cunningham, 1960).
Although 1982 was a severe drought year, the rainfall in south eastern coastal N.S.W. is not reliable. Rains causing germination are likely to be followed by an exceptionally dry period in any year and the ability of *E. sieberi* to survive dry conditions in the cotyledonary seedling stage may represent a specific adaptation for survival in the dry sclerophyll habitat. The structure and physiology of cotyledonary seedlings is described in Chapter 6.

This trial measured percentage establishment of seedlings from seed germinated on the surface, not percentage germination of seeds. Two features of the results indicated that the microclimatic conditions for germination may be very specific. First, establishment continued into October in granite soils suggesting that their surface was better able to retain water, or deliver it to the seed, or that the sites received marginally more effective rainfall, being at a higher altitude than the sedimentary sites. Second, the established seedlings in each plot reached the same stage of development at the same time, suggesting that they had germinated at one time, not throughout the wet September. Christensen and Schuster (1979) observed that karri, *E. diversicolor* F. Muell, did not germinate unless 50-75 mm of rain fell over a 3 day period, and it appeared that *E. sieberi* may have a similar relationship to rainfall incidence. The control of germination by seed/water relations is described in Chapters 7 and 8.
2.2 'CLOD' TRIAL

The glasshouse 'clod' trial tested the effect of compaction caused by Low, Intermediate and High levels of logging disturbance on the growth of newly established seedlings. A 4x3x2 factorial design was used with each treatment duplicated. Seedlings were grown in the four soils tested in the Field Trial at the three levels of disturbance sampled in it, under two different watering regimes:
(a) continually at or near field capacity (wet); and
(b) watered to field capacity weekly (dry).

Method

In October, soil was lifted by spade from the site of one 1 m² plot of each treatment in the Field Trial, when it was damp enough to lift without disturbing the structure. Each sample was brought to the laboratory. Light pressure on the soil caused it to break into clods, and homogenous clods in the order of 10x10 cm surface area and 7 cm deep were set in gypsum casts in 2 l plastic containers. When dry, the casts containing the clods were placed in larger 3 l containers and immersed in water to the level of the clod surface, to wet the clod through the plaster.

Approximately 50 seeds were broadcast on each clod and the container was covered with plastic film to simulate the high humidity conditions associated with rain. A preliminary experiment had shown that the 20-35 seedlings established at that rate of sowing did not compete during the trial; seedlings
growing 10 cm apart developed at the same rate as those 1 cm apart.

One week after sowing the film was removed from each container, exposing the germinated seedlings to 60% relative humidity in a whitewashed glasshouse with temperature fluctuating between 16° and 18°C. The 'wet' treatment clods were watered daily from above and a water table was maintained by standing the cast in 2 cm water. The 'dry' treatments were watered weekly and the cast was not stood in water.

Fewer than 20 seedlings became established in some clods. They were resown and the experimental schedule was adjusted for the 7 day delay.

Measurements of the diameter of the cotyledons, the length of laminae in the first seedling leaf pair and the stage of development of the second leaf pair were made on 20 seedlings chosen at random on the clods after 7, 21 and 40 days respectively.

Forty days after sowing, the casts were broken away and the clods immersed in warm water to facilitate removal of the seedlings. The root systems were washed free of soil and cut from the tops. Ten root systems and 10 tops chosen at random from each clod were bulked into 2 samples and oven dried at 90°C for 24 hours then weighed to assess growth by dry weight. Thus there were 4 samples of each treatment, 2 from each clod, and an analysis of variance using the GENSTAT package was performed to determine the significance of differences in seedling growth due to soil type, level of disturbance and water regime.
A further 2 clods of each soil were set in casts for measurement of soil strength with a Soilcrete penetrometer as an index of compaction. The moisture characteristic of 3 small clods of granite soil A and sedimentary soil C at each level of disturbance was determined as another index of compaction.

Results and discussion

1. Indices of compaction

(a) Soil strength

Soil strength or resistance to penetration is a measure of how tightly the particles are compressed together since tightly packed particles do not move under pressure from the penetrometer.

The level of logging disturbance was correlated with soil strength (Table 2.3).

Table 2.3 Effect of disturbance and water content on soil strength

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</table>
|            |             | Saturated | Dr...
In each soil the highly disturbed clods were stronger than the lightly disturbed clods whether the clods were saturated by standing the casts containing the clods in water up to the level of the clod surface, or air dry. Granite clods were stronger than sedimentary clods due to interlocking of the large, rigid quartz grains. The highly disturbed clods were so compacted that they resisted penetration even when saturated, and clods from disturbed sites resisted when dry, on a dry weight basis.

(b) Moisture characteristic

The moisture characteristic is a measure of the size, shape and distribution of the pores in the soil. Lightly compacted soils drain more quickly at high water potentials because the pores are large and interconnected.

The number of easily drained large pores in sedimentary soil C was markedly reduced in Intermediate and High compaction clods (Figure 2.7). The number of easily drained pores in Granite soil A was reduced by High compaction in comparison with Intermediate compaction. Although the lightly compacted granite clods drained rapidly, the saturated water content was low, possibly because there were no unweathered quartz pebbles forming large, rigid pores in the plots (Smith et al, 1978).
Fig. 2.5 Moisture characteristic of compacted clods
These results show that assessment of logging disturbance by eye as low, intermediate and high, correlates well with the degree of compaction of the soil in which seedlings grow.

2. Establishment on compacted surfaces

Establishment of seeds germinated on the soil surface depends on the ability of the radicle to enter the soil by penetrating the surface (Campbell and Swain, 1973). If the radicle does not, by chance, emerge from the seed over a pore large enough to enter, it must force an entry. In the eucalypts a ring of sticky hairs, around the collet (ie the junction between the hypocotyl and the radicle) anchors the newly germinated seedling, holding the radicle down as it exerts pressure against the resistant surface (Fig. 2.6). The mechanism is described in detail in Chapter 3.

Fig. 2.6 Mechanism of soil penetration in Eucalyptus
The effect of compaction on establishment is given in Table 2.4. Column 1 of the results shows the percentage of seedlings able to anchor to the surface, column 2 the percentage of those seedlings that were anchored firmly enough to allow the radicle to penetrate, and column 3 the percentage of viable seeds that produced established seedlings.

**Table 2.4** The effect of soil compaction on establishment of *E. sieberi* on saturated clods

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Comp. Strength</th>
<th>Establishment of seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg cm⁻²</td>
<td>anchored</td>
</tr>
<tr>
<td>Granite A</td>
<td>0.7</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>4.2*</td>
<td>60*</td>
</tr>
<tr>
<td>Granite B</td>
<td>0.3</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>2.5*</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>5.0†</td>
<td>55</td>
</tr>
<tr>
<td>Sedimen. C</td>
<td>0.4</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1.0†</td>
<td>40†</td>
</tr>
<tr>
<td>Sedimen. D</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1.0†</td>
<td>55†</td>
</tr>
</tbody>
</table>

*Significantly different from low level at 5% level
†Significantly different from intermediate level at 5% level

Except on granite soil B, the high strength, high compaction surfaces gave less anchorage and allowed fewer seedlings to penetrate than the lower strength surfaces. In soil B the low establishment was due to failure to anchor, not inability to penetrate when anchored.
These results show that at least 30% of *E. sieberi* seeds will produce established seedlings on most compacted sites in conditions that favour germination.

Radicles of anchored seedlings unable to penetrate grew along the surface while the R.H. was high but turned down into the soil when the seedling was exposed to 60% R.H., gaining a second chance to penetrate; a survival mechanism examined in Chapter 3.

3. Development of seedlings in compacted clods

Level of compaction had no effect on the expansion of the cotyledons (Table 2.5) and cotyledons reached the same size in 'wet' and 'dry' water treatments. High compaction severely reduced the growth rate of the first seedling leaf pair in dry treatments of all soil types and also in granite soil B and sedimentary soil D when wet. Results for development of the second leaf pair are similar in that it did not develop in highly compacted dry clods or in the highly compacted wet clods of soils A, B and D.

Plate 2.3 shows the effect of soil type and compaction level on the morphology of 40 day old seedlings. Root systems in granite soils were shallow and much branched compared with those in sedimentary soil. The most likely cause of the difference is soil structure. In granite soils tightly packed quartz grains prevent extension of the radicle and cause it to form numerous lateral roots. In the sedimentary soils, which have a plastic clay matrix
Table 2.5 Growth of seedlings in compacted soil

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Compaction</th>
<th>Cotyledon spread(\dagger) in cm (7 days)</th>
<th>Length 1st leaf in cm (21 days)</th>
<th>Development 2nd leaf pair (40 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Wet</td>
<td>Dry</td>
<td>Wet</td>
</tr>
<tr>
<td>Gran. A</td>
<td>Low</td>
<td>0.85</td>
<td>1.00</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>0.80</td>
<td>0.95</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.80</td>
<td>0.80</td>
<td>0.00*</td>
</tr>
<tr>
<td>Gran. B</td>
<td>Low</td>
<td>0.80</td>
<td>0.90</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>0.70</td>
<td>0.70</td>
<td>0.00*</td>
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<tr>
<td></td>
<td>High</td>
<td>0.70</td>
<td>0.70</td>
<td>0.00*</td>
</tr>
<tr>
<td>Sedimen. C</td>
<td>Low</td>
<td>0.90</td>
<td>1.00</td>
<td>0.60</td>
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<tr>
<td></td>
<td>Int.</td>
<td>0.80</td>
<td>0.95</td>
<td>0.60</td>
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<tr>
<td></td>
<td>High</td>
<td>0.70</td>
<td>1.00</td>
<td>0.30*</td>
</tr>
<tr>
<td>Sedimen. D</td>
<td>Low</td>
<td>0.85</td>
<td>1.00</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Int.</td>
<td>0.90</td>
<td>0.95</td>
<td>0.50</td>
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<tr>
<td></td>
<td>High</td>
<td>0.70</td>
<td>0.70</td>
<td>0.10*</td>
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*Significantly different from other treatments of same soil at same water content.

Key to development of 2nd leaf pair:
- none
+ <1 mm, folded
++ >1 mm, folded
+++ unfolding

when wet and few large quartz grains, the radicle continues to extend, and few laterals are formed (Scott Russel, 1977).

The ANOVA (Appendix A) for dry weight of tops and roots in each treatment shows that compaction, as indicated by soil strength and moisture characteristic, has a different effect on growth in the different soils (Fig. 2.7). In granite soil A, growth was the same in Low and Intermediate compaction whether the soil was wet or dry, but significantly less in High compaction. In granite soil B, growth in Intermediate and High compaction was significantly less than in Low compaction.
Fig. 2.7 Effect of soil disturbance on seedling growth (4 soils x 3 levels of disturbance x 2 water regimes)
Fig. 2.7 Effect of soil disturbance on seedling growth (4 soils x 3 levels of disturbance x 2 water regimes)
In sedimentary soil C, roots and shoots grew equally well at the three levels of compaction in wet soil. In dry soil their growth was significantly reduced in high compaction clods. In sedimentary soil D, root growth was reduced in intermediate and high compaction clods, whether wet or dry and shoot growth was reduced in high compaction clods.

Greacen and Sands (1980) stated that the effect of logging on the productivity of the regenerated forest would depend on complex interactions between compaction and the amount of water in the soil. This is apparent in the results of this trial. Nevertheless, there is a general relationship between compaction and seedling growth (Fig. 2.8). Top growth in drier conditions was reduced by approximately 22% in intermediate and 66% in high compaction clods and root growth by 33% and 60% respectively. In wet conditions top growth was not reduced in intermediate compaction clods but it was reduced by 17% in high compaction clods and root growth was reduced by 25% and 51%. These reductions are greater than those reported by Jacobsen (1983) and by Schuster (1979) and may indicate that seedlings are particularly disadvantaged by compacted soils.

2.3 GENERAL DISCUSSION

These results show that germination, establishment and growth of E. sieberi seedlings on logged sites depend on many factors. Establishment occurred on both soil types (granite and sedimentary) at all three levels of disturbance
Fig. 2.8 Effect of soil disturbance on seedling growth (3 levels of disturbance x 2 water regimes)
after germination in September (Table 2.1) but the variation between plots within treatments was as great as that between treatments. Clearly germination and establishment in the field depended on factors other than soil compaction. Survival and growth through the dry summer (Figs. 2.3 and 2.4) depended on factors which included soil colour (dark surfaces reducing survival and growth) and soil surface roughness (smooth surfaces reducing survival and growth).

The 'clod' experiment, in which the two soils at different levels of compaction were examined under more controlled conditions in the laboratory, allowed a more specific examination of the effects of soil compaction on germination, establishment and growth. Establishment occurred on all clods if the ring of sticky hairs around the collet of the germinating seedling anchored it to the soil sufficiently firmly to hold it down while the radicle exerted the force necessary for penetration of the surface. The ability of the seedling to anchor effectively was reduced with increasing soil compaction (Table 2.4).

Interactions between soil type, compaction, and the amount of water in the soil affected the growth of the seedlings established in the clods (Fig. 2.7). The results allow a more detailed interpretation of the results of the Field Trial. Growth in the sedimentary clods was significantly reduced in dry treatments and conditions were even drier in the field. This suggests that growth was confined, in the field, to the short periods when the soils were sufficiently wet to become plastic and allow the radicle to force a path through the soil. Few seedlings, even on light
coloured surfaces, survived over the summer because the dry soil was too cohesive for them to produce sufficiently long roots to reach moist soil. Seedlings surviving on the highly compacted plots of soil D were probably growing in cracks formed when the soil sheared under the weight of the logging machines. Growth in the dry granite clods was not generally less than growth in the wet clods. This suggests that radicles and lateral roots grew in less compacted micro-sites between quartz grains and continued growing as the soil dried, developing a more effective root system than do seedlings on the sedimentary soils.

These differences in root form in granite and sedimentary soils may explain the observation that regeneration on logged granite sites is always better than on logged sedimentary sites although the weather conditions and the nutrient status of the sites are comparable (Bridges, pers. comm.).

While the trial identified some of the factors affecting germination, establishment and growth of _E. sieberi_ on logged sites, it highlighted the seedling's ability to survive during and after germination on the surface of compacted seed beds exposed to drought conditions. This aspect of the biology of _E. sieberi_ is examined in the following chapters. The succeeding four chapters show how the newly germinated seedling penetrates the soil surface (Chapter 3), how it maximizes the chance of establishment by orientating the radicle vertically downwards in the preferred direction for rapid growth (Chapter 4), how it survives
desiccation between germination and penetration into the soil (Chapter 5) and how it responds to stress in the vulnerable period between penetration and establishment (Chapter 6). The last three chapters show how the seed reaches the point of germination by 'priming' during wet periods (Chapter 7), how the integuments of the seed interact with the microclimate to confine germination to periods when the soil is likely to remain wet long enough to support establishment (Chapter 8) and how the food reserves in the embryo are conserved so that several attempts to germinate may be made (Chapter 9).
CHAPTER 3
THE MECHANICS OF PENETRATION OF
THE RADICLE AND SEEDLING ESTABLISHMENT

INTRODUCTION

The condition for establishment of *E. sieberi* seedlings in compacted clods was that the anchorage of the 'clinging disc' to the soil be sufficiently stable to hold the seed down while the radicle penetrated the surface (Table 2.4). When the radicles of ineffectively anchored seedlings elongated, the seed was lifted until it toppled over, pulling the radicle tip away from the surface and exposing it to desiccation (Fig. 2.6).

Exposure and disorientation of the radicle, and disorientation of the elongating shoot commonly occur (Campbell and Swain, 1973) when seeds of plants without a stabilizing mechanism such as the 'clinging disc' germinate on the surface, rather than underground where pressure from soil around the seed prevents destabilization as the radicle pushes into the soil. Few of the *E. sieberi* seeds which germinated on the surface of the clods were disorientated because the anchorage was sufficient to hold each seedling down while the radicle was deflected and grew along the surface in contact with soil water and able to enter any large pore encountered.

Over 50% of seedlings penetrated into clods so strong that subsequent root growth was severely restricted
(Fig. 2.8), indicating that pores large enough for the radicle to enter and elongate in, were sparse. It appeared, therefore, that penetration of the surface was by force, rather than through a large pore situated, by chance, under the tip of the radicle as it emerged. The relationship between the structure of newly germinated seedlings and the mechanics of soil penetration is described in this Chapter.
3.1 PENETRATION OF THE SOIL SURFACE

E. sieberi seeds were germinated on weak (2%) agar and strong, penetration resistant (3%) agar surfaces to determine how the radicle penetrates the surface.

**Materials and method**

Microscope slides were covered with a 2 mm thick layer of 2% or 3% agar in water. A 1.5 mm wide slot was cut in the agar 1 cm from the end of the slide and 5 seeds of E. sieberi were inserted in the slot. A second slide was placed against the agar and the two slides were taped together to form a sandwich (Fig. 3.1) then placed upright in 1 cm water in a closed container in an 18°C constant temperature room. The seeds were held down by the agar above them while the radicle penetrated the surface and grew down into the agar.

The slides were removed after six days, some seedlings having penetrated the surface and grown several centimetres into the agar, others still penetrating into it. They were examined through crossed sheets of polaroid. Agar is an isotropic gel and when deformed by pressure, the stressed
zone shows, in polarized light, as a pale area against a dark ground. Thus the parts of the seedling exerting growth pressure against the agar appeared to be surrounded by a light zone.

Results and discussion

In 2% agar the radicle tips depressed the surface, penetrated, and grew into the agar. The zones of stress around them showed that the greatest pressure against the agar was being exerted by expansion in the zone of elongation and by forward movement of the shoulders of the tip into the agar (Plate 3.1). Plate 3.2 shows the zones of stress around a growing wheat root for comparison with the eucalypt. These results are similar to those obtained by X-ray analysis of the movement of soil particles under pressure from growing roots (Greacen et al., 1967) and show that the extent of the zone of stress observed under polarized light indicates the pressure being exerted on the agar by the seedling.

Plate 3.3 shows a one day old seedling penetrating a 3% agar surface, too strong for the radicle to depress and pierce. Growth of the upper side of the hypocotyl has forced the lower edge of the unformed 'clinging disc', and the radicle, into the agar, as shown by the large stress zone down the left side of the radicle. There is no stress zone ahead of the radicle tip, and little pressure has been exerted by the zone of elongation of the radicle. This indicates, by comparison with Plate 3.1, that there has not
Plate 3.1 Stress zones around the growing tip of an *E. sieberi* radicle.

Plate 3.2 Stress zones around the growing tip of a wheat radicle.

Plate 3.3 Stress zones around *E. sieberi* embryo penetrating the surface.
been significant growth of the radicle by meristematic activity.

These results show that the hypocotyl pushes the radicle into surfaces which resist penetration or lack pores large enough to admit it. Stress zones caused by the pressure of the hypocotyl against the agar were more extensive (Plate 3.3) than those around the growing tip of the radicle (Plate 3.1) indicating that the hypocotyl could exert more pressure against the surface than the radicle, and hence have more capacity for breaking or separating strong or cohesive aggregates at the soil surface. To test this further the pressure exerted by the two organs against masses placed directly above them as they grew, was compared.

Materials and method

The force exerted by a hypocotyl or radicle was measured by setting up a lever system with a mechanical advantage of 2 by having the organ press against the edge of a cover slip resting on a pivot an equal distance away from the centre of gravity of the mass holding the cover-slip down (Fig. 3.2).

The coverslip rested on a ring of cellotape 1.5 cm in diameter with the adhesive surface facing inwards. Eight 3 mm long E. sieberi seeds were fixed onto the inside of the strip 5 mm apart, each with the micropyle up so that the emerging hypocotyl and radicle would grow vertically upwards and press against the edge of the coverslip. To
Before germination

After germination

Fig. 3.2 Lever system used to test pressure exerted by growing organ

test the hypocotyls the strips were 3 mm wide so they contacted the slip as they emerged from the seed. To test the radicle the strips were 5 mm wide, leaving sufficient space above the micropyle for the hypocotyl to complete its extension and the radicle to begin active growth.

Four rings of seeds were placed on the plate of each of 6 tensiometers set at -5 kPa matric potential and each ring was filled to 2 mm with fine sand. The seeds were held firmly vertical by adhesion to the tape, by resting against the tensiometer plate and by pressure from the sand around them. Three tensiometers held narrow rings testing the hypocotyl against 6, 7 and 8 g masses, as preliminary trials had shown that the hypocotyl would lift a 1 cm cube of iron, sp.gr. 6.8 but not of lead, sp.gr. 11.2. Three tensiometers held wide rings testing the radicle against 2.5, 3.0 and 4.5 g as preliminary trials had shown that radicles lifted an aluminium cube, sp.gr. 2.8. After loading the systems by placing a weight in the centre of each coverslip the tensiometers were closed with plastic film raising the relative humidity to ensure good germination.
The position of the weights was noted twice daily as they slipped sideways when the growing organ levered up the coverslip. In any group of 8 seeds, one germinates 12 hours before the next and the second 12 hours before the next 5, which tend to germinate together. Therefore the first 2 seeds to germinate could be tested independently of the others. Eight seeds were used instead of 1 or 2 to ensure that the vigour of the seed population was tested in each replicate and also to allow for the proportion of misaligned or dislodged seeds that would not press directly against the coverslip.

Results and discussion

In no case did the radicle of the first seed to germinate raise the 2.5 g weight (Table 3.1). The radicles were deflected by the coverslip and grew out of the cellotape ring beneath it, without dislodging it. In two cases the radicle of the second seed to germinate raised the weight, and the third did so in the other two rings. No single radicle raised the 3 g weight, although two together did.

The force exerted by the radicle was therefore of the order of $\frac{2.5}{2} g = 1.25 \times 10^{-3} \text{ N}$.

Hypocotyls apply their growth force for a finite period only, stopping after approximately 1 mm growth. There was no occasion on which two hypocotyls exerted maximum growth force together. The first or second hypocotyl raised the 6.0 g weight but no hypocotyl raised the 7 g weight. The
Table 3.1 The force exerted by penetrating organs of *E. sieberi*

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of seedling lifting mass</th>
<th>Mass lifted up</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.5 g</td>
</tr>
<tr>
<td>Radicle</td>
<td>2nd</td>
<td>3rd  &amp;  4th</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>4th  &amp;  5th</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>4th  &amp;  5th</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>3rd  &amp;  4th</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0 g</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>1st</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>-</td>
</tr>
</tbody>
</table>

hypocotyls were not deflected. They remained stunted, and the 'clinging disc' adhered to the coverslip preventing the radicle from elongating.

The force exerted by the hypocotyl was in the order of $\frac{6.0}{2} = 3.0 \times 10^{-3}$ N.

Thus the hypocotyl exerts twice as much penetrating force against a resistant surface as the radicle, under these conditions.

3.2 STABILIZATION OF THE SEEDLING

Extension of the hypocotyl places the radicle tip in the soil if it penetrates the surface, or against the soil if it does not penetrate. As hypocotyl extension slows, active growth of the radicle in the zone of elongation starts and the coleorhiza expands into the 'clinging disc',
anchoring the seedling to the soil by long hairs within 24 hours of germination.

The cotyledons are raised when the disc has expanded, whether or not the radicle has grown into the soil fabric. They are raised by straightening of the hypocotyl. This lifts them off the surface and their weight applies a torque to the seedling axis until the hypocotyl is erect. The radicle is too short to counterbalance the weight of the cotyledons and it is not bound to the soil, since it has no root hairs so soon after its growth started. Nevertheless, the seedlings do not topple under the weight of the cotyledons.

This experiment was designed to test the mechanism by which the seedling is stabilized while the cotyledons are raised.

**Materials and method**

The radicles of newly germinated seedlings of *E. sieberi* were killed by touching them with a hot needle without damaging the unopened coleorhiza around them. Seedlings so treated could not stabilize themselves by extension of the radicle into the substrate. Treated seedlings were placed on wet 4% agar blocks some of which were covered with fine sand set into the surface. The hairs growing from the 'clinging disc' formed by the open coleorhiza could not penetrate the 4% agar to anchor the seedling. Nor could they adhere to the wet surface as the mucilage by which they cling is not adhesive until it is
dry. They could, however, adhere to the rough surfaces of the sand grains and anchor the seedlings.

The agar blocks were placed in closed containers providing a saturated atmosphere for continued post germinative development of the seedlings.

**Results and discussion**

All the seedlings raised the cotyledons without toppling, whether or not the hairs were clinging to the sand grains on the surface. Plate 3.4 shows the disc of a seedling lying on the surface of the agar.

The inference is that the 'clinging disc' acts as a stabilizing flange. A crane remains stable during lifting if its loaded centre of gravity lies within the area of the base. The eucalypt seedling remains stable because the disc reaches far enough under the seed to prevent the weight of the cotyledons toppling it, not because the hairs anchor it to the surface.

There is no stress on the hairs of the disc, and no stress on the radicle, since they are not pulled away from the substrate by the weight of the cotyledons as they are raised. Unanchored seedlings, and those anchored to loose aggregates or light pieces of organic matter, raise the cotyledons as effectively as anchored seedlings. Thus stabilization of the seedling by mechanics rather than adhesion of the 'clinging disc' to the soil obviates the need for the anchorage to be firm while the cotyledons are raised, protects the clinging hairs from damage, and ensures that the radicle remains in or on the soil surface.
instead of being pulled from it.

Although anchorage of the 'clinging disc' to the substrate is not necessary for stabilization of the seedling while the cotyledons are raised, it is necessary for establishment of the seedling in strong soils (Table 2.4). The role of the 'clinging disc' in stabilizing the seedling while the radicle becomes established in the soil after penetration and when it is deflected along the surface was examined in this experiment.

Materials and method

Microscope slides were covered with 2 or 3% agar in water as described in experiment 3.1, except that the seeds sown were not held down by a strip of agar above them. Seeds were also sown on the surface of blocks of 4% agar with sand grains embedded in the surface as in experiment 3.2. Germination proceeded on these three surfaces in high relative humidity conditions in closed containers at 20°C.

Results and discussion

(a) 2% agar

After penetration of the radicle, the peripheral hairs on the 'clinging discs' grew into the agar and the radicle continued to grow down into the agar as freely as if the seed had been held down, as in experiment 3.2.

(b) 3% agar

The clinging hairs of some seedlings grew into the
Plate 3.4 The 'clinging disc'.

Plate 3.5 The 'clinging disc' as a reaction force.

Plate 3.6 Penetration of radicle tip growing along the surface.
agar and held the seedling down. The hairs of other seedlings did not enter the agar; growth in length of the radicle continued but the tip could not grow down into the resistant agar and the increase in length of the radicle caused the disc to lift off the surface, as shown in Plate 3.5.

(c) 4% agar

Many seedlings penetrated 4% agar when the collar hairs were attached firmly to sand grains embedded in the agar surface. Others did not penetrate, and the radicles were deflected along the surface (Plate 3.6). The deflection was always away from the seed and in line with its long axis because the seed-side shoulder of the radicle tip contacted the agar first as it grew from the angled edge of the hypocotyl. The line taken by the deflected radicle ensured that the 'clinging disc' hairs in contact with the agar were not disturbed by its elongation as it did not pass beneath them.

These results show that the 'clinging disc' hairs stabilize the seedling in the early stages of establishment when the radicle requires anchorage against forces tending to lift the seedling out of the ground. The most likely source of such forces is strong soil, which restricts forward growth of the radicle.

3.3 PENETRATION BY DEFLECTED RADICLE

The tip of the radicle deflected along the surface of 4% agar shown in Plate 3.6 had turned down. The
symmetrical pattern of reflections around the tip shows that pressure was being exerted downwards, making a cone shaped depression in the agar surface. Weaker agar would have yielded to penetration. This abrupt turn down occurred in all seedlings soon after they were exposed to a reduction in relative humidity (R.H.) by removal of the lid of the container in which they germinated.

Control of this abrupt turn down into a smooth surface without pores to admit the tip was examined in the following experiments.

(i) **Effect of reduction in R.H.**

**Materials and method**

Four rows of 20 *E. sieberi* seeds were sown on wet filter paper folded over 12, 15 cm square styrofoam blocks floating on water in 3 l plastic containers. The paper dipped into the water and remained wet throughout the experiment. The containers were closed with plastic lids to raise the R.H. sufficiently to allow germination. When the seeds germinated, the seedlings were stabilized by the disc clinging to the filter paper and each radicle was deflected by the impenetrable styrofoam beneath it. The containers were in a glasshouse at 16-18°C and R.H. approximately 60%.

After 8 days, when most seeds had germinated, the lids were removed from 8 of the 12 containers and water was added to 4 of these until the styrofoam block floated level with the top of the container. Thus, 3 R.H. treatments
were imposed on the seedlings, R.H. >99% in closed containers, R.H. >60% in still air in the bottom of open containers and R.H. 60% in a moving air stream above the full containers. The radicles exposed to the R.H. treatments varied from 1 day old (2 mm long) to 5 days old (20 mm long).

Results and discussion

Regardless of age, or length, each radicle completed an abrupt turn downwards within 12 h of lowering the R.H. about it (Table 3.2). The few radicles turned down in >99% R.H. had penetrated the wet filter paper above a wide gap in the surface of the styrofoam block.

Table 3.2 Effect of lowering R.H. on orientation of deflected radicle tips

<table>
<thead>
<tr>
<th>R.H.</th>
<th>% of radicles turned downwards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>99%</td>
<td>3</td>
</tr>
<tr>
<td>60% still air</td>
<td>100</td>
</tr>
<tr>
<td>60% moving air</td>
<td>100</td>
</tr>
</tbody>
</table>

When the turn occurred in short radicles too young to be attached to the filter paper by root hairs, the whole radicle was levered off the surface but the seedling remained attached to the filter paper by the 'clinging disc' (Plate 3.7a). When the turn occurred in longer, older radicles, that part of the radicle without root hairs
Plate 3.7 The effect of lowered R.H. on the radicle tip
lifted off the filter paper and the continued extension of the zone of elongation caused a loop to form between the tip and that part of the radicle still attached to the paper (Plate 3.7b). Numerous slow growing lateral roots emerged from the portion of the radicle attached to the filter paper (Plate 3.7c). These results show that a saturation deficit in the atmosphere around a seedling of *E. sieberi* produces a positive geotropic growth curvature of the radicle similar to that produced when the radicle tip encounters a pore wide enough to enter.

Plate 3.7d shows deflected radicles growing along the surface and radicles which have reached the edge of the block and turned down. The latter are longer than the former although they are the same age, suggesting that radicles forced to grow horizontally may elongate more slowly than those growing vertically.

(ii) **Effect of reduction in matric water potential**

**Materials and method**

Fifteen seeds of *E. sieberi* were sown in lines on the plates of 4 Büchner funnels connected to hanging water columns (Haines Apparatus, H.A.) set at matric water potential of -5 kPa. The seeds germinated in a R.H. of >99% obtained by covering the funnels with plastic film. The 'clinging discs' of germinated seedlings anchored them to the plates of the funnels and the radicles were deflected. When all radicles were 1.5 cm long (or longer, depending on their age) and attached to the plates by root hairs, matric
potentials of -7.5, -10 and -15 kPa were applied to 3 plates and the other plate remained at -5 kPa.

The radicles were measured against a translucent scale without removing the plastic film controlling R.H. before the matric potential was altered, and again after 1 day.

Results and discussion

Root extension growth was maintained on the plate in which the matric potential continued at -5 kPa (Table 3.3). Growth ceased in the -15 kPa treatment and in the other treatments (-7.5 and -10 kPa) the roots continued to grow but did not extend along the plate because the tips had turned downwards. Thus, a drop in matric potential that

Table 3.3 The effect of reducing matric potential on radicle growth

<table>
<thead>
<tr>
<th>Radicle extension 1 day after dropping $\psi$ from -5 kPa</th>
<th>-5 kPa</th>
<th>-7.5 kPa</th>
<th>-10 kPa</th>
<th>-15 kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

does not restrict growth induces positive geotropic growth curvature in deflected radicles of *E. sieberi*.

Both increase in atmospheric saturation deficit and decrease in matric water potential, experiments 3.3 (i) and (ii), caused deflected radicles of *E. sieberi* seedlings to make an abrupt positively geotropic curvature, although the
surface lacked pores large enough for the radicle to enter.

An experiment was designed to determine whether the water potential of the seedling, as controlled by its water relations with the surroundings, affected the positive geotropic curvature of the radicle.

(iii) Effect of seedling water potential on orientation of radicle

Materials and method

Seedlings were grown on filter paper folded over styrofoam blocks as in experiment (i). Opaque lids were used to close the containers in which the blocks floated, to reduce light intensity inside them and induce the hypocotyls of the seedlings to grow long enough for insertion through the diaphragm of a pressure bomb used to measure seedling water potential. After 10 days two levels of stimulus to curvature were applied, namely, increase in saturation deficit of the atmosphere (applied by opening the container and filling it with water to raise the block above the lip exposing the seedlings to the atmosphere in the laboratory which reduced the R.H. from >99% to 70%) and increase in saturation deficit coupled with decrease in matric water potential (applied by lifting the block out of the water and allowing the filter paper to dry in the 70% R.H. of the laboratory).

The two levels of stress imposed on the seedlings to induce curvature were applied for 0 h, $\frac{1}{2}$ h, 1 h to 7 hours by half hourly increments, giving a 2x15 factorial
design. Thirty seedlings, all growing in two rows on one styrofoam block, were exposed to each stimulus at each time. After exposure and sampling of 3 seedlings for water potential, the blocks were refloated on water in plastic containers for continued growth. The containers were closed with transparent plastic film so that the radicles could be observed making the geotropic growth response.

Results and discussion

Seedlings exposed to 70% R.H. after growing at >99% responded with a drop in water potential of -0.3 MPa within half an hour, and remained stable at -0.6 MPa during continued exposure (Fig. 3.3). They were not visibly wilted. Seedlings exposed to an increase in saturation deficit and decrease in matric water potential dropped -0.6 MPa within half an hour, then they maintained a water potential of approximately -0.9 MPa for three hours after which they wilted severely, and their water potential dropped steadily.

All the seedlings recovered rapidly when replaced in wet conditions and the root tips were growing downwards within 24 h. The tips of seedlings which had not been exposed to drying conditions did not turn downwards except where they grew over a wide gap in the styrofoam block and made the normal geotropic response.
Fig. 3.3 Water potential of seedlings exposed to desiccation
These results indicate that the turn is initiated as the seedling water potential starts to fall. If growth continues, the tip may penetrate a surface. If not, the tip will be in the correct orientation for penetration when rain raises the seedling water potential.

3.4 GENERAL DISCUSSION

The purpose of this investigation into the mechanics of soil penetration by *E. sieberi* was to demonstrate how seedlings become established in seed beds too strongly compacted to provide cracks or holes large enough to permit ready entry of the radicle. The results have shown that penetration is possible because the seedling has structural adaptations appropriate for small light seeds germinating on the surface. That is, the relatively broad, rigid hypocotyl penetrates the surface (3.1) rather than the less forceful radicle; the seedling is stabilized by the disc while the radicle enters the soil (3.2) and anchored down by the clinging hairs when the radicle meets resistance from the soil. These morphological features compensate for the lightness (<2.0 mg) of the seed by holding it down and thus obviating the need for burial, and for the smallness of the embryo by using the strongest part of the axis, the hypocotyl, to break into the soil surface.

Penetration of the surface must precede establishment of the seedling. However, establishment can occur only if the hairs of the radicle bind to the soil aggregates to make contact with the water and mineral resources of the soil and
to anchor the radicle so that it may exert pressure against resistant soil, for the 'clinging disc' is an evanescent structure.

Binding of the radicle after penetration may be achieved in four ways:

(i) The radicle, or the hypocotyl in the case of the eucalypt, may emerge from the seed directly into a crack or hole made by a root or an animal. Successful establishment then depends on the diameter of the hole in relation to that of the radicle. If the hole is too wide, hairs on the side of the radicle lying close to the wall will bind (Dexter, 1978) but the other side will be in a void, unable to absorb nutrients. The radicle will be prone to desiccation as the air in the crack dries. Radicles of *E. sieberi* penetrating large pores are likely to encounter this difficulty as the seedbed often dries soon after the seed germinates.

(ii) The radicle may emerge from the seed between two soil aggregates and push them apart, making a crack of the same diameter as the radicle and allowing all the root hairs to bind. The tilth of agricultural seedbeds is adjusted to ensure that radicles of crop plants bind in this way. The aggregates must be less than 1 mm diameter if they are to be moved by roots less than 0.5 mm wide, even in friable beds (Whiteley and Dexter, 1984(a)). *E. sieberi* seedlings germinating on naturally cohesive or compacted beds may not
bind in this way since the radicle is only 0.2 mm wide, but the 0.7 mm wide hypocotyl may displace aggregates and penetrate.

(iii) The radicle may emerge from the seed, or hypocotyl, against an aggregate, split it, and make a crack of the same diameter, allowing the root hairs to bind all around the circumference when they develop. If the seed is held rigidly by its own weight or by the soil around it, the radicle is able to exert a growth pressure of 1000 to 1200 kPa (Taylor and Ratliff, 1969) against the aggregate. The eucalypt radicle may be able to bring this pressure to bear if it is held down firmly by the 'clinging disc' until the root hairs of the radicle bind.

(iv) The radicle may emerge, encounter an aggregate that cannot be split or moved, and be deflected. If the aggregate has a void beside it, some hairs will bind to the aggregate but others will be unable to bind. If the aggregate is embedded in the matrix of the soil, the radicle may be able to force a path between the aggregate and the soil by cylindrical expansion in the zone of elongation (Plate 3.1) if that expansion is strong enough to displace the soil particles laterally. In some soils the lateral displacement of the particles causes a reduction in soil strength ahead of the advancing root tip, facilitating further extension (Whiteley et al., 1981). The radicles of *E. sieberi* seedlings in compacted sedimentary soils
C and D (Plate 2.3) grew through the soil by deforming the clay matrix in this way.

A radicle approaching the soil surface for penetration is free in the air and one growing in a void in the soil is free in an air gap. A free radicle becomes a rod which has a tendency to buckle or bend if it encounters resistance on the opposite side of the gap, because it is not confined laterally or bound by root hairs. It buckles if it meets the resistant surface at right angles, bends if it approaches at an angle (Dexter, 1978). The radicle may buckle before it is able to exert its full growth pressure against the aggregate. The force at which it buckles, the buckling stress, depends on the elasticity of the radicle and its diameter.

The rigidity of the tissue is determined by turgor and cell wall elasticity, which vary along the radicle. When the variation is accounted for, a generalized elastic modulus (M) can be determined for any root (Whiteley and Dexter, 1981). It will indicate the buckling stress that limits the ability of the root to break an aggregate encountered at the end of an air gap. Two narrow roots measured by Whiteley and Dexter, lucerne, diameter 0.28 mm, and medic, diameter 0.27 mm had an M of 2.27 and 1.07 MPa respectively, while the widest root, soybean, diameter 0.95 mm, had an M of 20.8 MPa. Even at full turgor the narrow roots had little resistance to bending. The narrow diameter, 0.2 mm, radicle of *E. sieberi* may have a low resistance to bending, compared with the broader hypocotyl as suggested by the results of experiment 3.1.
The pressure a root exerts before buckling or bending depends on the length of unconfined root between the tip and the anchorage as well as on the generalized elastic modulus. Whiteley, Hewitt and Dexter (1982) allowed roots to grow through air gaps from 3 to 15 mm long before impinging directly on a non-slip surface. For air gaps longer than 3 mm they found a linear relationship between the logarithm of buckling stress ($\sigma_b$) and the logarithm of the length of gap ($l$). This relationship can be expressed:

$$\ln \sigma_b = a + b \ln l,$$

where $a$ and $b$ are the elasticity parameters determined experimentally for each root type, and $b$ is negative. The smallest diameter roots tested, rape, $d = 0.4$ mm, had a buckling stress of 140 kPa when the gap was 3 mm and only 20 kPa for a 10 mm gap. The large diameter pea root, $d = 0.92$ mm, had a buckling stress of 300 kPa at 3 mm and 50 kPa at 15 mm. For E. sieberi seed germinating on the surface, the advantage of placing the radicle in or against the surface, without any air gap, is clear. The narrow radicle, with its low resistance to bending is able to exert its full growth pressure against the aggregate it must enter.

When a root approaches an aggregate at or near 90° to its surface, the probability of entering it remains above 70% (Dexter, unpub.) for aggregates of strengths up to 600 kPa, beyond which the probability falls rapidly to zero. Roots approaching at less than 45° have a high probability of entering low strength aggregates but at soil strengths between 100 to 300 kPa there is an almost linear
relationship between strength and probability of entering the aggregate, the probability being very low at 300 kPa. Thus there is an advantage in placing the radicle at right angles to the aggregate by positive geotropic growth of the hypocotyl, as in *E. sieberi*. The radicle so placed has a high probability of entering the aggregate and binding effectively.

The alternative method of establishment used by *E. sieberi* is penetration by the deflected radicle when it makes a geotropic growth curve in response to a negative change in the seedlings water relationships.

Deflected radicles typically penetrate the surface when the tip passes over a soil pore wide enough to admit it. The tip 'sees' the pore when it is bent down by gravity as it extends over the edge. The bend initiates the gravity response (Jackson and Barlow, 1981), causing cells on the top side of the zone of elongation to elongate relative to those on the lower side, accentuating the initial bend into the pore. If the forward growth pressure of the tip is greater than its buckling strength, it will not 'see' the pore because it will not bend as it extends over the edge, and the gravity response will not occur. Therefore, by inference, the size of the pores a root enters is related to its buckling strength. Narrow roots, with low buckling strength such as those of *E. sieberi*, are more likely than broad roots to penetrate consolidated surfaces with small pores.

Apparently, deflected radicles of *E. sieberi* continue to grow along surfaces without pores large enough to admit
them, while forward growth pressure is maintained by unrestricted water supply. When there is a negative shift in the water relations of the seedling the radicle loses turgor, becomes less rigid and buckles, initiating the gravitropic growth response that may effect penetration. The response is so sensitive to incipient water stress that most radicles deflected by an impenetrable seedbed surface will be stimulated to turn soon after they begin to elongate as the rain which caused germination clears. Few may penetrate on consolidated or compacted seedbeds, but the response to failure to penetrate is precocious and vigorous production of lateral roots, any of which may penetrate.

This study has shown how well the young seedling is adapted, morphologically, for establishment after germinating on the soil surface. The 'clinging disc' compensates for the lightness of the seed, the hypocotyl compensates for the weakness of the radicle in penetration, and the placement of the radicle compensates for its low buckling strength. The advantage of the narrow radicle, its ability to enter small diameter pores, is not compromised by inability to enter strong aggregates. Direct entry to the soil by splitting an aggregate or forcing aggregates apart facilitates seedling establishment because all root hairs bind to the walls of the channel made by elongation and expansion of the radicle, giving it secure anchorage, maximum contact with soil resources and protection from desiccation.
CHAPTER 4
THE EFFECT OF GRAVITY ON GERMINATION AND ESTABLISHMENT

INTRODUCTION

Two aspects of the response of the newly germinated seedling to gravity are examined in this Chapter: the effect of orientation of the radicle on growth and the control of the positive geotropic curvature of the hypocotyl that places the radicle in the soil.

Radicles growing vertically down the edge of styrofoam blocks (Plate 3.7d) grew faster than those deflected horizontally along the surface of the block, suggesting that seedlings with radicles constrained by the strength of the soil to grow at angles other than vertically downwards, may be disadvantaged in comparison with those whose radicles grow vertically. The effect on seedling growth of displacement of the radicle from the vertical is examined in section 4.1.

Positive geotropic growth curvature of the collet region of the hypocotyl places the radicle directly downwards into the soil allowing it to exert maximum growth pressure against resistance, and the completed curvature aligns the 'clinging disc' with the soil surface, allowing hairs all round the disc to adhere to the soil and stabilize the young seedling. These consequences of the positive geotropic curve are of such significance to establishment that the place and time of its initiation and the effect of changing the orientation of the embryo during
germination were examined further in parts 4.2 and 4.3.

4.1 THE EFFECT OF DEFLECTION OF THE RADICLE ON SEEDLING GROWTH

The experiments described below examine the significance of growing vertically downwards to the successful establishment of *E. sieberi* seedlings, and compare the response of *E. sieberi* seedlings with carrot seedlings (*Daucus Carota* L).

(i) *E. sieberi*

**Materials and method**

Forty eight microscope slides were covered with a 2 mm thick film of 4% agar in water to provide an impervious substrate for the seedlings. The slides were placed on scaffolds at 90°, 85°, 70°, 55°, 40° and 25° from the horizontal, eight slides at each angle. These angles forced the radicles to experience a gravity component of 1 x gravity 0.99, 0.93, 0.82, 0.65 and 0.42 x gravity (gr.) respectively, since the component experienced is determined by the sine of the angle the root makes with the horizontal.

Variability between seeds was reduced by using long, heavy seeds which germinated on day 4 after sowing. Five germinated seeds were placed in a line 1 cm from the end of each slide and the slides, on scaffolds, their lower ends immersed in water, were placed in plastic containers. The containers were covered with translucent plastic film which admitted light from a fluorescent tube on a 12 h photoperiod and maintained a R.H. close to saturation. The

* This physiological comparison is between two species with small seeds, epigeal germination and narrow radicles.
experiment was carried out at a constant temperature of 18°C.

The radicles were measured daily against graph paper inserted behind the slides on the scaffolds. When the longest roots reached the bottom of the slide the experiment was terminated. Slides were wrapped in aluminium foil and placed in a deep freezer. This procedure destroyed the agar gel structure making it possible to lift the radicle away from the agar without tearing the hairs and the cortex away. The seedlings were washed, oven dried at 85°C overnight, and weighed.

Results and discussion

Radicles experiencing 1 x gravity grew 9 mm per day and growth rate decreased with decreasing gravity (Table 4.1) those experiencing 0.4 gr. growing less than 5 mm.

Table 4.1 Effect of deflection from vertical on growth of E. sieberi radicles

<table>
<thead>
<tr>
<th>Growth rate of radicle in mm/day</th>
<th>Component of gravity acting on radicle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
</tr>
</tbody>
</table>

Tracings of representative slides from each treatment, (Fig. 4.1) show that radicles experiencing 1 x gr. grew straight, but deflected radicles took an increasingly wavy path down the slide and radicles at angles closer to the horizontal produced lateral roots at the bends. The shorter radicles
Fig. 4.1 Effect of gravity on morphology of *E. sieberi* seedlings

appeared thicker than those experiencing a greater component of gravity but examination with a binocular microscope showed them to be the same width, 0.2 mm. The thickened appearance was due to profuse, long, root hairs starting close behind the tip, and to the shape of the tip, pointed in radicles experiencing close to 1 x gr. and rounded in severely deflected radicles.

The analysis of variance of length of roots after 5 days' growth was carried out using the S.P.S.S. Package, Licence No. 9275. The 0.42 x gr. treatment was excluded because many radicles grew in short, tight spirals in the agar and too few grew down the surface for significance. Table 4.2 shows that displacement of the radicle from the vertical has a significant effect on radicle length. Table 4.3 shows that small reductions in the component of
Table 4.2 Analysis of variance for angle of deflection

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>S.S</th>
<th>M.S.</th>
<th>F.Ratio</th>
<th>F.Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angles</td>
<td>4</td>
<td>18.23</td>
<td>4.57</td>
<td>27.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Within angles</td>
<td>45</td>
<td>7.57</td>
<td>.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>25.86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3 Effect of deflection on radicle length

<table>
<thead>
<tr>
<th>Angle</th>
<th>Gravity component</th>
<th>Mean length of radicle in cm</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°</td>
<td>0.65</td>
<td>2.80</td>
<td>a</td>
</tr>
<tr>
<td>55°</td>
<td>0.82</td>
<td>3.28</td>
<td>a</td>
</tr>
<tr>
<td>70°</td>
<td>0.93</td>
<td>3.61</td>
<td>b</td>
</tr>
<tr>
<td>85°</td>
<td>0.99</td>
<td>4.21</td>
<td>c</td>
</tr>
<tr>
<td>90°</td>
<td>1.00</td>
<td>4.46</td>
<td>d</td>
</tr>
</tbody>
</table>

*Significantly different means denoted by different letter (0.05 level).

Gravity experienced are associated with significantly shorter radicles when the slope is between 90° and 55° but that the length is not affected as the slope decreases from 55° to 40°.

The total amount of growth, on a dry weight basis, made by the seedlings was the same in all treatments (Table 4.4). However, the allocation of growth to roots and shoots differed between treatments and the root/shoot ratios show that seedlings exposed to increasing gravity had grown more shoot relative to root. Seedlings experiencing 0.65 xgr. allocated photosynthate to production of lateral roots and root hairs at the expense of shoot growth.
Table 4.4 The effect of gravity on growth of seedlings with deflected radicles - *E. sieberi*

<table>
<thead>
<tr>
<th>Gravity component</th>
<th>Dry weight mg*</th>
<th>Total</th>
<th>Shoot</th>
<th>Root</th>
<th>R/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.80</td>
<td>1.02a</td>
<td>.80a</td>
<td>.77</td>
<td></td>
</tr>
<tr>
<td>0.99</td>
<td>1.48</td>
<td>.85a</td>
<td>.63a</td>
<td>.74</td>
<td></td>
</tr>
<tr>
<td>0.93</td>
<td>1.58</td>
<td>.85a</td>
<td>.73a</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>0.82</td>
<td>1.99</td>
<td>.96a</td>
<td>1.00b</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>0.65</td>
<td>2.04</td>
<td>.80b</td>
<td>1.24b</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>

*Values with subscript 'a' are significantly different from those with 'b' by t-Test, at 5% level.

Thus any deflection of the radicle from the vertical reduced its rate of extension, and deflections reducing the gravity component to 0.8 and below caused the *E. sieberi* seedlings to make more root growth relative to shoot growth.

(ii) D. Carota

**Materials and method**

The carrot, like the eucalypt, regenerates from small, light, seeds germinating on the surface and producing a narrow radicle. Therefore it was chosen for comparison with the eucalypt.

Carrot seeds were soaked in water for one day then transferred to agar-covered slides as described for *E. sieberi*. A pilot experiment had shown that carrot radicles were not sensitive to change in angles between 90° and 80° and that they would grow along a 10° slope. Therefore the angles used for this experiment were 85°, 50°, 30° and 10°. Thirty seeds were subjected to each angle.

Growth records were kept by placing an indian ink mark beside the tip of each radicle each day. The root
hairs of these seedlings were not embedded in the agar and after the radicles were lifted off the agar the daily growth increments were measured directly from the ink dots.

Results and discussion

The reduction in radicle extension caused by decrease in the gravity component to which it was exposed is shown in Plate 4.1. Carrot radicles subjected to 0.99 x gr. grew 9.5 mm per day (Table 4.5) while those subject to 0.17 gr. grew 4.7 mm per day.

Table 4.5 Effect of deflection from vertical on growth of D. Carota radicles

<table>
<thead>
<tr>
<th>Component of gravity acting on radicle</th>
<th>.99</th>
<th>.76</th>
<th>.50</th>
<th>.17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate of radicle in mm/day</td>
<td>9.5</td>
<td>7.8</td>
<td>5.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Carrot radicles did not produce lateral roots and exhibited only a small degree of wavy growth down the slide. However, radicles subjected to less gravity produced numerous long root hairs and the shape of the tip was rounded as in the eucalypt.

The analysis of variance for length of roots in 7 days (Table 4.6) and the comparison of means of treatments (Table 4.7) show that each increase in displacement caused a significant decrease in radicle extension.

Seedlings with radicles exposed to 1x gr. grew best (Table 4.8) in contrast to the eucalypt in which total growth was unaffected by deflection of the radicle. The
Plate 4.1 Effect of deflection from the vertical on growth of carrot radicles
Table 4.6 Analysis of variance for angle of deflection

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.Ratio</th>
<th>F.Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angles</td>
<td>3</td>
<td>206.26</td>
<td>68.75</td>
<td>768.95</td>
<td>0.0001</td>
</tr>
<tr>
<td>Within angles</td>
<td>116</td>
<td>10.37</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>216.63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.7 Effect of angle of deflection on radicle length

<table>
<thead>
<tr>
<th>Angle</th>
<th>Gravity component</th>
<th>Mean length of radicle cm</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°</td>
<td>0.17</td>
<td>3.32</td>
<td>a</td>
</tr>
<tr>
<td>30°</td>
<td>0.50</td>
<td>4.05</td>
<td>b</td>
</tr>
<tr>
<td>50°</td>
<td>0.76</td>
<td>5.52</td>
<td>c</td>
</tr>
<tr>
<td>85°</td>
<td>0.99</td>
<td>6.71</td>
<td>d</td>
</tr>
</tbody>
</table>

*Significantly different means denoted by different letters (0.05 level).

Table 4.8 The effect of gravity on growth of seedlings with deflected radicles - D. Carota

<table>
<thead>
<tr>
<th>Gravity component</th>
<th>Dry weight 20 plants*</th>
<th>R/S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total mg</td>
<td>Shoot mg</td>
</tr>
<tr>
<td>0.99</td>
<td>17.42a</td>
<td>11.24</td>
</tr>
<tr>
<td>0.76</td>
<td>15.12b</td>
<td>9.95</td>
</tr>
<tr>
<td>0.50</td>
<td>15.40b</td>
<td>10.75</td>
</tr>
<tr>
<td>0.17</td>
<td>14.61c</td>
<td>10.58</td>
</tr>
</tbody>
</table>

*Values with subscript 'a' are significantly different from those with 'b' and 'b' from 'c' at 5% level, by t-Test.
root/shoot ratio in carrot decreased with decrease in the gravity component rather than increasing as it did in *E. sieberi*.

The effect of the gravity component on extension growth of the radicle of *E. sieberi* and *D. Carota* is similar (Fig. 4.2). Radicles of both species extended faster when growing vertically.

In overall growth, the response to gravity of an *E. sieberi* seedling differs markedly from that of a carrot seedling. With carrot, deflection from the vertical caused shoot growth to be maintained while root growth decreased. This may represent the most effective partitioning of available energy for a species which germinates in cool temperate conditions of relatively low light intensity and generally favourable water availability. With the eucalypt deflection from the vertical caused root growth to increase while shoot growth decreased; the R/S ratio increased rather than decreasing as in carrot. This may represent the most effective partitioning of available energy for a species which germinates under conditions of high light intensity and uncertain water availability.

Eucalypt seedlings whose radicles are able to grow vertically exhibit good shoot growth while extending their radicles deep into the soil. Seedlings with radicles deflected from the vertical use a greater proportion of total assimilate for production of a branched root system in the surface soil. The role of the hypocotyl in forcing the radicle to enter the soil vertically therefore has significance both for the mechanics of soil penetration and
Effect of gravity on root growth
growth rate mm / day

Fig. 4.2 Effect of gravity on radicle extension
for the continued growth and establishment of the seedling.

4.2 RESPONSE OF THE HYPOCOTYL TO GRAVITY

Placement of the radicle vertically into the soil by a positive geotropic curvature of the collet end of the hypocotyl was shown to facilitate soil penetration and subsequent establishment of the seedling in Chapter 3 and the first part of this Chapter. It was of interest to determine when the gravity stimulus is perceived, as seed germinating on the surface may be rolled over during germination, negating the value of a response during extension of the hypocotyl before it breaks the seed coat. Another point of interest was whether the positive response to gravity is confined to the collet end of the hypocotyl, as the apical end elongates in a negatively geotropic, or a positively phototropic, direction if stimulated to do so.

Materials and method

_E. gummifera_ (Sal ex Gaertner) Hochr seed was used to examine the response of the hypocotyl to change in orientation as it elongated to break the seed coat, and to determine whether light influenced the response. _E. gummifera_ seed is sufficiently large (4 to 6 x 4 mm) to be manipulated easily and the micropylar end, into which the hypocotyl fits, can be distinguished easily from the wing end. The wrinkled hilum is unmistakable so the seed can be orientated accurately, hilum up or down.
Three groups of 10 viable seeds of *E. gummifera* were sown on the plates of 8 tensiometers (Haines Apparatus, H.A.) at -0.5 kPa matric potential and covered to maintain a saturated atmosphere, in diffuse natural light or in the dark under an aluminium foil cover. The temperature fluctuated diurnally between 13° and 18°C.

The seeds in one group in each H.A. were horizontal with the hilum up, those in one group were horizontal with the hilum down, and those in the last group were vertical, this orientation being achieved by standing them in a cylinder of fine sand resting on the tensiometer plate. The seeds in some H.A.s were maintained in the same orientation throughout the experiment; in other H.A.s the orientation of seeds was changed before germination.

The initial orientation (0 hours) of each group in each H.A. is shown in Table 4.9 where the symbol, —L—, indicates the seed is horizontal with the hilum up, —l—, with the hilum down and |, vertical. This table also shows the re-orientation regime in each H.A. and the light to which each group of seeds was exposed. For example, in H.A. 1 the seeds were retained in the original orientation throughout germination, in the dark, while in H.A. 7 the seeds were sown vertically, but one group was laid horizontally after 12 h, one after 36 h and one after 76 h when the first seed was due to germinate.

When half the seeds in each group had germinated the remaining seeds, which were all close to germination, were re-orientated by changing —L— to —l— and vice versa,
and to test the effect of the change on hypocotyls already in the process of rapid expansion.

**Results and discussion**

Every seed, irrespective of the original orientation, which was in a horizontal position at 76 h after sowing germinated by breaking the seed coat underneath the micropylar end (column 2 of results, Table 4.9). The hypocotyl curved towards gravity while still within the seed and emerged through a pale area between hilum and micropyle if the seed lay hilum down, ; through the ridge between curved surfaces of the seed if it lay hilum up, (Fig. 4.3). Seeds in the vertical position germinated through the micropylar end regardless of whether this was orientated upwards or downwards, in light or dark conditions, showing that light does not influence the direction of emergence of the hypocotyl.

The right hand column in Table 4.9 shows that all the seeds rotated through 180° or laid horizontally after being vertical while the hypocotyl was elongating rapidly prior to breaking the seed coat, germinated through the micropyle. These were the seeds not yet germinated at 148 h, but close to germination. An explanation for emergence through the micropyle rather than the side of the seed is given in Fig. 4.4. As the hypocotyl had started to bend before re-orientation it had to be straightened before bending in the opposite direction. That straightening was accompanied by elongation, and the hypocotyl emerged through the micropyle.
Table 4.9: Response of germinating hypocotyl to gravity

<table>
<thead>
<tr>
<th>H.A. No.</th>
<th>Dark (h)</th>
<th>Position of hilum at time given</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dark</td>
<td>0 hrs</td>
<td>12 hrs</td>
<td>36 hrs</td>
</tr>
<tr>
<td>2</td>
<td>Dark 36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Light 36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Although the direction of emergence of the hypocotyl was unaffected by light; seeds germinated more rapidly in the dark H.A.s, 1 and 3. Almost all had germinated within 148 h from sowing, while approximately half had germinated in the light H.A.s, 2, 4, 5 and 6. Twenty percent germinated within 148 hours in the light H.A.'s 7 and 8 in which
the initial orientation was vertical. A possible explanation for the delay was that the germinative processes of the seeds were disrupted temporarily when they were removed from the sand, brushed clean, and repositioned on the H.A. plate, for there was no indication from the other H.A.s that vertically orientated seeds are slow to germinate.

These results show that the collet of the hypocotyl responds precisely to gravity while elongating during germination and compensates rapidly for any change in the orientation of the seed. The curvature ensures that the hypocotyl emerges from the seed as close as possible to the soil surface, minimizing the time the radicle is exposed to desiccation between germination and soil penetration. In _E. gummifera_ the hypocotyl curves within the seed and the radicle is placed beneath it for penetration. In _E. sieberi_, however, there is insufficient room within the rigid seed coat for the hypocotyl to turn before germination and the coat splits when sufficient pressure is applied by the growing hypocotyl. If the seed lies in any orientation other than vertical the seed coat splits above the micropyle where the turning hypocotyl exerts pressure. Thus the hypocotyl of seeds lying almost vertically must make more growth before penetration than that of seeds lying horizontally (Fig. 4.5). Seeds lying vertically micropyle down produce a short hypocotyl through the micropyle as do those lying vertically micropyle up, and in the latter case soil penetration depends on a positive geotropic response of the radicle when it extends from the hypocotyl.
To examine whether gravity perception is confined to the collet region, germinating seeds of *E. gummifera* were clipped to shorten the hypocotyl within, and their subsequent germination was observed.

**Materials and method**

Three hundred seeds of *E. gummifera* were placed horizontally on wet filter paper and turned through 180° every twelve hours so that the hilum was up for 12 h and down for 12 h. This procedure induced the hypocotyls to grow straight towards the micropyle of the seed instead of turning towards gravity within it, as explained in the previous experiment.
The capacity of 3 different parts of the hypocotyl to respond to gravity, at 3 different times in the germination period and 3 different orientations in both light and dark were tested using a 3x3x3x2 factorial design.

The three different parts of the hypocotyl were tested by clipping the seed at 3 distances from the micropylar end. Seed clipped close to the end lost the radicle tip and some of the coleorhiza. Seed clipped 1 mm from the end lost all the radicle but retained the collet, and seed clipped 2 mm from the end lost all but the apical end of the hypocotyl. The effects of clipping are indicated diagrammatically in Fig. 4.6.

![Intact seed and clipping treatments](image)

- Intact seed
- Coleorhiza removed
- Radicle tip removed
- Basal end of hypocotyl removed

Fig. 4.6 'Clipping' treatments for testing response of hypocotyl to gravity

Ninety seeds were clipped 24 hours after sowing, 90, 72 h after sowing as the seed was due to germinate, and 90 were clipped as the hypocotyl broke the seed coat.

Thus 30 seeds were clipped to each length at each time. Five of these seeds were placed horizontally
with the hilum up on the plate of an H.A. set at -0.5 kPa matric potential and covered to maintain a saturated atmosphere. Another 5 were placed horizontally hilum down and 5 were placed vertically as in the previous experiment. The other 15 seeds were placed in the same 3 orientations in another H.A. and the whole was wrapped in aluminium foil to exclude light.

Results and discussion

Every horizontally orientated (↔ or −) seed responded to gravity by positive geotropic curvature of the hypocotyl except for those which became infected with mould during germination, reducing the numbers responding to gravity below 100% in some treatments (Table 4.10). No vertically orientated hypocotyls (↑) turned down.

Table 4.10 The effect of foreshortening the hypocotyl during germinative period of E. gummifera

<table>
<thead>
<tr>
<th>Position of cut in hypocotyl</th>
<th>Germinating in light</th>
<th>Germinating in dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut at 24 h</td>
<td>Cut at 72 h</td>
<td>Cut germinating</td>
</tr>
<tr>
<td>Cut at 24 h</td>
<td>Cut at 72 h</td>
<td>Cut germinating</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Germinating in light</th>
<th>Germinating in dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>95 95 0 85 80 0 100 100 0 90 100 0 90 100 0 95 100 0</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td>100 80 0 100 100 0 100 100 0 90 85 0 100 80 0 95 95 0</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td>100 100 0 100 90 0 100 90 0 100 95 0 100 100 0</td>
<td></td>
</tr>
</tbody>
</table>

This result shows that gravity perception is a property of the hypocotyl, all parts of which respond in the same manner. The apical end of the eucalypt hypocotyl, which turns upwards during establishment of a developing
seedling is not intrinsically negatively geotropic, as it turns down if the collet end is removed. The negative geotropism exhibited by this part of the hypocotyl was acquired soon after germination as the cotyledons of seeds planted vertically with the micropyle up and the cotyledons down emerged upright from the sand within days of germination.

Seeds from which the radicle tip only was removed produced normal seedlings after the remaining part of the radicle elongated for 2 mm, and lateral roots grew from it. Seedlings developed from seeds in which the coleorhiza was completely removed were unable to form a 'clinging disc' for stability while the cotyledons were raised, as discussed in experiment 3.2. However, they became established when the apical end of the hypocotyl lengthened with a negative geotropic curvature, raising the cotyledons, and adventitious roots emerged from the collet end of the severed hypocotyl. Thus *E. sieberi* is capable of establishment from an embryo lacking the radicle and much of the collet end of the hypocotyl.

These two experiments show that the hypocotyl compensates rapidly for changes in orientation of the seed during germination, thus minimizing the time between germination and penetration, and facilitates seedling establishment after loss of the radicle by completing the positive geotropic curvature and entering the soil, where it may produce adventitious roots.
4.3 THE EFFECT OF SEED ORIENTATION ON GROWTH RATE OF SEEDLINGS

It was noted (Table 4.9) that all vertical seeds in the dark germinated within 148 h of sowing while some horizontal seeds were yet to germinate. Seventy percent of vertical seeds germinated in the light while less than 50% of horizontal seeds germinated within 148 h. These observations suggested that hypocotyls growing under 1 g gravity may extend faster than those experiencing a component of gravity, as radicles do, 4.1.

Wheat grains were used to gain some indication of the influence of seed orientation on rate of embryo growth. The wheat embryo is visible on the surface of the grain and all grains in a sample germinate within hours of each other, in contrast to the eucalypt in which the embryo is hidden and the time to germination varies from 4 to 8 days.

In a preliminary experiment wheat grains were placed horizontally, vertically scutellum end up, and vertically scutellum end down, for germination. The last orientation is the 'correct' one since the first seminal root points down and the plumule up. 'Correctly' orientated seeds germinated earlier than others because both coleorhiza and coleoptile elongated faster at that orientation. Subsequent seedling growth from 'correctly' orientated seeds was also faster than that from seeds placed upside down, the latter seedlings attaining only 65% of the total size of the former after 4 days growth. Grains placed horizontally produced seedlings intermediate in size,
apparently due to the grain slipping from the horizontal towards the vertical orientation before and during germination.

Wheat grains subjected to 1 x gravity germinated more quickly than those subjected to a component of gravity if 'correctly' orientated, not if inverted. This effect of seed orientation is examined further below.

Materials and method

Lines of 20 wheat grains were laid 1 cm below the edge of 16 strips of wet blotting paper, 20 cm x 5 cm. The grains on 8 strips were placed with the scutellum down in the 'correct' orientation and those on the other 8 strips were placed with the scutellum up. Each line of grains was covered by a 1 cm x 20 cm strip of wet blotting paper and the two strips were pressed together to hold the grains in place in a paper seedbed (Fig. 4.7). Each strip was rolled into a coil inside thick brown paper and placed in a 100 ml beaker holding 20 ml tap water to keep the blotting paper wet. The beakers were covered with black lids to exclude light and maintain a high atmospheric humidity.

Eight hours after sowing two rolls of grain in each orientation were opened and the seeds were turned through 180° to reverse their orientation. This procedure was repeated for additional pairs of rolls, 24 h and 32 h after
sowing, when the first seminal root was emerging from the coleorhiza of 'correctly' orientated grains in the unturned controls. Two rolls contained 'incorrectly' orientated unturned controls.

The shoots and the first seminal roots of all grains were measured 72 h after sowing. The embryos were removed from the empty husks, divided into root and shoot, oven dried, and then weighed.

**Results and discussion**

The growth of roots and shoots was significantly decreased by incorrect orientation of the grain in the first 24 h of germination (Figs. 4.8 and 4.9).

Extension growth and dry weight gain of shoots and roots sown scutellum up, the 'incorrect' orientation, was doubled if the grain was turned to the 'correct' orientation 8 h after sowing and significantly increased by turning 24 and 32 h after sowing as it was germinating. Likewise, the extension growth and dry weight of shoots and roots sown scutellum down was halved when the grain was turned to the incorrect orientation after 8 hours, and reduced almost as much when turned at 24 or 32 hours.

Grain sown in the 'correct' orientation germinated more rapidly than that sown scutellum up because the embryo elongated more quickly. Earlier germination gives seedlings from correctly orientated seeds an advantage in establishment, as they are able to utilize the conditions allowing the seed to germinate to grow larger and extend further into the soil.
Effect of reorientation of grain before germination

1. Length

Initial Orientation

Shoot down
Shoot up
Root up
Root down

Length of time in initial orientation

Fig. 4.8 Effect of reorientation of germinating grain on seedling length

Effect of reorientation of grain before germination

2. Weight

Initial Orientation

Dry weight in mg.

Length of time in initial orientation

Fig. 4.9 Effect of reorientation of germinating grain on seedling weight
While the eucalypt does not necessarily gain an advantage from orientation of the micropyle downwards, it is interesting to note that the collet end of the hypocotyl turns into the 'correct' orientation before germination and may thus increase its growth rate and hence the seedling's chance of establishment. The turn is possible in eucalypts because the collet end of the hypocotyl is free of the remainder of the embryo. If the shoot and seminal roots of a wheat embryo turned in grain lying horizontally one or the other must grow through the endosperm before emerging from the seed, rather than directly into the seedbed. The morphology of the eucalypt seed enables it to turn in the direction of gravity before germination.

4.4 GENERAL DISCUSSION

Vertical orientation of the radicle in the soil by the hypocotyl of *E. sieberi* confers an advantage in establishment on the seedling because that is the preferred direction for extension growth as well as the most effective direction for penetration.

Roots deflected by a discontinuous barrier revert to the original direction when possible, even if continued growth in the deflected direction is equally possible (Scott Russel, 1977). Whiteley and Dexter (1984b) examined the effect of displacing radicles of safflower and rape from the preferred vertical direction by forcing them to grow along cracks in compacted soil. They found the rate of root elongation to be consistently low and independent
of soil strength, and suggested that normal growth processes are disturbed when radicles grow at angles to the vertical. In eucalypt and carrot radicles (experiment 4.1) displacement from the vertical caused foreshortening of the tip and prolific growth of long root hairs in addition to reduced rate of extension and, in *E. sieberi*, early production of lateral roots. These changes in root morphology commonly accompany restriction of the forward growth of the root by compact soil, but in that case increase in root diameter also occurs, allowing the root to enlarge the space in which it grows (Greacen, *et al.*, 1969) and to shear the soil ahead of it in some soils, allowing further extension (Whiteley, *et al.*, 1981). The morphological changes observed in the eucalypt and carrot facilitate penetration of the slope along which the disorientated radicle grows by anchoring it firmly and shortening the unanchored tip, thus increasing the buckling strength of the radicle relative to that of a poorly anchored radicle extending rapidly in the preferred direction. The disorientated radicle, so modified, may gain sufficient strength to resume the preferred angle.

The mechanism by which orientation influences root extension is not known, although many authors have reported the effect, including Hering (1904), Ahrens (1933) and Larsen (1953). Larsen turned radicles through 180° from vertically downwards to vertically upwards then returned them to the preferred direction. The radicles were inverted for too short a time to start a geotropic growth curvature,
which in itself alters growth rate. He showed that inverted radicles extended less after reinversion than control radicles which were not inverted, and concluded that gravity retarded growth when acting in the direction opposite to that 'preferred'.

McDonald (1976) compared the length of radicles of 4 day old cress seedlings grown at different angles in light and dark and showed that radicles growing vertically were less inhibited by light than those deflected from the preferred direction. The light inhibition was also overcome by an axial force applied by centrifugal motion (McDonald and Gordon, 1978) and the conclusion from these results was that any longitudinal force up to 1 x gr., enhances extension. Jackson and Barlow (1981) in a reexamination of the role of growth regulators in gravitropism stated that growth enhancement is associated with reduction in gravi-reactivity: that is with decreasing stimulus to turn towards the preferred orientation.

_Eucalyptus sieberi_ and carrot radicles exhibited this enhancement but the response of the eucalypt to incorrect orientation may be as significant to establishment as the rapid extension of correctly orientated radicles, for the lateral roots it produces may be able to take up their preferred orientation and extend rapidly while soil conditions are favourable. It is interesting that _E. sieberi_ produces lateral roots from the radicle when the component of gravity is 0.6 x gr. (experiment 4.1) while carrot radicles experiencing 0.2 x gr. did not produce them, and
the response has not been reported in other species in relation to gravitropism.

McDonald and Gordon (1978) noted that growth of the shoots of seedlings whose radicles grew in the preferred direction appear healthier than those of seedlings with deflected radicles and referred to this as a 'tonic' effect. However experiment 4.3 indicates that growth of the wheat shoot is enhanced by correct orientation while contained within the seed, apart from any tonic effect due to root orientation. It is possible that the positive geotropic growth curvature of the collet of *E. sieberi* may facilitate establishment by orientating both hypocotyl and radicle in the preferred direction for rapid extension, and for a 'tonic' effect.

These experiments have shown that *E. sieberi* embryos and seedlings respond rapidly and precisely to the gravity stimulus compensating for reorientation of the seed during germination and for deflection of the radicle during establishment. Both contingencies occur in its habitat where water, wind or animals may roll the seed as it lies on the surface and cohesive or compacted soils may prevent radicle extension in the preferred direction.
CHAPTER 5
SURVIVAL OF SEEDLINGS BETWEEN GERMINATION AND ESTABLISHMENT OF THE RADICLE

INTRODUCTION

One aspect of the biology of *E. sieberi* revealed by these studies is its capacity for establishment with little or no radicle in the soil. Observed instances include:

(i) the survival of seedlings with deflected radicles on dry compacted clods in experiment 2.2;
(ii) extension of the hypocotyl and opening of the cotyledons before the radicle has produced the root hairs necessary for good contact with soil resources and for anchorage, experiment 3.2, and
(iii) establishment of seedlings without radicles by means of adventitious roots from the hypocotyl, experiment 4.2.

In addition, the seed coat is broken by the hypocotyl, which is a cuticle-covered aerial organ, so the radicle, which has no cuticle, is not exposed directly to the atmosphere in the early post-germinative stages.

These observations suggested that the newly germinated seedling may tolerate a degree of water stress between germination and establishment of the radicle in the soil. The water supply to the newly germinated seedling is examined in 5.1, its tolerance to desiccation in 5.2, and its response to loss of the radicle in 5.3.
5.1 ROLE OF 'CLINGING DISC' IN SEEDLING WATER RELATIONS

In this experiment the capacity of the long hairs on the periphery of the 'clinging disc' to supply water for the seedling is examined.

Materials and method

Twenty seeds of *E. sieberi* were sown in each of twelve different water regimes, namely combinations of four different matric water potentials and three different relative humidities (R.H.). These regimes were established in tensiometers made by connecting Büchner funnels to hanging columns of water (Haines Apparatus, H.A.). The four matric water potentials used were -0.5, -2.0, -5.0 and -10.0 kPa while the three levels of R.H. used were 99.9 to 100% (funnel covered with plastic film impervious to water), 98-99% (funnel partly covered) and 60-65% (funnel open to ambient R.H. of the glasshouse). As the open funnels were not in equilibrium the R.H. varied with distance from the tensiometer plate. The value given for the partly closed funnel is that of the air 1 cm above the plate, measured with a psychrometer.

The seedlings were measured individually as they grew by means of a scale inside the Büchner funnels. After 12 days the seedlings were removed from the H.A.s and the 5 largest in each treatment, all of which had germinated on days 3 and 4, were weighed, oven dried and reweighed to assess the growth after 8 or 9 days. Younger seedlings
were weighed to assess growth after shorter periods and at different stages of development.

Results and discussion

No seeds germinated in the lowest R.H. treatment or at -10 kPa in 98% R.H. (Table 5.1).

Table 5.1 Effect of R.H. and $\psi$ on seed germination

<table>
<thead>
<tr>
<th>$\psi$ kPa</th>
<th>99.9% R.H.</th>
<th>98% R.H.</th>
<th>60% R.H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.5</td>
<td>100</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>-2.0</td>
<td>100</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>-5.0</td>
<td>100</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>-10.0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The growth of seedlings after 8 or 9 days was greater in R.H. >99% than at 98% at the same matric potential (Table 5.2).

Table 5.2 Effect of matric water potential and R.H. on growth

<table>
<thead>
<tr>
<th>R.H.</th>
<th>Substrate matric potential</th>
<th>Mass of seedling in g after 8 or 9 days' growth ± S.E.$\bar{x}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.5 kPa</td>
<td>Wet wt</td>
</tr>
<tr>
<td>&gt;99%</td>
<td></td>
<td>Wet wt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Seedlings grown at -2 kPa matric potential grew more (wet weight basis) than those at -0.5 kPa.

The relationship between size, shape and weight of seedlings at different stages of development was determined from measurements of seedlings younger than 8-9 days old on day 12 (Fig. 5.1). This relationship was used to plot the course of development of the seedlings in each treatment from their length measurements during growth and the final weight measurement (Figure 5.2).

All seedlings gained weight in two phases (Fig. 5.2). In the first, rapid phase to day 7 or 8 after sowing, the hypocotyl straightened, the cells in the collet end elongated and the cotyledons expanded sufficiently to displace the seed coat. Extension of the hypocotyl was greatest in the collet region, for Indian ink dots placed there in earlier investigations of hypocotyl growth were elongated into dashes while those placed on the apical end remained circular. However, in the wetter treatments (-0.5 kPa) elongation of the apical as well as the collet end occurred, together with greater expansion of the cotyledons. In the slower second phase of growth beginning
Effect of Matric water potential and R.H. on Growth of seedlings

Fig. 5.2 Effect of $\psi$ and R.H. on seedling development
on days 7 or 8 the hypocotyl completed its extension, the cotyledons in drier treatments continued their expansion and the radicle lengthened.

Seedlings growing at -0.5 kPa did not enter the second phase of growth and the radicle did not lengthen. Although the seedlings at -0.5 kPa were large the radicle was not in contact with the sintered glass plate of the funnel (Plate 5.1a). During the time of growth of these seedlings (ie the first three days after germination) the 'clinging disc' hairs were in contact with the wet plate but they did not adhere to it. When the radicle began to extend the seedlings were not held firmly to the plate and they toppled. This caused the radicle, and most 'clinging disc' hairs to lose contact with the plate. The seedling obtained sufficient water for the hypocotyl to lengthen and for the cotyledons to expand from the hairs that remained in contact with the plate after the seedling toppled. However, when the hypocotyl curved in an attempt to raise the cotyledons the seedling was brought to a point of balance with all the hairs raised from the plate.

With seedlings grown at -5 kPa the hairs clung firmly to the sintered glass plate and were not dislodged by growth of the radicle (Plate 5.1b). The radicle, being unable to penetrate the plate, grew along it and the seedling could obtain water from the adhering clinging hairs and also, possibly, the radicle. This allowed growth to continue in the second growth phase and by day 12 the seedlings attained the same total growth as those at
-0.5 kPa. The clinging hairs of seedlings at -2 kPa also adhered firmly to the plate and they grew faster than those at -5 kPa surpassing the growth of those at -0.5 kPa by day 12.

When grown at -10 kPa, some seedlings developed sufficiently long clinging hairs to hold them to the plate when the radicle began to grow (Plate 5.1c). In these seedlings the cotyledons were raised normally and the seedlings grew at much the same rate, during phase 2, as seedlings on moister surfaces. Some seedlings, however, did not produce sufficiently long clinging hairs to resist the pressure of the radicle and the hairs were torn off the plate and withered. These seedlings had obtained sufficient water through their short hairs for the collet to unbend but not to straighten fully, so the cotyledons remained inside the seed which was tilted up off the sintered glass plate. The radicle of these seedlings was deflected along the surface and grew slowly. However the apical end of the hypocotyl did not grow, and the cotyledons remained within the seed coat for the last 4 or 5 days of the experiment.

These results indicate that the hypocotyl straightens and the cotyledons are raised independently of water uptake by the radicle. Cotyledons were lifted at -0.5 kPa where the radicle was not in contact with water and lifting began at -10 kPa, before the radicle grew, but ceased when the collar hairs were torn from the plate, and did not resume for the duration of the experiment even though the radicle grew.
Plate 5.1 Effect of water availability on seedling development

(a) $\psi - 0.5 \text{ kPa}$

(b) $\psi - 5.0 \text{ kPa}$

(c) $\psi - 10.0 \text{ kPa}$
The role of the 'clinging disc' is to raise the cotyledons quickly and to enable the seedling to grow rapidly if water is available before the radicle is established; that is, to take full advantage of the favourable conditions that caused germination. The disc hairs are the primary source of water for raising the cotyledons, a supplementary source after the radicle is established, and if they remain intact they may support the seedling for some time after the radicle is lost.

5.2 TOLERANCE OF NEWLY GERMINATED SEEDLING TO DESICCATION

The development of *E. sieberi* seedlings at -10 kPa in the previous experiment showed that the growth of the peripheral hairs on the 'clinging disc', which stabilize and supply water to the seedling, is restricted by low matric potential. The consequence is that seedlings may not complete the establishment of the radicle in the soil. They may be subject to interruption of their development while water conditions are unfavourable. In these experiments the tolerance of the newly germinated seedling to desiccation is assessed.

(i) The emerging hypocotyls of seeds germinating on the surface were subject to cycles of wet and dry periods as they carried the radicle to the surface.

**Materials and method**

Twenty four groups of 10 seeds of *E. sieberi* were sown on sintered glass plates of H.A.s held at -5 kPa
matric potential and were allowed to imbibe water in a saturated atmosphere at 18°C in diffuse light for one day. They were then dried by reducing the R.H. to 70% for one day by removing the plastic film covering the H.A.s. The R.H. was then raised to saturation by replacing the film. The initial wetting and drying 'primed' the seed so that most of the population would germinate within three days of rewetting, compared to the eight day period of germination required in 'non-primed' populations (Chapter 7).

After two days the 'primed' seed was dried again by removing the film from the H.A.s, kept dry for 3 days and then rewet for one day by replacing the film. This long dry/short wet cycle was repeated until the short wet cycle ceased to induce further seeds to germinate. The remaining seeds were left in wet conditions.

The number of seeds germinated in each short wet period was recorded when the cover was removed to end the period, and the number of seedlings with raised cotyledons was recorded before the cover was replaced at the end of each dry period and these seedlings were removed from the H.A.s. They were seedlings whose 'clinging discs' had made sufficient contact with the sintered glass plate in the wet period to continue their post-germinative development in the dry period, by using water from the plate of the H.A.
Results and discussion

In each of 5 cycles following the 'priming' cycle approximately a quarter of the seeds remaining on the plate germinated during the wet period (Table 5.3).

Table 5.3 The effect of wetting and drying on germination and establishment

<table>
<thead>
<tr>
<th>No. of wet/dry cycles</th>
<th>Priming</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of seeds germinated</td>
<td>0</td>
<td>25</td>
<td>24</td>
<td>26</td>
<td>24</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>% of germinated seeds 'established'</td>
<td>0</td>
<td>92</td>
<td>63</td>
<td>65</td>
<td>17</td>
<td>64</td>
<td>0</td>
</tr>
</tbody>
</table>

In the first of these cycles 92% of these seedlings developed sufficiently during the wet period to make effective contact with the plate and continue growth, but fewer did so in the succeeding cycles.

Since initial 'priming' of the seed would ensure that all seeds were ready to germinate by cycle 3 (a further 2 or 3 days of wet conditions) all seeds after cycle 3 were subjected to drying while the embryo was actively growing, whether or not the hypocotyl had broken the seed coat.

Seeds which had not germinated by the end of cycle 7 germinated 'atypically' (Bachelard, 1967) after several days in wet conditions, when the apical end of the hypocotyl elongated and forced the cotyledons through the seed coat. A radicle grew from some of these seedlings but it
was not carried to the surface of the plate by growth of the hypocotyl and the coleorhiza did not develop into a 'clinging disc'. On others of these seedlings the radicle did not grow, but adventitious roots were produced by the hypocotyl.

Some seedlings assessed as 'germinated' at the end of a wet period were 'not germinated' after the following dry period. Drying had caused the tip of the hypocotyl to retract inside the seed coat, concealing the split made at germination. Some of these seedlings germinated and became established normally in the succeeding wet period. Others lost the capacity for normal establishment after several false starts, and produced adventitious roots.

Thus seedlings whose development is arrested by drying before the hypocotyl completes its extension into the soil, resume their development on rewetting. Seedlings which experience several dry periods lose the ability to germinate normally, but they do not lose viability, and they germinate 'atypically' after an extended wet period. The hypocotyl has a degree of tolerance to drying even when subjected to a sudden drop in R.H. while growing rapidly.

(ii) The hypocotyls and elongating radicles of seedlings yet to raise the cotyledons were subjected to severe desiccation in this experiment.
Materials and method

One hundred seeds of *E. sieberi* were 'primed' on H.A.s to ensure uniform germination in the way described previously. After 'priming' the seeds were removed from the H.A.s and air dried on filter paper at 70% R.H. After two days they were replaced in the H.A.s at -5 kPa matric water potential in a saturated atmosphere at 18°C, for 2½ days. This dry/wet cycle was repeated three times after which the seedlings were kept under wet conditions.

Results and discussion

After 'priming' and subsequent exposure to air drying for 2 days, then rewetting for 2½ days all the seeds germinated and 'established' on the glass plate by the 'clinging disc', and their radicles began to grow. Radicle growth ceased on exposure to 70% R.H. and resumed after the second cycle of air drying and rewetting in only 30 of the seedlings. However, root hairs grew from the 2 to 3 mm of radicle adjoining the collet in the 70 seedlings whose radicle tip did not resume growth. Following a further exposure of seedlings to the air dry/wet cycle, radicle growth resumed in only 3 of the seedlings and after a fourth cycle it ceased in all seedlings.

During the prolonged wet period that followed the fourth air dry period 55 of the seedlings continued their establishment and all of these were seedlings that had not expanded the cotyledons during a previous wet period. Those that had expanded the cotyledons did not recover. Thirty
of the 55 living seedlings produced adventitious roots from the hypocotyl after the cotyledons expanded but the remainder died from fungal infection attacking the tip of the radicle.

Thus seedlings which experience desiccation after the radicle begins to grow but before the cotyledons expand are capable of establishment on rewetting. If the tip of the radicle is unable to grow the collet end of it produces root hairs, and would produce lateral roots subsequently. If the entire radicle is unable to grow the hypocotyl produces adventitious roots after the cotyledons expand.

These two experiments show that the hypocotyl tolerates drying and continues the establishment of the seedling on rewetting, if the cotyledons have not expanded.

The collet end of the radicle also tolerates drying to a degree. This suggests that the early extension of the radicle that occurs as it is being placed in the soil is different from growth in the zone of elongation after meristematic activity begins (see experiment 3.1). Further evidence for this conclusion is that the first 2 to 3 mm of radicle are always at right angles to the clinging disc, (Plate 5.2). This must occur if the original cells in the radicle primordium extend without response to gravity. The radicle turns abruptly into the preferred orientation after the initial extension.

The morphology of the embryo and newly germinated seedling of *E. sieberi* is appropriate for germination on seedbeds with an unreliable water supply. The hypocotyl
Plate 5.2  Effect of orientation on early growth of the radicle. Note that in each case the radicle has extended in line with the hypocotyl for a short distance before turning into the positively geotropic direction.
and collet end of the radicle, both of which have a degree of tolerance to desiccation, are the only parts of the seedling exposed to the atmosphere during the early post-germinative development of the seedling.

5.3 RECOVERY AFTER LOSS OF THE RADICLE

E. sieberi seedlings are capable of establishment without the radicle or the 'clinging disc' (experiment 4.2) and able to recover from conditions that debilitate the collet end of the hypocotyl by 'atypical' germination (experiment 5.2). In these cases the hypocotyl produces adventitious roots to replace the radicle and the biology of the process is examined in the experiments in this section.

Materials and method

Seedlings were germinated and allowed to grow on sintered glass plates at -5 kPa matric water potential until the cotyledons were fully expanded. Under these conditions seedlings remain in the cotyledonary stage unless provided with nutrients.

The seedlings were lifted from the plates by the radicle without damaging the hypocotyl, and were cut into sections. Ten different types of section were used (Fig. 5.3). Some radicle tissue was retained on types 1 and 2 whereas in types 3, 4, 5, 6 and 7 all radicle tissue was removed but cotyledonary tissue was retained. In types 8, 9 and 10 all radicle and cotyledonary tissue was removed.
<table>
<thead>
<tr>
<th>Response of 10 types of section in light</th>
<th>in dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>6 9</td>
</tr>
</tbody>
</table>

Fig. 5.3 Response of *E. sieberi* seedlings to loss of the radicle and the collet
Ten sections of each type were placed vertically, collet end down, 10 vertically, collet end up and 10 were laid horizontally in 2% water agar in the top of a 10 cm Petri dish. The dishes were covered by taping a second top to the first, and placed in a shaded glasshouse at 18°C. Preliminary experiments had shown that intact seedlings remained alive for many months under these conditions.

A further 10 sections of types 6 and 9 were placed in the three orientations described above and the dishes were covered in aluminium foil to exclude light and placed with the other dishes.

All dishes were opened after 10 days and the seedling sections removed.

**Results and discussion**

All sections appeared turgid and healthy and all replicates of a particular type of section responded in the same way (Fig. 5.3, drawn to scale).

All sections which retained some cotyledonary tissue produced one or two adventitious roots from the hypocotyl if it was present and radicle tissue absent. Sections with two cotyledons produced two roots whereas those with one cotyledon produced only one root.

The adventitious roots were formed immediately behind the severed end of the hypocotyl, distal to the cotyledons. The roots grew through the cut end of hypocotyls which were placed vertically, collet end down, and through the epidermis of those placed vertically, collet end up and
those placed horizontally (Plate 5.3). Dissection showed that the roots had arisen on opposite sides of the procambium, directly beneath the insertion of the cotyledonary petioles, and had grown out through the cortex in the direction of gravity.

The hypocotyls of seedlings kept in the dark elongated and produced adventitious roots. These roots were not connected to the procambial tissue of the hypocotyl by continuous xylem strands, as were those of the sections grown in the light, and broke away from the hypocotyl readily.

Sections on which some radicle tissue was retained (types 1 and 2) did not produce adventitious roots but formed lateral roots if the radicle tissue was in contact with the agar and cotyledonary tissue present, and dense root hairs if the radicle tissue was not in contact but the cotyledonary tissue was in contact with the agar.

Loss of the radicle always preceded adventitious root formation in seedlings. The following experiment tested the effect of loss of the radicle before germination.

**Materials and method**

Seeds were plated out on 2% water agar in closed Petri dishes in an 18°C constant temperature room under a 60 Watt fluorescent light. In these conditions seeds germinated at 72 h and at 84 h the coleorhiza opened to expose the radicle.

Batches of 40 seeds were removed from the dishes at 6, 12, 24 and 48 h after sowing and the embryos were
(a) From severed hypocotyl
(b) From healthy tissue behind the damaged collet. Note callused swelling distal to roots.

Plate 5.3 Emergence of adventitious roots from the hypocotyl.

Plate 5.4 Replacement of radicle by adventitious roots.
dissected from the seeds and cut into tip, intercalary
and apical sections. The apical sections included the
cotyledons. The radicle was burned from the tip sections
using a hot needle. All sections were returned to 2% agar
in closed Petri dishes and their growth was observed daily.
The germinating seeds (72 h) and seedlings (84 h) were not
sectioned but their radicles were burned from the end of
the hypocotyl without damaging the coleorhiza, and they
were replaced in the Petri dishes.

Results and discussion

Removal of the radicle and sectioning before germina-
tion had no effect on the growth pattern of each section
(Fig. 5.4). All tip sections which included the coleorhiza
anchored themselves to the agar independently of when they
were excised. After 96 h the bend in the hypocotyl had
straightened in exactly the same manner as occurs in intact
seedlings and embryos from which the radicle was removed,
and the hypocotyls lengthened in proportion with the amount
of tissue present. No tip sections produced adventitious
roots.

All intercalary segments curved downwards as in the
tip sections but since these sections lacked the coleorhiza
they could not anchor themselves to the agar. Subsequent
straightening and lengthening of these sections resulted
in a curved section, as shown. None produced roots.

All apical sections turned downwards as in intact
seeds or embryos. As these sections attempted to straighten
the cotyledons held them down and lengthening of the
<table>
<thead>
<tr>
<th>Time from sowing in hours</th>
<th>6</th>
<th>48</th>
<th>72</th>
<th>84</th>
<th>96</th>
<th>120</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tip section</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercalary section</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical section</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 5.4** Germination of excised sections of the embryo
sections caused the hypocotyl to curve upwards. All these sections produced two adventitious roots.

These results confirm that the conditions for adventitious root formation are loss of the radicle and presence of the cotyledons. In addition they show that the pre- and post-germinative extension of the hypocotyl and its response to gravity is independent of the radicle and the cotyledons and the apical meristem.

The failure of hypocotyls to form adventitious roots until they had extended and the cotyledons had expanded suggested that mature, metabolically active cotyledons of germinated seedlings provided the resources for adventitious root formation. Control of the number of roots by the number of cotyledons supported this conclusion. The possible role of the cotyledons in providing metabolites and auxin for adventitious root formation was examined in this experiment.

**Materials and method**

Eighty 10 mm long, stiff, intercalary sections of *E. sieberi* hypocotyls from 15 day old seedlings grown in a light glasshouse at 20°C were surface sterilized with 1% hypochlorite solution. Each was placed vertically, physiological end down in a vial containing 5 ml of 1.5% Bactoagar, with one of several combinations of metabolites, according to the schedule in Table 5.4. There were 10 hypocotyls in each treatment.

Metabolites used in the schedule were 2% sucrose (S) and the basal mineral nutrient medium of Murashige and
Skoog (1962). The growth hormone used was indole acetic acid at $1.0 \, \text{mg} \, \text{l}^{-1}$. A preliminary experiment indicated that some hypocotyls would produce an adventitious root while lying on the surface of agar with sucrose, mineral nutrients and IAA at 0.5, 1.0 and 5.0 mg $\text{l}^{-1}$, hence the use of 1.0 mg $\text{l}^{-1}$ for this experiment.

The vials were incubated at 18°C for 14 days, after which the hypocotyls were removed and examined under a dissecting microscope.

**Results and discussion**

Hypocotyls without sucrose did not produce callus or roots (Table 5.4) and the red anthocyanin pigment in the epidermis became discoloured.

Table 5.4 The control of regeneration from the hypocotyl

<table>
<thead>
<tr>
<th>Basal Medium</th>
<th>% hypocotyls with callus or roots</th>
<th>Lower</th>
<th>Higher</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 mg $\text{l}^{-1}$ IAA</td>
<td>Callus</td>
<td>Callus + roots</td>
</tr>
<tr>
<td></td>
<td>1.0 mg $\text{l}^{-1}$ IAA</td>
<td>Callus</td>
<td>Callus + roots</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>20</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>M+S</td>
<td>10</td>
<td>10</td>
<td>80</td>
</tr>
</tbody>
</table>

Hypocotyls with sucrose retained the red colour and most of those with IAA produced callus while some of those without IAA produced both callus and several short, weak, adventitious roots from the cut end of the hypocotyl. The callus was produced in a zone several mm behind the damaged end of the hypocotyl as shown in Plate 5.3b.

The IAA and sucrose provided for cell multiplication in these hypocotyl sections entered it through the cut end and callus formed in a broad zone across the hypocotyl, as it did after adventitious roots were initiated in
hypocotyl sections with cotyledons. This suggests that the cotyledons supply IAA and sucrose, and that the manner, or the concentration in which it reaches the physiologically active, undamaged, zone of the hypocotyl determines whether callus or roots form first.

Adventitious root formation in eucalypts cultured in vitro requires a favourable balance of hormones supplied to the hypocotyl. Callus formed in 40% of intact seedlings of *E. alba* in White's medium with coconut milk, but addition of 1 mg l$^{-1}$ IAA and 0.5 mg l$^{-1}$ 2,4-D caused callus to form on 90% of the seedlings and this latter callus produced adventitious roots when subcultured (Kitihara and Caldas, 1975). Cotyledon and hypocotyl explants of *E. citriodora* in Murashige and Skoog's basal medium with Gamborg's vitamin supplement and 5 mg l$^{-1}$ IAA produced long strong roots from compact, red callus (Sita, 1979). Callus formed readily on the cut ends of hypocotyl segments of *E. globulus* in Schenck and Hildebrandt's medium with vitamins and $10^{-5}$ M IAA and adventitious roots grew from callus formed on the apical segments of hypocotyl and from the procambium of basal segments (Seibi Oka Yeung and Thorpe, 1982). In these cases sucrose was provided and callus formed before, or together with, adventitious roots, while in the present experiment callus formed, but roots did not form within 14 days where IAA was provided at 1 mg l$^{-1}$.

An explanation for the formation of an adventitious root below each cotyledon can be derived from these results. A limited amount of sucrose and IAA enter the
hypocotyl through the cotyledonary petioles and the sucrose moves down the hypocotyl in the phloem until its passage to the radicle is blocked by tissue damage. The sucrose collects in the procambium adjacent to the phloem in sufficient quantity to provide energy for the production of a root if IAA moving by polar transport is available. The adventitious root makes a direct connection with the vascular system of the hypocotyl. Further sucrose from the cotyledons moves into the root rather than across the hypocotyl where it would be used to form callus as occurred when sucrose and IAA were supplied directly to the base of the hypocotyl.

The few hypocotyls that produced callus and roots without added IAA apparently contained a quantity of IAA appropriate for the formation of callus or roots. The results suggest that roots form if the concentration of IAA is low and callus forms if it is higher.

Thus the distribution of resources into the hypocotyl may control the formation of adventitious roots. If the seedling is intact resources pass into the radicle, and if the radicle tip is destroyed they initiate and support lateral root formation. If the radicle is lost the resources collect in the basal end of the hypocotyl and two adventitious roots form in the procambium and emerge through the epidermis directly downwards. The roots grow symmetrically and diverge on entering the substrate (Plate 5.3) bracing the seedling, and replacing the radicle effectively.
The formation of two adventitious roots is so predictable (Plate 5.4) that it may be regarded as a germination strategy for establishment of seedlings likely to lose the radicle. It may be more effective than production of adventitious roots from callus, for it takes less time, produces roots with a direct connection to the stele of the hypocotyl and divides resources evenly between two roots, rather than amongst many, allowing the two roots to grow rapidly.

5.4 GENERAL DISCUSSION

The most likely risk to survival between germination and establishment of the radicle is failure of the hypocotyl to gain sufficient water to complete its placement of the radicle in the soil. Experiment 5.2 showed that the germinating embryo, and the newly germinated seedling, recover from desiccation and continue their establishment normally if undamaged, or by atypical development if the radicle and collet are damaged during the dry period. Survival of the radicle is not critical to establishment of the seedling.

Experiment 5.1 indicates that the newly germinated seedling is dependent on the hairs of the 'clinging disc', not the radicle, for water while the cotyledons are raised. A consequence of the transfer of water absorption from the radicle to the 'clinging disc' hairs is that the development of the radicle can be delayed for some time after germination as it is carried towards the soil surface inside the
unopened coloerhiza. This strategy is appropriate for germination on the surface of seedbeds with an unreliable water supply as it protects the radicle while growth of the hypocotyl carries it towards the soil.

The rapid and orderly production of adventitious roots after loss of the radicle, experiment 4.3, is another feature that is appropriate for such seedbeds as the roots may replace the radicle and the 'clinging disc' if they are lost to desiccation in a dry period between germination and establishment of the radicle in the soil.

There is an interesting parallel between the germination strategy of *E. sieberi* described here and that of the desert chenopod *Salsola inermis* Forsk. *Salsola* germinates by elongation of both the hypocotyl and the radicle, but the latter remains protected within the membranous folds of the persistent fruit while the hypocotyl arches up and raises the cotyledons. Active growth of the radicle does not begin until heavy rain leaches a root growth inhibitor from the fruit. Seedlings whose development is arrested by desiccation before the radicle begins active growth germinate normally on rewetting, but those whose radicles were growing actively before desiccation do not regenerate after rehydration (Negbi and Evanari, 1959). In lettuce seed the radicle begins active growth soon after the seed imbibes water after sowing and there is no tolerance to desiccation. Young and Evans (1972) found that the initial elongation of the radicle was caused by a reversible physical response to imbibition of water, not by growth, which is irreversible. Results presented in experiment 4.2
for *E. sieberi* suggest that the initial extension of the radicle may be by imbibitional swelling rather than growth following meristematic activity for it is not positively geotropic and it has a degree of tolerance to desiccation, as in the chenopod. The eucalypt, however, is able to use the 'clinging disc' hairs for rapid establishment before the radicle grows, and although radicle growth is not delayed until heavy rain brings good growing conditions, the radicle is replaced if lost.

The morphology of the embryo of newly germinated seedlings was shown, in Chapters three and four, to be appropriate for germination and establishment on the surface because it facilitates soil penetration and subsequent elongation of the radicle. These experiments relate the morphology and physiology of the embryo and the newly germinated seedling directly to survival and establishment in seedbeds with an unreliable water supply.
CHAPTER 6
FACTORS INFLUENCING THE SURVIVAL OF THE COTYLEDONARY SEEDLING

INTRODUCTION

The germination strategy of *E. sieberi* seeds is consistent with germination on the surface of seedbeds with an unreliable water supply, for the seedling recovers on rewetting if it is desiccated between germination and soil penetration (Chapter 5). However, this stage of development is followed by some days in which the seedling is vulnerable to desiccation (Jacobs, 1955) because the cotyledons are raised off the soil and depend on the 'clinging disc' hairs for water until the late developing radicle lengthens in the soil, experiment 5.1.

Seedlings surviving the vulnerable stage become established as cotyledonary seedlings and many survived drought conditions for some months in the field (experiment 2.1). These seedlings produced a pair of triangular seedling leaves 2-3 mm long, which were the first parts of the seedling to die as conditions became severe. The cotyledons dried from the margin inwards. Dead seedlings remained erect, the cotyledons held horizontally. Death appeared to have been sudden, due possibly to heat shock, after a long period of survival without wilting or drooping of the cotyledons. Some seedlings survived the drought and the dead plumule was replaced by axillary shoots from the cotyledonary node after rain.
These observations suggested that the seedling might have a means of avoiding desiccation during the vulnerable period, and that it might have xeromorphic features consistent with its survival during establishment. Both possibilities are examined in this Chapter.

6.1 RESPONSE TO LIGHT AT EMERGENCE

Exposed seeds germinate, and early post-germinative development continues only in high relative humidity conditions. If such conditions persist for a day after germination, development progresses to the opening of the 'clinging disc' and its adherence to the soil prior to supplying water for raising the cotyledons. The seedling enters the few days in which desiccation is the prime cause of death. Any signal that the wet conditions prevailing will not continue may allow a response that increases the ability of the seedling to survive the dry period. The most likely signal is an increase in light intensity as the sky clears of cloud. In this experiment the effect of light intensity on the post germinative development of *E. sieberi* seedlings was examined.

**Materials and method**

Large seeds of *E. sieberi* were sown in three rows of 20 seeds on wet filter paper folded over each of 8 styrofoam blocks floating on water in 3 l black painted plastic containers. The containers were closed with a black lid to exclude light and to raise the relative humidity to
saturation and were placed in a controlled growth cabinet at a temperature of 20°C and R.H. 70%. After 2½ days the containers were emptied and refilled after 1 day to dry and rewet the seed. This treatment 'primed' the seed for uniform germination on days 5 and 6 after sowing.

On day 7, the time at which most seedlings would raise their cotyledons, the lids on each of 2 containers were replaced by 0, 1, 2 or 3 layers of sarlon shade cloth to expose the seedlings to varying light intensities. Light in the cabinet was provided by fluorescent tubes supplemented with incandescent bulbs exposing the seedlings to 1000, 500, 200 and 100 μE m⁻² s⁻¹, with a 12 h photoperiod.

After 3 days exposure the seedlings were observed and measured and the treatments were interchanged as follows: 1000→200, 500→100, 100→500 and 200→1000 μE m⁻² s⁻¹ and the seedlings were exposed for 5 days.

The experiment was repeated for measurement of the anthocyanin content of seedlings in each treatment. The anthocyanin content of 0.03 g wet weight of seedling tops (approximately 5 seedlings) relative to a blank was measured at 530 nm in a U.V. spectrometer after extraction into 4 ml of a solution of 0.1 M HCl in methanol. Thick free-hand sections of the cotyledons were cut to determine the distribution of anthocyanin.

Results and discussion

Seedlings receiving 1000 μE m⁻² s⁻¹ initially produced small, red cotyledons on a short hypocotyl. After transference to 200 μE m⁻² s⁻¹ the hypocotyls elongated, the
Table 6.1 The effect of light intensity $\mu\text{m}^{-2}\text{s}^{-1}$ on seedling development

<table>
<thead>
<tr>
<th>Light intensity</th>
<th>Days 0-3 in light</th>
<th>Days 4-8 in light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypocotyl length</td>
<td>Cotyledon spread</td>
</tr>
<tr>
<td></td>
<td>mm ± SEX</td>
<td>mm ± SEX</td>
</tr>
<tr>
<td>1000</td>
<td>7.5±0.4</td>
<td>5.9±0.06</td>
</tr>
<tr>
<td>500</td>
<td>12.5±1.6</td>
<td>8.1±0.7</td>
</tr>
<tr>
<td>200</td>
<td>18.8±0.6</td>
<td>10.2±0.03</td>
</tr>
<tr>
<td>100</td>
<td>24.6±0.8</td>
<td>10.3±0.04</td>
</tr>
</tbody>
</table>
cotyledons expanded and became slaty green in colour with a red outer rim, and the first leaf pair developed (Table 6.1). Seedlings initially at $500 \mu\text{Em}^{-2}\text{s}^{-1}$ responded similarly with all becoming red but in this treatment some cotyledons expanded and elongated. After transference to $100 \mu\text{Em}^{-2}\text{s}^{-1}$ the smaller cotyledons expanded and became grey green in colour and the first leaf pair developed. Seedlings grown at $100 \mu\text{Em}^{-2}\text{m}^{-1}$ initially, produced fully expanded green cotyledons and a first leaf pair, and on transference to $500 \mu\text{Em}^{-2}\text{s}^{-1}$ they became red. Those grown at $200 \mu\text{Em}^{-2}\text{s}^{-1}$ initially, expanded fully, produced the first leaf pair, and became light green. On transference to $1000 \mu\text{Em}^{-2}\text{s}^{-1}$ these seedlings reddened.

By the end of the initial exposure the amount of anthocyanin in the seedlings was directly proportional to the intensity of light received (Fig. 6.1). On transference from high to lower light intensities the cotyledons expanded and the concentration of anthocyanin fell, while on transference from low to high intensities there was no change in the size of the cotyledons but the concentration of anthocyanin rose.

High light intensity stimulates anthocyanin production and reduces hypocotyl, cotyledon and seedling leaf growth.

Distribution of anthocyanin in the cotyledon tissues after each light treatment is summarized in Fig. 6.2. Cotyledons developing in higher light intensities contain anthocyanin in the large vacuoles of the epidermal cells, (Fig. 6.2a) and appear red. On transference to lower light
Effect of Light intensity on Anthocyanin Content

Absorbance at 530 nm.

Light Intensity \( \mu \text{E m}^{-2}\text{s}^{-1} \)

Fig. 6.1 Effect of light intensity on anthocyanin content of seedlings
the epidermal and mesophyll cells expand. The mesophyll cells contain little anthocyanin and the expansion of the epidermal cells both dilutes the anthocyanin in the vacuole and produces a cytoplasm filled border without anthocyanin around the vacuolate part of the cell (Fig. 6.2b). Thus the anthocyanin is confined to small patches on the surface and the cotyledons change from red to slaty or grey green.
Cotyledons developing in low intensity light contain little anthocyanin in mesophyll or epidermal cells both of which expand rapidly (Fig. 6.2c). On transfer to high light both epidermal and mesophyll cells produce anthocyanin and the cotyledons become purple-red in colour (Fig. 6.2d).

_E. sieberi_ seedlings respond to high light intensity at germination by slowing the extension of the hypocotyl and the expansion of the cotyledons, and by delaying the production of the first leaf pair. These responses prevent increase in the volume and the surface area of the seedling and may, as a consequence, enable the 'clinging disc' hairs to maintain an adequate water supply to the seedling if the seedbed dries. The anthocyanin produced in response to high light intensity may protect the tissues from U.V. damage as anthocyanin absorbs strongly in that wave length. Anthocyanin is produced on top of the positive geotropic curve of the hypocotyl when it emerges from the seed and the colour spreads down to the collet, then up to the apical end of the hypocotyl and then into all the cells of the lower epidermis of the cotyledons as they are exposed to light when the seed coat is levered off. These observations, considered with the experimental results, suggest that anthocyanin production is a localized response to light on the epidermis.

The two responses, reduction in growth rate and production of anthocyanin are an example of acclimation to conditions prevailing in the seedbed at the time the seedling emerges, (Smith, 1982).
6.2 ABSORPTION OF WATER BY COTYLEDONS

Many seedlings which survived the first few days in the field (experiment 2.1) remained alive in conditions which prevented development of the plumule and confined the radicle to the dry surface soil. Observations indicated that water from passing rain storms, or mist, remained on the cotyledons for some time after it dripped or evaporated from seedling leaves of more advanced seedlings and of surrounding herbs and shrubs. This suggested that eucalypt seedlings may obtain water through the cuticle while the cotyledons are wet.

These experiments examined the absorption of water by wet cotyledons and compared it with absorption by seedling leaves.

(i) The effect of absorption on seedling water potential

Materials and method

Seeds sown on wet filter paper folded over styrofoam blocks floating on water were enclosed in 3 l black plastic containers and placed in a glasshouse at 18° to 20°C. After 10 days the top of the container was removed to harden the seedlings by exposing them to light and to an air stream of 60% R.H. for 5 days. After 15 days these seedlings were still in the cotyledonary stage.

Further seeds were sown on blocks floating in half strength Aquasol mineral nutrient solution and these seedlings had produced the first seedling leaf pair after 15 days.
After hardening, 20 seedlings received each of the treatments described below, (a) to (g), and the water potential of each seedling was measured in a pressure chamber.

(a) Cotyledonary seedlings were severed at the collet and the water potential measured immediately.

(b) Cotyledonary seedlings were wet by placing a water droplet on the cotyledons, left for two hours in situ, severed and then measured.

(c) Cotyledonary seedlings were severed, maintained in air at 70% R.H. for two hours to cause wilting and then measured.

(d) Cotyledonary seedlings were severed and allowed to wilt as in (c). The cut ends were then sealed with vaseline and the seedlings floated for four hours with the upper surface on water at 18°C. Water did not enter the floating cotyledons directly through the stomata since a bubble of air was trapped in the opening of each, and there was no loss of water vapour from the cut xylem vessels since they were sealed. After four hours the hypocotyls were cut above the seal and the water potential measured.

(e) Seedlings with leaves were treated as in (a).

(f) Seedlings with leaves were treated as in (c).

(g) Seedlings with leaves were treated as in (d) except that the cotyledons were covered with vaseline and the seedling immersed in water.
(ii) The effect of absorption on seedling weight

Materials and method

Seedlings for this experiment were grown on sintered glass plates in Büchner funnels covered with plastic film at a matric water potential of -5 kPa. The seedlings were exposed to diffuse natural light and a temperature between 15° and 20°C in a laboratory. After 10 days from sowing 32 seedlings with hypocotyls 3 mm long were removed and severed at the collet.

Each seedling was weighed and allowed to wilt for 1 hour at a R.H. of 70% before reweighing. Eight seedlings were then allocated to each of four treatments, namely:

(a) supported in a loop of cotton from the cap of a vial containing 2 ml water (Fig. 6.3) with no part of the seedling touching the water or the vial, and reweighed after 12 hours.  
[b]Fig. 6.3 Measurement of cuticular water absorption

(b) supported in a loop as above but with the cotyledons floating on the water, removed after 12 hours, dried by pressing firmly against tissue and reweighed.

(c) as in (b) but dried by touching gently against tissue.

(d) as in (b) but maintained in the vial for 10 days.

The vials containing the seedlings were placed in a constant temperature room at 20°C under a 65 watt fluorescent light on a 12 h photoperiod.
Results and discussion

The water potential of cotyledonary seedlings decreased markedly when wilted (treatment c) but the seedlings recovered to the water potential of intact seedlings with wet cotyledons (b) after floating on water (d), Table 6.2. Seedlings with wet cotyledons (treatment b) had higher water potentials, on average, than those with dry cotyledons (treatment a).

Table 6.2 Effect of surface water on seedling water potential

<table>
<thead>
<tr>
<th>Mean water potential of seedlings in kPa (and SE of 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledons (a)Unwet (b)Wet (c)Wilted (d)Rewetted (e)</td>
</tr>
<tr>
<td>-320</td>
</tr>
<tr>
<td>± 30</td>
</tr>
</tbody>
</table>

The water potential of seedlings with leaves in water was not greatly increased by immersion for 4 h after wilting (treatment g) but seedlings did recover after 24 h. Water on wet cotyledons was used to raise the water potential of E. sieberi seedlings, but leaves did not wet sufficiently easily for water to be absorbed rapidly.

All seedlings lost from 8 to 10% of their original weight during wilting before allocation to the treatments and the wilted weight was used as a base for calculation.
of water gain through the cotyledons (Table 6.3).

Table 6.3 Effect of wet cotyledons on seedling water content

<table>
<thead>
<tr>
<th>% Increase in weight of wilted seedlings</th>
<th>± SE of 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry cotyledons</td>
<td>Wet (b)</td>
</tr>
<tr>
<td>1.07 ± 0.01</td>
<td>12.45 ± 0.47</td>
</tr>
</tbody>
</table>

The control seedlings which were suspended in a saturated atmosphere increased slightly in weight but did not regain turgor. The weight gain may have been due to the deposition of a surface film of water rather than to uptake. All seedlings with cotyledons resting on water recovered turgor and those dried roughly (treatment b) gained approximately the same amount as they lost during wilting, a 10% loss on wilting replaced by a 12% gain. Those dried gently (treatment c) appeared to gain considerably more, indicating that water was retained on the surface of the cotyledons.

These results show that water was absorbed through the cotyledons. Some cotyledons had not expanded fully before wilting and these seedlings gained more weight than those with fully expanded cotyledons.

The seedlings left suspended for 10 days all developed two adventitious roots from the end of the hypocotyl as described in experiment 5.3.
These experiments indicated that *E. sieberi* seedlings may take up water through the cotyledons. Possible effects of this water uptake on growth were tested in this experiment.

**Materials and method**

The radicles of 30, 10-day old seedlings grown in Büchner funnels, as described in the previous experiment, were removed and the hypocotyls were inserted through a 1 mm diameter hole in a 23 mm diameter washer cut from plastic foam sealed on both surfaces. The washer was pushed into the mouth of a 20 mm diameter vial and it sealed against the vial and the hypocotyl (Fig. 6.4). This procedure isolated the hypocotyl in the dry vial.

A drop of water was applied to the cotyledons of 20 seedlings each morning and the cotyledons were exposed to a relative humidity of 60% under a 65 watt fluorescent tube with a 12 h photoperiod. Under these conditions the cotyledons dried within 12 hours. The remaining 10 seedlings were not wetted.

**Results and discussion**

Seedlings receiving no water wilted within 12 hours and died. Seedlings receiving water produced adventitious roots and showed no visible sign of wilting even when the
cotyledons were dry. Absorption of water through the surface of the cotyledons was sufficient to raise the humidity in the vials rapidly, as a 15° reduction in temperature caused dew to form in vials containing seedlings with wet cotyledons but not in the control vials. High relative humidity and water from the cotyledons allowed the roots to grow vigorously.

These experiments show that placing a water drop on the upper surface of the cotyledons of a seedling severed from its root is effective in restoring turgor and maintaining growth. Thus the water retained on the cotyledons of *E. sieberi* seedlings after rain storms, mist or dew fall, may be a factor in the survival of cotyledonary seedlings and older seedlings which retain the cotyledons for at least six months.

6.3 SURFACE CHARACTERISTICS OF THE COTYLEDONS

The cotyledons of *E. sieberi* appear able to survive exposure and to absorb water better than the first seedling leaves. In this investigation the surface structure of the cotyledons was examined as it may be a factor in light reflection, reduction of transpiration, retention of water droplets and cuticular absorption of water.

**Materials and method**

Fully expanded cotyledons and 10 mm long seedling leaves were selected from 6 week-old seedlings grown on styrofoam rafts on half-strength Aquasol solution in a
naturally lit glasshouse (temperature range 160-20°C, relative humidity ca 60%). Six cotyledons and 6 leaves were picked at random, cut into triangular pieces (5 mm side), laid on a metal surface, frozen by immersing the metal in liquid nitrogen and freeze dried in a Dynavac Cold Stage Freeze Dryer. After coating in gold in a Dynavac Evaporative Coating Unit the surfaces were examined in a Cambridge Stereoscan 180 scanning electron microscope.

The same procedure was applied to unexpanded cotyledons from day old seedlings and unexpanded seedling leaves from within the apical bud of the older seedlings.

Results and discussion

Each epidermal cell on the upper surface of the cotyledon has a papilla with longitudinal striations on the sides and convoluted cuticular ornamentation on the apex (Plate 6.1a). The margins of the mature epidermal cells are thickened into a ridge (Plate 6.1b). Scattered plates of wax crystallites occur on these older cotyledons.

The surface characteristics of the cotyledons differ markedly from those of the seedling leaves, which have gently convex epidermal cells without papillae. Unexpanded seedling leaves have a coating of smooth wax (Plate 6.5) and older seedling leaves are densely covered with plate wax crystallites (Plate 6.2). These differences are consistent with the observation that the cotyledons are wettable while the leaves are not (Experiment 6.2). Holloway (1969) states that non-wettable leaves are, in general, covered with a fine micro-relief of hydrophobic
Plate 6.1 Upper surface of expanded cotyledon (L. and R.)

- Cuticular ornamentation
- Cuticle ridge
- Striated cuticle
- Fissure between adjacent cells

Plate 6.3 Stoma of cotyledon.

- Flange
- Cylindrical plinth

Plate 6.4 Upper surface of unexpanded cotyledon.
Plate 6.2 Upper surface of seedling leaf
(a) Surface relief.

Plate 6.5 Surface of unexpanded seedling leaf
(b) Detail of wax plates.
wax tubes or plates which hold water away from the wettable cuticle. This condition occurs on the older seedling leaves of *E. sieberi* which do not become wet when floated on water or absorb water readily when immersed. On the other hand, wettable leaves, in general, have non-crystalline waxes and low micro-relief or portions of the cuticle exposed. The cotyledons of *E. sieberi* have large portions of the cuticle exposed, although there are scattered wax crystallites (Plate 6.1b) and they absorb water readily (Experiment 6.2).

The relief of the *E. sieberi* cotyledons may influence the retention of water droplets by holding the water by capillarity in the grooves of the cuticular ornamentation and in the fissures between adjacent cells (Plate 6.1b). Retention of water by cotyledons dried gently on tissue in Experiment 6.2 supports this contention, as does Plate 6.7 which shows light reflecting from water around the edges of the epidermal cells after the cotyledon had been shaken free of a water droplet. Thus the surface structure of the cotyledons, unlike that of the seedling leaves, is appropriate for retention of water and absorption through the exposed cuticle.
Plate 6.6 Retention of water droplets on E.sieberi seedling cotyledons (leaf surface almost vertical).

Plate 6.7 Water held in grooves above anticlinal walls of the epidermal cells of the cotyledons. (Bright reflections indicate water, lesser reflections indicate apices, no reflection indicates cuticle).

Plate 6.8 The effect of water stress on the orientation of seedling leaves.

LHS - Unstressed seedling.
RHS - Stressed seedling with leaves folded.
The cotyledons of *E. sieberi* appear to reflect light (Plate 6.7) from the angles and surfaces on the cuticular ornamentation on the top and sides of the papillae while absorbing it through the smooth cuticle surrounding the papillae. Cuticular ornamentation on eucalypt leaves is genetically determined (Carr et al., 1971) and there is some evidence that the provenances of *E. tereticornis* Sm that occur in drier habitats have more strongly developed papillae than those from more temperate habitats (Gibson, 1979). It is possible that the surface roughness on the papillae of *E. sieberi* cotyledons is a factor in reducing the heating of the cotyledons by reflecting insolation on the difficult, exposed seedbed.

Unexpanded cotyledons, including those prevented from expanding by high light intensity (Experiment 6.1) present the highly ornamented apices of the papillae to the sun (Plate 6.4), because the apices of adjacent papillae have not been separated by the spreading of the margins of the cells between them. The cuticle on the apices is more reflective than that on the sides of the papillae so the unexpanded cotyledons may be particularly well able to reflect insolation (Plate 6.7). If so, failure to expand in high light intensity conditions constitutes an adaptation of considerable value in the vulnerable stage of a seedling's life between germination and the establishment of the radicle.
6.4 GENERAL DISCUSSION

In the absence of comparative data on the structure and function of cotyledonary seedlings of eucalypts from habitats other than the dry sclerophyll forest it is not possible to relate the factors described in this chapter directly to the survival of *E. sieberi* seedlings. However, the structure of the cotyledons (6.3) appears to be consistent with, and to complement the seedling's response to light (6.1) and to precipitation (6.2). Together these three factors may enhance the seedling's chance of surviving the vulnerable period between germination and establishment. The possible significance of each factor is discussed below.

Production of juvenile anthocyanin and suppression of cell expansion may protect the young seedling from damage caused by high irradiance. Ultra violet light absorbed by a blue absorbing photo-receptor elicits a high irradiance response which includes the stimulation of anthocyanin synthesis (Gaba *et al.*, 1984). Anthocyanin may protect tissues from high irradiance damage because it absorbs strongly in the UV. Möhr and Drumm Herrel (1983) cite indirect evidence that it does so. They describe a correlation between the amount of UV penetrating the ozone layer at a given latitude, and the production of anthocyanin in seedlings native to exposed sites at that latitude. The *E. sieberi* seedlings may be protected from UV damage by anthocyanin and from heat damage by the highly ornamented, reflective cuticle on the papillae.
Hajibagheri et al. (1983) consider that absorption of water through plant cuticles is controlled by the distribution of impervious wax lamellae embedded in the hydrophylic cutin. The lamellae lie parallel to the surface of the cell and when the cuticle is dry and shrunken they overlap, preventing the passage of water in or out of the cell (Schrönherr, 1976). However, when the cuticle is wet it swells, pulling the overlapping lamellae apart and forming channels for water movement. The lamellae dip down along the contour of the cell where it adjoins another, forming a direct pathway along the lamellae and thus making the cuticle above cell junctions more permeable than that over the external walls (Martin and Juniper, 1970). Thus, by analogy the water collected in the deep fissures between the epidermal cells of *E. sieberi* cotyledons may have a direct pathway for entry into the tissue of the cotyledons. This may explain the readiness with which the cotyledons absorb water.

Kay et al., (1980) showed that light incident on the epidermis at 90° is refracted into flat epidermal cells while that incident at low angles is reflected. On the other hand light incident at 90° on a papillate surface is refracted into the apex and reflected from the sloping sides of each papilla, while light incident at low angles is refracted into the sloping sides. Therefore, it may be that light incident on the horizontal, papillate cotyledons of *E. sieberi* at 90° is reflected from the apices of the papillae by the cuticular ornamentation and from the sides of the papillae, so that the cotyledons are protected from the midday sun. Likewise, light incident at low angles
might be refracted into the cotyledons allowing them to photosynthesise at the ends of the day when the ambient water conditions are likely to be most favourable.

These features of *E. sieberi* seedlings are consistent with establishment in harsh conditions. Newly germinated seedlings remain undeveloped if exposed to high light intensity on emergence and produce anthocyanin which may protect the tissue from UV damage. If not exposed to high light intensity the cotyledons, and then the first seedling leaf pair, expand rapidly, but exposure causes formation of anthocyanin in both types of leaf and should the seedling water potential fall towards wilting point the seedling leaves fold up, exposing only the red underside to the sun (Plate 6.8). The plumule is protected inside the folded leaves, but the cotyledons are exposed to the sun and to precipitation. The seedling may recover from this folded position when the cotyledons absorb water from passing rain storms, fog or dew.
Should the plumule die or its development be delayed, the cotyledonary seedling may survive for many months, during which time the reflective surface of the cotyledons may enable the seedling to avoid heat stress, while the papillae enable it to refract light incident at low angles while the ambient water conditions allow photosynthesis.

Thus the rigid, horizontal cotyledons of *E. sieberi* may be specifically adapted for survival of the seedling in seedbeds subject to drying during its establishment.
CHAPTER 7
FACTORS AFFECTING GERMINATION

INTRODUCTION

Eighty percent of viable seeds germinated within a week of sowing on saturated clods of soil with rough surfaces, under conditions of high atmospheric relative humidity (Table 2.4). Further germination ceased when the clods were exposed to 70% R.H. and the soil matric potential fell below saturation. Twenty percent of seeds germinated on clods with smooth surfaces under the same wet conditions and no further seeds germinated after the clods were exposed to drier conditions. These observations suggested that the availability of water to the seed and its protection from loss due to evaporation are of primary importance for seed germination.

Seeds whose germination was stopped by removing the cover of the H.A. and exposing them to lower R.H. germinated within a day of rewetting (experiment 5.2) indicating that the embryo was not damaged by the sudden cessation of growth.

In this Chapter the water conditions necessary for germination of E. sieberi seeds and the response of seeds exposed to desiccation during germination are examined.
7.1 WATER CONDITIONS REQUIRED FOR GERMINATION

(i) Matric water potential

The effect of soil matric water potential on germination of seeds of *E. sieberi* species was examined.

**Materials and method**

Matric water potentials of -0.5, -1.0, -2.0, -3.0, -5.0 and -10.0 kPa were established in tensiometers made by attaching hanging columns of water to Büchner funnels with sintered glass plates of pore size 5-10 μm (Haines Apparatus, H.A.). Two H.A.s were established at each water potential.

Twenty black seeds of *E. sieberi*, varying in size from 1mm to 1x1x3mm, were sown directly onto the sintered glass plate of each H.A. and the funnel was covered with plastic film to maintain a saturated atmosphere. The experiment was carried out in a glasshouse at a temperature of 16-18°C.

The number of seeds germinated was counted daily and the experiment was terminated when most seeds had germinated. Ungerminated seeds were squashed to determine their viability and the percentage germination of viable seeds was calculated.
Results and discussion

All the seeds were viable and germination was complete after eight days. The time course of germination at 0, -1, -2 and -3 kPa matric potential was the same, 50% of the seeds germinating by day four. One sample of seeds at -5, and one at -10 kPa took a day longer to reach 50% germination. Thus a reduction in matric potential from 0 to -10 kPa did not significantly affect germination.

This result was inconsistent with that from the Clod Trial where many seeds failed to germinate at very high matric potentials on the smooth surfaced clods (Table 2.4). The inconsistency suggested that seeds on the clods may have been prevented from germinating by loss of water to the atmosphere in the less confined conditions of the containers compared with that in the H.As. in this experiment. This possibility was examined in the next experiment.
(ii) Matric water potential and relative humidity

Materials and method

Twelve different water potential treatments were established in Haines Apparatus by combining 3 relative humidities with 4 matric potentials. The matric potentials were -0.5, -2, -5 and -10 kPa. The atmospheric R.H.s in the H.A.s were >99% (H.A. covered with plastic film), 98% (H.A. partly covered) and 60% (ambient R.H. of the glasshouse, temperature 16-18°C, in which the experiment was conducted). The R.H. given for the open funnels is that of the air 1 cm above the plate. These funnels were not in equilibrium, and thus they represented the natural conditions under which seeds lying on the surface germinate.

Twenty seeds of E. sieberi were sown in each H.A. and germination was recorded daily. After 8 days the H.A.s at 98% and 60% R.H. were covered with plastic film to bring the R.H. to >99%.

Results and discussion

At the highest R.H. (>99%) germination at the two higher matric potentials (-0.5 and -2 kPa) commenced by day 4 and was virtually complete by day 5. At the lower matric potentials (-5 and -10 kPa) germination commenced by day 5 but was not complete until day 9 (Fig. 7.1 I). The final germination attained in all treatments at R.H. >99% was 100%.

At 98% R.H. germination at the two higher matric (-0.5 and -2 kPa) commenced by day 4 and germination ceased
Fig. 7.1 Effect of $\psi$ and R.H. on germination of *E. sieberi* seeds.
by day 5 or 6 (Fig. 7.1 II). In these treatments, however, the final germination percentages were only 60% and 80% respectively. Seed at -5 kPa commenced germination on day 5 and completed it on day 7 after only 25% had germinated, and no seeds at -10 kPa germinated.

At the lowest R.H. no seeds germinated (Fig. 7.1 IIIa). On day 8 after sowing all seeds exposed to R.H.s of 98 and 60% were covered to increase the atmospheric R.H. All seeds that had not germinated at R.H. 98% and matric potentials -0.5 and -2 kPa germinated within 3 days and those at -5 kPa within 6 days. Germination of seeds at -10 kPa commenced within 3 days after covering (Fig. 7.1 II b). Seeds exposed for 8 days at 60% R.H. at -0.5 and -2 kPa matric potentials commenced germination within 2 days of raising the R.H. and those at the lower matric potentials (-5 and -10 kPa) within 3 and 4 days respectively, (Fig. 7.1 IIIb).

Freshly sown seed took 4 days to commence germination in >99% R.H. but all the seeds that did not germinate at lower R.H.s took less than 4 days to commence germination after the R.H. was raised. Some seeds at the higher matric potentials (-0.5 and -2 kPa) germinated within a day. These results suggested that seed at low R.H. and low matric potential commenced the germination process and seeds at high matric potential almost completed germination even at low R.H.s. An increase in R.H. allowed the seed to continue from the point reached before the low R.H. stopped the germination process.
7.2 EFFECT OF DRYING SEEDS DURING THE GERMINATION PERIOD ON GERMINATION

Results from the previous experiment suggested that seeds may commence the germination process even under conditions (low R.H. and low matric potential) which prevent completion of the process. The seeds may then be able to pick up where they left off when conditions become more favourable for germination. This possibility was examined.

Materials and method

Five groups of 10 viable seeds of E. sieberi were sown on each of 5 H.A.s at a matric potential of -5 kPa. The H.A.s were set up in a laboratory under diffuse light at a temperature of 15-20°C and were covered with plastic film to raise the R.H. One H.A. remained covered throughout whilst the plastic film was removed from one of the other four after 30, 42, 54 and 66 hours respectively. Removal of the cover exposed the seeds to drier air (R.H. 70%) to prevent the seed from germinating.

When 50% of the seed in the covered control H.A. had germinated the other H.A.s were closed again to raise the R.H. The number of seeds germinated was recorded every twelve hours.

Results and discussion

Fifty percent of the freshly sown seeds had germinated after 120 hours in the covered H.A. at -5 kPa matric potential (Fig. 7.2). However, seeds which had been wetted
Fig. 7.2 Conservation of progress towards germination
for 30 h in a closed H.A. and then allowed to dry at 70% R.H. for 90 h reached 50% germination after 42 h of 'rewetting' (i.e. after the H.A. was closed again). After 54 h 'prewetting' followed by drying, 50% of seeds germinated after 21 h of 'rewetting'. The longer the period of 'prewetting', the faster the seeds germinated after 'rewetting'.

The average time taken for the first seed in each of the 5 groups in each treatment to germinate, (time to initial germination, Fig. 7.2) became progressively closer to the time required for 50% to germinate as the length of the 'prewetting' period increased. This suggested that the germinative energy of the seed population was increased by the wet period.

Thus, seed which was wetted for too short a period to complete germination picked up where it left off, or conserved the progress it had made towards germination and appeared to have greater germination energy when rewetted. The increase in vigour was examined further.

Materials and method

Ten groups of 10 seeds were placed in each of 10 H.A.s at -5 kPa matric potential and the funnels were closed with plastic film to raise the R.H. Seeds in one funnel were left undisturbed. Seeds in the second funnel were removed after 3 h and placed on dry filter paper in an open Petri dish. Seeds in the 8 succeeding funnels were removed and air dried after 6, 12, 18, 30, 42, 54, 66 and 78 h respectively.
Five of the 10 groups of 10 seeds removed from a funnel, were replaced in their original position after 24 h and the funnel was covered. The other 5 groups were replaced after 120 h. Thus there were 20 treatments comprised of 10 time periods of initial wetting ('prewetting') and two lengths of time of air drying. Five groups of 10 seeds were assigned to each treatment.

The number of seeds germinated in each group of 10 was counted every 6 h until most had germinated, then twelve hourly.

**Results and discussion**

Germinative energy, or vigour, is measured by the times to germination of the seeds, with the more vigorous seeds germinating in a shorter time period. A proportional hazards regression model for survival analysis developed by Cox (1972), the 'Cox model', is the most appropriate analysis for the results from this experiment (R.B. Cunningham, pers. comm.). The model requires the assumption that the risk of germination for a given treatment, relative to some standard value, is constant at all times (proportional hazards), and therefore that the treatments have some common underlying distribution of times to germination. The assumption is valid for this experiment because almost all the seed was viable and able to germinate, in time. The statistical model is fully described in Cunningham, et al (1981) but the essence of it is that the conditional probability that a seed will
germinate at a particular time can be regarded as being binomially distributed, so the model belongs to the class of Generalized Linear Models which can be fitted by using the computer program GENSTAT (Aitkin, 1980). The analysis of deviance table, Table 7.2, provides information on the effects of the treatments.

Table 7.2 Summary of analysis of deviance for times to germination of *E. sieberi* seed

<table>
<thead>
<tr>
<th>Model terms</th>
<th>d.f.</th>
<th>Residual deviance</th>
<th>Change of deviance</th>
<th>d.f.</th>
<th>Mean change</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>787</td>
<td>1230.388</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time air dry</td>
<td>786</td>
<td>1230.327</td>
<td>0.061</td>
<td>1</td>
<td>0.061</td>
<td>n.s.*</td>
</tr>
<tr>
<td>Time prewetted</td>
<td>777</td>
<td>933</td>
<td>296.955</td>
<td>9</td>
<td>32.995</td>
<td>s.</td>
</tr>
<tr>
<td>Interaction</td>
<td>768</td>
<td>918</td>
<td>15.297</td>
<td>9</td>
<td>1.700</td>
<td>n.s.†</td>
</tr>
</tbody>
</table>

*χ², for significance = 3.84  
†χ², for significance = 16.92

The length of the air dry period, 24 or 120 h, did not affect the vigour of germination, and there was no interaction between drying and the length of time 'prewetted'. The length of time 'prewetted' was significant and the plot of Cox constants which measure the times to germination shows that the longer the time 'prewetted' the shorter the time to germination (Fig. 7.3). A long wet period increased the vigour of the seed relative to that of seed germinating after a short wet period.
The effect of drying and rewetting on
Time to germination

- 24 h. dry
- 120 h. dry

Length of time "prewetted" in hours

Fig. 7.3 Effect of length of dry period on germination

The effect of "prewetting" on the germination of E. sieberi seeds

Hours from initial germination

Fig. 7.4 Effect of drying and rewetting on vigour of germination
Figure 7.4 shows all the germination curves fitted by the model after combining the data from 24 and 120 h dry periods. Each curve starts at the time the first seed germinated in the treatment, so this figure shows the effect of 'prewetting' on vigour but not on time to initial germination. The vigour of germination in seeds dried after 30, 42 and 54 h of 'prewetting' was similar, although overall the trend was for increasing length of 'prewetting' to increase vigour. This anomaly also occurred in a preliminary experiment and it suggested that seed received a setback if drying occurred in the middle of its progress towards germination.

Hypocotyls from seeds 'prewetted' for 18 and 66 h were examined using a scanning electron microscope after freeze drying and coating in gold. Early in germination both the hypocotyl and the coleorhiza had thin walls which caused the cells to collapse on freeze drying (Plate 7.1a) but as the seeds progressed further towards germination the hypocotyl cells retained their integrity while the coleorhiza cells collapsed (Plate 7.1b). The junction between hypocotyl and coleorhiza is distinct at the collet suggesting that the solidification of cuticle on the hypocotyl cells made them sufficiently rigid to resist collapse on freeze drying. (The presence or absence of cuticle distinguishes the aerial hypocotyl from parts that remain underground). While seeds are in the transitional stage they may suffer some damage during drying and thus show less vigour than expected when 'rewetted'.
(a) Early in 'priming', 18 h after sowing.

(b) Late in 'priming', 66 h after sowing.

(c) At germination, 72 h after sowing.

Plate 7.1 Surface structure of *E. sieberi* hypocotyl during 'priming' and at germination.
Figure 7.5 shows representative germination curves fitted by the model. Each curve starts at the time the first seed in each treatment germinated after replacement on the H.A. plate, or placement on the plate in the case of the control seeds which were not wetted then dried and rewetted. Therefore the figure shows both the vigour of the seeds and the advance in time to initial germination.

The first of the control (no 'prewetting') seeds germinated at 60 h, 50% germinated in 136 h and germination was complete at 192 h. The first of the seeds 'prewetted' for 3 h, germinated in 48 h, 50% in 120 h and all in 100 h. Thus the seed gained 12 h after being wet for 3 h. When wet for 6 h the first seed to germinate gained 18 h, 50% germinated in 102 h and germination was virtually complete in 148 h. (Seventy-five percent of seeds 'prewetted' for 24 h germinated within 120 h (5 days) of 'rewetting' whereas 170 h (7 days) were required for that proportion to germinate if not 'prewetted'. This shortening of the germination period by 'prewetting' was used to facilitate comparison between treatments throughout this project).

Shortening the germination period has significance for seed germinating in the field. If the seedbed dries before it germinates or, in the context of this study, if the humidity above exposed seeds drops below 98%, seed will respond quickly to rewetting and gain sufficient time to germinate before the seedbed again dries. Moreover, seeds may progress towards germination in successive short wet periods, which is particularly advantageous for *E. sieberi* germinating on seedbeds with an intermittent
The effect of "prewetting" on germination when rewetted

Fig. 7.5

The effect of "prewetting" on the germination of seeds after 8 months

Fig. 7.6
water supply.

The course of germination of 'prewetted' seed after an 8 month storage period was examined.

Materials and method

'Prewetting' treatments were imposed on seeds in H.A.s as before, using 'prewetting' times of 3, 6, 12, 18, 24, 36, 48, 60 and 72 h. Forty seeds were subject to each treatment. After 'prewetting' the seeds were stored in open glass vials in a laboratory with a temperature fluctuating diurnally between 13 and 20°C and a R.H. of approximately 70% for 8 months. The seeds were 'rewetted' as before and the course of germination was determined from the 4 groups of 10 seeds in each treatment.

Results and discussion

The increase in vigour of 'prewetted' seeds was maintained for 8 months (see Fig. 7.6 for representative curves), although the times to initial germination in each treatment were delayed by 12 to 30 h in 60 and 3 h 'prewetting' treatments respectively, when compared with seed rewetted within 5 days of drying.

Seed 'prewetted' for 3 h germinated between 80 and 170 h after rewetting, while seed 'prewetted' for lengths of time between 18 and 48 h (24 h shown) germinated between 60 and 120 h. Seed 'prewetted' for 60 and 72 h germinated earlier (after 48 h), at 48 h, than seed 'prewetted' for 18 to 48 h, but took the same time (60 h) between initial and final germination. Thus, seed surviving an extended dry period after 'prewetting' for sufficient time to complete the formation
of cuticle on the hypocotyl would start to germinate at much the same time as fresh seed (60-70 h) but reach 50% germination in 100 h while the fresh seed would take 130 h, and seed ready to germinate when dried would germinate 12 h earlier than fresh seed and reach 50% germination after 72 h.

Seeds surviving a long dry period may become established in the first wet period even if it is too short to allow fresh seed to germinate and only 'prewets' them, in preparation for the next wet period.

7.3 GENERAL DISCUSSION

The water conditions necessary for germination of *E. sieberi* on a surface, are high matric water potential and an atmospheric R.H. of >98%, but these conditions need not persist unchanged from the initial imbibition to germination. The seeds are able to progress towards germination during short wet, 'prewetting' periods and conserve that progress, with undiminished vitality, through dry periods as long as 8 months, germinating more vigorously than fresh seed when rewetted.

The process of increasing the vigour of seed populations so that they germinate in a relatively short period is used in agriculture to overcome limits to establishment (Heydecker, 1973). Vigour is increased by allowing seeds to imbibe sufficient water for the germination process to start but insufficient for it to go to completion. Water supply to the seed is limited osmotically by soaking it in
salt or polyethylene glycol solutions) or by calculating the quantity of water needed for full imbibition and supplying a lesser amount to the seed. The process is referred to as 'priming'. Osmotically 'primed' soy bean seed will germinate in an unseasonably early warm period, gain time in the vegetative period and yield more grain than 'unprimed' plants while the growing season is short (Knyp1, 1983).

Rye grass seed 'primed' by wetting in a restricted amount of water germinates in half the time taken by 'unprimed' seed, allowing pastures to be established where seedbeds remain wet for too short a period for 'unprimed' seed to germinate (Lush and Groves, 1981). The coleorhiza and the coleoptile of 'primed' rye grass seed protrudes due to enlargement of the cells, but there is no growth by cell division (Lush, et al., 1981). Seedlings which progress past cell enlargement to growth during the 'priming' period are unable to continue normally when rewetted after drying; the radicle rehydrates but does not grow (cf. Salsola, Chapter 5), and secondary roots arise from the nodes of the shoot. Thus, establishment proceeds in much the same way as in E. sieberi where desiccation prevents radicle growth, and adventitious roots arise from the hypocotyl.

Watt (1978) showed that Queensland blue grass was 'primed' at low soil water potentials by enlargement and protrusion of the coleorhiza without cell division and Lush and Groves (1984) found 'natural priming' in Clematis and related it to lengthening of the embryo axis by cell enlargement. In E. sieberi it is related to lengthening of
the collet end of the hypocotyl and to formation of the cuticle on the hypocotyl.

*E. sieberi* 'primes' in the sense that any progress made towards germination in successive wet periods is conserved through dry periods, a process referred to as STOP GO germination by Groves (pers. comm.). This process differs from conventional 'priming' in which development proceeds to a specific point, ie the end of cell enlargement, when there is insufficient water to complete germination (Hegarty, 1978). In such conventional 'priming' all seeds germinate together, and all seedlings may die if seedbed conditions deteriorate before establishment. In STOP-GO priming some seeds are ready to germinate on rewetting but others are less advanced and germinate in the following wet period.

'STOP-GO priming', shown here for *E. sieberi*, is appropriate for seeds germinating on seedbeds with intermittent water supply. Effective 'STOP-GO priming' depends on three factors. Firstly the seeds must GO in, or use, short wet periods, which requires that they imbibe readily. Bachelard (1985) has shown that *E. sieberi* germinates at lower soil matric potentials than other eucalypts in its habitat, and ascribes this to the micro-relief of the seed coat (Plate 8.2) which may allow better seed-soil contact. *E. sieberi* seed was less affected by low matric potentials on sintered glass plates than the other species tested in experiment 7.1, supporting this conclusion. Secondly, the seed must STOP when a change in the ambient conditions
presages an approaching dry period. The change sensed is a reduction in R.H. below 98% (experiment 7.1) and the biology of the response to it is examined in Chapter 8. Thirdly, the seed must conserve progress made during a 'GO' period, through the 'STOP' period. This has been demonstrated in experiments 5.2, 7.1 and 7.2, and appears to be due to the tolerance of the hypocotyl, the only part of the embryo to extend during germination, to desiccation. The extension of the hypocotyl is examined further in Chapter 8.
INTRODUCTION

The capacity of *E. sieberi* seed for 'stop-go' germination depends on a rapid response to changes in the ambient water conditions and on the tolerance of the embryo to desiccation during germination. Response to a lowering of the atmospheric relative humidity enables the seed to 'stop' germination when rainfall ceases, even though the soil water potential remains high, thus avoiding germination on a seedbed likely to dry before the seedling is established. Response to an increase in the relative humidity enables the seed to 'go' when rainfall resumes and thus allows the seedling to establish before the seedbed dries again.

The water relations of the germinating seed and their bearing on 'stop-go' germination are described in this Chapter.
8.1 CONTROL OF GERMINATION AFTER 'PRIMING'

Germination of seeds on sintered glass plates, (experiment 7.1(ii)) depended on an interaction between relative humidity and matric water potential. However, all seeds at matric water potentials down to -10 kPa germinated if the atmosphere was saturated while some at -0.5 kPa failed to germinate at 98% R.H. although they were 'primed', suggesting that germination occurs only if the R.H. is very high. These experiments examined the control of germination in 'primed' seeds.

(i) Relative humidity

Materials and method

Five rows of 20 seeds of E. sieberi were sown on wet filter paper folded over a 10 cm² styrofoam block floating on water in a 3 l plastic container. The paper dipped into the water and remained at 0 kPa matric potential throughout the experiment. Four of the rows on each of two replicates were covered with wet filter paper to saturate the atmosphere surrounding the seeds. The fifth row remained uncovered and the blocks were placed in a growth cabinet at 95% R.H. and 22°C. After 1 day the wet strip was removed from one row of seeds and the second and third strips were removed on the second and third days to expose the seed to 95% R.H. The last strip was removed as germination began on day 4.

Germination was recorded for 14 days, after which the containers were closed to raise the R.H.
Results and discussion

Few seeds germinated in the first 14 days (Table 8.1) but all germinated within 2 days of raising the R.H. showing that they had 'primed' during the first 14 days of the experiment.

Table 8.1 Germination of seeds at 95% atmospheric R.H.

<table>
<thead>
<tr>
<th>Length of time at 95% R.H.</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>at &gt;99% R.H.</td>
<td>Block 1</td>
</tr>
<tr>
<td></td>
<td>14 day</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

24 48 72 96

Hours from sowing

During the first 14 days the only seeds to germinate were large, long seeds (1 x 1 x 3 mm) which occurred at random amongst smaller cubic seeds (1 mm³). All long seeds germinated, so the percentage germination in all treatments at 14 days indicates the proportion of long seeds in each sample (Plate 8.1).

These observations indicate that embryos within long seeds retained sufficient water to complete germination at 0 kPa and 95% R.H. while those within cubic seeds did not.
Plate 8.1 Variation in size of *E. sieberi* seeds

a) Long seed lying on flat side: good soil/surface contact
b) Cubic seed lying on curved dorsal surface: poor contact
c) Cubic seed lying on ridged ventral surface: poor contact
The most likely reason for longer seed germinating is that its contact with the water source enables it to replace water lost to the atmosphere readily. Several factors may contribute to the advantage of the long seed. The shape of long seed (1x1x3mm) indicates that 3mm² of the total surface area of 14mm is in contact with the wet surface, a ratio of 4.7:1, exposed area : contact area. The cubic seed has a ratio of 6:1 exposed area : contact area, ie it has relatively less area in contact with the wet substrate. In addition the curvature of the surfaces of cubic seeds is greater than that of the long faces on which the long seeds lie, so that the ratio of exposed area to contact area is wider than the theoretical ratio given above, which assumes that the faces are flat. The micro-relief of the surface of long seeds also differs from that of cubic seeds in that the greater proportion of the surface of long seeds has longitudinal channels (ca. 30 μm wide) whereas the cubic seeds have a higher proportion of tubercles (Bachelard, 1985). The shape of the long seeds ensures they would generally present the channels to surface soil water which may enable them to maintain a continuous film of water over the contact surface better than the cubic seeds.

Germination was preceded by formation of a large drop of water, stained yellow-green by compounds leached from the outer integument, on the micropylar end of the seed. Droplets did not form on cubic seeds until the R.H. was raised to enable germination to occur. It was noted subsequently that the formation of a water drop always precedes germination.

The experiment demonstrated that completion of germination beyond the 'primed' stage depends on the
Plate 8.2 Surface structure of *E. sieberi* seed.
(Micrographs by courtesy of E.P. Bachelard).

Plate 8.3 Structure of the inner integument of an *E. sieberi* seed.
capacity of the seed to retain more water than that required for 'priming'. Water is withheld from the seed by the matric potential and the osmotic potential of the substrate. The effect of the former was examined in experiment 7.1 and that of the latter is examined below.

(ii) Osmotic potential

**Materials and method**

Five lines of 20 seeds (ie 100 seeds) were sown in Petri dishes on filter paper wetted by osmotic solutions over the range 0 to -7,000 kPa NaCl determined by molality of the solution and 0 to -4,000 kPa Dextran, determined by psychrometer measurements. The Petri dish lids were sealed to the bottoms with cellophane and the dishes were placed in a 20°C constant temperature room under fluorescent light. Seed germination was checked daily. At completion of germination in the 0 kPa treatment (7 days after sowing), ungerminated seeds at the low osmotic potential treatments were washed three times in distilled water and placed in covered H.A.s established at -0.5 kPa matric potential to determine whether the seed had 'primed'.

**Results and discussion**

No significant differences were observed between the 5 replicate rows of 20 seeds in any one treatment and the results are summarized in Tables 8.2 and 8.3.

The course of germination in NaCl solution at -5 kPa osmotic potential was the same as at 0 kPa (Table 8.2) but
Table 8.2 % Germination of E. sieberi seed on molal salt solutions

<table>
<thead>
<tr>
<th>Osmotic Potential -kPa</th>
<th>Molality NaCl</th>
<th>Days from sowing on osmotica</th>
<th>Days from sowing after removal from osmotica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>10 25 80 95 100</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>10 20 85 98 98</td>
<td></td>
</tr>
<tr>
<td>2200</td>
<td>0.5</td>
<td>0 0 0 0 0 0</td>
<td>38 83 94 94 98</td>
</tr>
<tr>
<td>4500</td>
<td>1.0</td>
<td>0 0 0 0 0 0</td>
<td>0 27 61 87 100</td>
</tr>
<tr>
<td>7000</td>
<td>1.5</td>
<td>0 0 0 0 0 0</td>
<td>0 25 66 85 98</td>
</tr>
</tbody>
</table>

Germination was prevented at osmotic potentials of -2200 kPa and less. Seeds exposed to -2200 kPa 'primed', 40% germinating on day 2 after washing and placement in the H.A., and 94% germinating by day 4. Seeds at -4500 and -7000 kPa also 'primed' but to a lesser extent with 30% of the seeds germinating on day 3 and all by day 6.

Table 8.3 % Germination of E. sieberi seed on Dextran solutions

<table>
<thead>
<tr>
<th>O.P. Dextran -kPa</th>
<th>Days from sowing on osmotica</th>
<th>Days from sowing after removal from osmotica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>0</td>
<td>10 25 80 95 100</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7 20 83 97 97</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10 31 87 92 100</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>5 22 80 93 98</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>0 0 0 10 15 87 100</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td>0 0 0 0 0 0 0</td>
<td>0 0 23 75 90 95</td>
</tr>
</tbody>
</table>

Results from the Dextran solutions are similar to those for NaCl. Germination was unaffected by osmotic...
potentials down to -1000 kPa and at -2000 kPa some seeds germinated slowly. All seeds 'primed' completely over the 7 day period for all germinated within a day of removal from Dextran. Seeds at -4000 kPa commenced 'priming', and germination occurred more rapidly after removal from Dextran than in the controls.

It was of interest to determine whether all seed would germinate at -2000 kPa if given more than 7 days since all the seed was 'primed' by then. In an experiment using 3 lines of 20 seeds in each of two replicates under the same conditions as before, 20% of the seeds germinated within 15 days but no further germination occurred in the following twelve weeks. Thus an osmotic potential of -2000 kPa prevented rather than delayed germination.

The relative humidity above solutions of osmotic potential -2000 kPa is about 98.5%, the critical level for germination of exposed seeds. These results support the conclusion that germination occurs only if the R.H. is sufficiently high to restrict loss of water gained from the substrate, while 'priming' occurs at lower levels of available water.

Although seeds 'primed' at -2200 kPa osmotic potential, and a few seeds germinated, growth of seedlings at this osmotic potential did not develop beyond the early post-germinative stage. Development ceased before the radicle produced root hairs or the cotyledons expanded. Positive geotropic growth of the hypocotyl carried the radicle to the surface and the coleorhiza expanded but its diameter
was greatly reduced. The hypocotyl straightened to raise the cotyledons but did not extend in length so the seedling was stunted in appearance. Thus seeds best able to obtain water may germinate in conditions that do not support establishment, but the seedlings do not reach the vulnerable stage between the onset of active growth of the radicle and its establishment in the soil (experiment 5.2) and many recover from desiccation.

These results are consistent with the conclusion that the germination of 'stop-go primed' seeds exposed to the atmosphere is stopped when rain ceases and the relative humidity falls below 98%, even though the soil water potential is unchanged, because the seed is unable to retain sufficient water for hypocotyl extension.

8.2 CONTROL OF PRIMING

Germination of *E. sieberi* seeds is controlled by the interaction between matric water potential and atmospheric relative humidity and occurs only in very wet surroundings, but 'priming' occurs at lower levels of available water. The extent to which 'priming' progresses depends on the availability of water. In this experiment the effect of the level of available water on 'priming' was examined.
Materials and method

The experiment was established in Haines Apparatus (H.A.) at three matric potentials, -2, -5 and -10 kPa, and two atmospheric relative humidities, >99% in closed H.A.s and ca 70% in open H.A.s. The matric potentials used were known to influence the percentage germination (experiment 7.1) and the lower R.H. was known to prevent germination (experiment 8.1).

Eight groups of 10 seeds of *E. sieberi* were sown in each of 6 H.A.s, one of which was open and one closed at each matric potential, and exposed to natural diffuse light in a laboratory with a temperature range of 13-18°C.

After 12, 24, 36, 48 and 72 h each group of seeds was removed from the H.A., weighed and returned to its original place. This procedure took approximately 3 minutes. After 96 h four of the eight groups in each treatment were replaced in the original H.A. but the other four groups were transferred to the alternate R.H. at the same matric potential.

The number of seeds germinated in each group was counted from the onset of germination at 96 h and all funnels were closed after 108 h.

Results and discussion

All seeds reached a water content of about 30% or more, expressed as a percentage of oven dry weight, within 12 h of sowing (Fig. 8.1). Seed in high R.H. reached 50-60% by 36 h, while seed in low R.H. reached only 40%. After reaching a
maximum, the water content of seeds in high R.H. dropped rapidly between 36 and 48 h but stabilized at 30% (-10 kPa) or above. Seeds in low R.H. dropped below 30% and continued to fall with time. The fall in water content occurred at the time the seeds produced a sticky mucous and may reflect a change in the seed-surface contact with the plate of the H.A. as well as loss of water in the mucous, which remained on the forceps used to move the seeds for weighing. The water supply in high R.H. treatments may have been sufficient for seeds to regain their water content, despite disruption and drying of the mucous layer between weighings, while that on the low R.H. treatments may have been insufficient. Some support for this explanation is provided by the large standard errors for low R.H. treatments after 36 h. They may be due to some seeds having made good contact on replacement in the H.A. and others poor contact. Bachelard (1985) has suggested that the mucous coat affects seed water relations in other eucalypts.

In seeds transferred from high to low R.H. at 96 h the water content dropped from 30-40% to 10-20% within 12 h (Fig. 8.2). Water content of seeds transferred from low to high R.H. at 96 h rose from 10 to 20-25% - a slower rate of increase than in the initial 12 h imbibition period at high R.H. (Fig. 8.1). Water content of seeds remaining in the original R.H. did not change between 96 and 108 h although seeds in the high R.H. treatments started to germinate between 72 and 96 h (Table 8.4).
Fig. 8.1 Effect of \( \psi \) and R.H. on water content of germinating seeds

Fig. 8.2 Effect of change in R.H. on water content of germinating seeds
Fifty percent of seeds in high R.H. had germinated before transference to low R.H. at 96 h. No further seeds germinated during the 12 h at low R.H. while the percentage germination of the seeds remaining at high R.H. increased by about 5% during that period. There was no difference in percentage germination due to matric potential in this experiment, in contrast to experiment 7.1, where the R.H. was lower and the temperature higher. However the water content of the seeds at -10 kPa is lower than the other two treatments at high R.H. but still sufficient to permit germination. No germination occurred in the 12 h following transfer of seeds from low R.H. to high R.H. and the water content of the seeds did not reach 30% in that time (Fig. 8.2).

Table 8.4 Effect of low R.H. on germination

<table>
<thead>
<tr>
<th>Matric potential (kPa)</th>
<th>% germination</th>
<th>R.H.</th>
<th>Hours from sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>108</td>
</tr>
<tr>
<td>-2</td>
<td>High throughout</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>High → low (96 h)</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Low throughout</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-5</td>
<td>High throughout</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>High → Low (96 h)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Low throughout</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-10</td>
<td>High throughout</td>
<td>55</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>High → low (96 h)</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Low throughout</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Since matric potential did not affect germination the counts for all seeds in a particular R.H. regime were
combined, and Fig. 8.3 shows that seed maintained at high R.H. (>99%) throughout took 192 h to complete germination. Ungerminated seeds maintained at high R.H. for 96 h, dried for 12 h and then rewetted by closing the H.A., started to germinate within 6 h of rewetting, 50% germinated within 12 h, and germination was complete in 84 h from rewetting, showing that it had been 'primed' by the 96 h of 'prewetting'. Seeds maintained at 70% R.H. for 96 h before wetting had not germinated (Table 8.4), but they commenced germination 24 h after wetting. However, it took 78 h for 50% to germinate and germination was complete after 134 h. This seed was partially 'primed' and showed a similar increase in vigour to that observed with seed maintained at >99% R.H. for 48 h in the priming experiment, 7.2. Thus, seed in 70% R.H. 'primed' for 48 h, the length of time the water content remained at or above about 30% (Fig. 8.1).

These results indicate that *E. sieberi* seeds 'prime' if the water content remains above about 30%, but do not germinate unless the R.H. is about 99%.

The effect of matric water potential on seed water content and 'priming' was tested further, at -17 kPa matric potential, under the same conditions of temperature (13-18°C) and light. Eight groups of 10 seeds were maintained in high R.H. conditions in this experiment for comparison with seeds at -10 kPa in the previous experiment. Seeds exposed to -10 kPa matric potential reached 30% water content within 12 h, and the first seed germinated
Fig. 8.3 Effect of low R.H. on 'priming'

Fig. 8.4 Effect of low matric water potential on seed water content
60 h later (Fig. 8.4). Seed exposed to -17 kPa matric potential took 48 h to reach 30% water content and the first seed germinated 60 h later. These results confirm that a seed water content of above about 30% is needed for the seed to 'prime' (ie progress towards germination) and its progress towards germination depends on whether atmospheric conditions allow it to maintain that water content.

In the context of 'stop-go' 'priming' the seed 'goes' when its water content is above about 30%.

8.3 INTERNAL WATER RELATIONS OF 'PRIMING' EMBRYO

Two points of interest arose from the study of control of germination (8.1) and of 'priming' (8.2). 'Priming' occurred at a seed water content of about 30%, which is unusually low (McDonough, 1975). Germination also occurred at a seed water content of about 30%, there being no significant increase in seed weight during germination (Fig. 8.1), but a high R.H. was required.

These features of the germination of *E. sieberi* seeds were examined further by studying the water relations of the embryo in the experiments described below.

(i) Water potential

**Materials and method**

Large seeds of *E. sieberi* were placed to germinate on wet filter paper in closed Petri dishes at 20°C and on the sintered glass plates of closed H.A.s established at -5 and -15 kPa matric potential at 13-18°C. At varying periods
after sowing the water potential of whole seeds at 0 and 
-5 kPa and of the hypocotyl of excised embryos at all 
three matric potentials were measured in a Wescor Thermo-
couple psychrometer. Large seeds were used to reduce 
variation in time to germination since all germinate on 
days 3 or 4 after sowing, and to reduce variation in the 
dimensions of the hypocotyls all of which were about 1.5 mm 
long and 0.5 mm in diameter when dry. Three hypocotyls 
were used for each determination of water potential. They 
were protected from desiccation during excision by the 
cotyledons around them and were transferred to the psych­
rometer chamber within 30 s of exposure to the atmosphere 
at 18°C and 70% R.H.

Results and discussion

The water potential of whole seeds stabilized within 
24 h at -0.6 MPa (Fig. 8.5) and water was readily expressed 
from them. (The high water potential of the seed was due 
to water in the seed coat under the high R.H. conditions of 
this experiment). The water potential of hypocotyls rose 
rapidly to about -3 MPa, then fell and stabilized at -4.5 
MPa until germination (72 h), when it rose to -1.0 MPa. 
Sap could not be expressed from the hypocotyl until it 
extended after germination and reached the high water 
potential. The embryos at -15 kPa matric potential were 
slower to reach the high water potential after imbibition, 
but germinated at the same water potential as those at -0 
and -5 kPa. The water potential rose rapidly from -4.5 to 
-1.0 MPa during germination. The hypocotyls slipped
Fig. 8.5 Water potential of seed and embryo during 'priming' and germination
Fig. 8.6 Water relations of the germinating embryo
readily from the cotyledons at this stage whereas they had not previously. This observation suggested that the turgor of the hypocotyl rose at germination, relative to that during 'priming'.

(ii) Osmotic potential

In this experiment the water status of the hypocotyl as it emerged through the seed coat was examined and compared with that of the cotyledons, which appeared less turgid than the hypocotyl.

Materials and method

Seed was sown in Petri dishes at 0 kPa matric potential as in experiment (i) but sowing was staggered in time to allow thirty determinations of osmotic potential at the time the water potential rose from -4.5 kPa ('priming') to -1.0 kPa (germination).

Three hypocotyls, excised as before, or one cotyledon, were frozen in liquid nitrogen in a fine nylon mesh bag for 60 s before enclosing in a psychrometer chamber to thaw and equilibrate for 40 min for measurement of water potential. Freezing destroys cell structures, releasing the cell sap, so the water potential measurement is a direct measurement of osmotic potential of the sap.

Results and discussion

There was no significant difference between the water potential and the osmotic potential of the hypocotyl until after germination at 72 h (Fig. 8.6b) and of the cotyledons until after they were raised at 110 h (Fig. 8.6c). Thus
the turgor potential of the whole embryo did not alter during germination suggesting that the swelling of the collet that breaks the seed coat is localized (Fig. 8.6c).

An interpretation of these results is that the osmotic potential of the localized cells responsible for breaking the seed coat decreased at the end of 'priming' (72 h) sufficiently to extract water from the surrounding cells and expand. The change in osmotic potential presumably could not be detected in whole hypocotyls as it was confined to a small section of the hypocotyl tissue. After germination in the wet conditions of this experiment water potential and osmotic potential in the hypocotyl rose and a turgor potential of 0.5 MPa developed in the cells. These cells expanded, carried the radicle to the surface and formed the 'clinging disc'.

Turgor potentials developed in the cotyledons after they were raised at 110 h and then increased as they expanded. A subsequent fall in turgor potential in the cotyledons stabilized them at the same level (0.5 MPa) as in the hypocotyl.

These results suggest that the low water content of 'priming' E. sieberi seeds is due to the low water potential maintained by the embryo even when the seed coat is saturated. They show that in high R.H. conditions the hypocotyl is able to break the seed coat at the end of 'priming' without setting up a water potential gradient between the absorbing cotyledons and the substrate, to supply the expanding hypocotyl. The significance of this
feature of the germination of *E. sieberi* is discussed in Chapter 9 and the mechanism for it in the next section.

8.4 THE MECHANISM OF GERMINATION

(i) Inner integument

Results presented in experiment 8.3 were interpreted in terms of germination occurring when a localized group of cells overcame a restraint to water absorption imposed by a force other than full turgor in the cells of the embryo. The seed coat of *E. sieberi* consists of two separate integuments (Gauba and Pryor, 1958), namely a thick, black outer integument which surrounds the seed loosely, and a thin, heavily suberized, inner integument which envelops the embryo so tightly that it is difficult to remove without damaging the embryo. In this experiment the role of the inner integument in restricting entry of water into the embryo is examined.

**Materials and method**

Seeds of *E. sieberi* were soaked overnight in order to soften and swell the outer integument and so facilitate removal of the embryo in the inner integument from the outer integument. One hundred and sixty embryos were removed from the outer integument and were then placed to germinate on wet filter paper in closed Petri dishes at 20°C for 48 h, ie until all seeds were in the 'priming' phase.
Twenty embryos, within the inner integument were blotted dry, weighed individually and replaced in the Petri dish. The inner integuments were removed from 20 embryos and these 'peeled' embryos were weighed individually and replaced in the Petri dish. After 18 h while the seeds were still in the 'priming' period, the embryos were reweighed to determine whether removal of the inner integument, 'peeling', affected the water content. The weighed embryos were then returned to the Petri dish to germinate.

Sixty of the remaining 120 embryos were 'peeled' and 60 were left in the inner integuments and the water potential of these embryos was measured from 3 hypocotyl samples as before, 20 h after peeling, during, and after germination.

Embryos damaged during removal of the inner integument ('split' embryos) were allowed to germinate.

Results and discussion

The average weight of 'peeled' embryos increased by 0.43 mg after 18 h in water after the integument was removed, while that of the 'unpeeled' embryos did not change (Fig. 8.7). In both cases the slope of the regression and the regression coefficient approached 1.

There was no change in the water potential of 'unpeeled' embryos, but that of 'peeled' embryos rose to -1.3 MPa (Table 8.5). Embryos at -1.3 MPa were visibly swollen and those inside split inner integuments protruded
Effect of "peeling" on fresh weight of embryo.

Fig. 8.7 Effect of removing the inner integument on seed weight.
from the split. These results indicate that the inner integument prevents water absorption and maintains the water potential of the 'priming' seed at -4.5 MPa.

Table 8.5 Water potential of excised embryos of *E. sieberi* during germination

<table>
<thead>
<tr>
<th>Embryo excised</th>
<th>Water potential in MPa and SE×</th>
<th>Pregeneration</th>
<th>Germination</th>
<th>Germinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ inner integument</td>
<td>-4.5±0.06</td>
<td>-4.5±0.05</td>
<td>-1.3±0.04</td>
<td></td>
</tr>
<tr>
<td>- inner integument</td>
<td>-1.2±0.07</td>
<td>-1.3±0.03</td>
<td>-1.2±0.06</td>
<td></td>
</tr>
</tbody>
</table>

When 'unpeeled' embryos germinate the inner integument tears in random directions away from the swelling hypocotyl, rather than splitting precisely across the top of the collet as it does in intact seeds. This indicates that germination in intact seeds occurs when a localized swelling in the collet presses the inner integument against the resistant outer integument, splitting both, and allowing water to enter the embryo.

Observation of the germinating embryos showed that hypocotyls emerging from 'unpeeled' embryos were broader and emerged later than those emerging from intact seed (Table 8.6). This suggested that water was supplied to the hypocotyl inside the inner integument of the excised embryo to allow it to grow sufficiently to tear the inner integument, after the initial partitioning of water into the localized swelling failed to cause germination.
Table 8.6 Effect of removal of outer integument on course of germination

<table>
<thead>
<tr>
<th>Integuments</th>
<th>Hypocotyl width mm ± SEx</th>
<th>% Germination</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0.7±0.07</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>1.0±0.05</td>
<td>1</td>
<td>35</td>
</tr>
</tbody>
</table>

After air drying the inner integuments of the 'peeled' embryos were wedged in the barrel of a 1 ml syringe. The syringe was weighed and 0.1 ml water was drawn into it to saturate the atmosphere around the integuments. After 12 h the water was expelled, the plunger was removed, and the syringe was weighed as the integuments dried. The weight of the integuments increased 30% while they were exposed to the saturated atmosphere and fell to the air dry weight within minutes of exposure to drier air. Water was not absorbed into the suberized cells of the inner integument (Plate 8.3) but adsorbed onto the cuticle covering the inner surface of the integument (Gauba and Pryor, 1958). The cuticle is deeply fissured, having formed between the cells of the nucellus, and has a velvety appearance. When it is wet, water can be squeezed from it by the touch of a needle, leaving a dark, wet mark on the compressed surface.

These observations are consistent with the suggestion that initial expansion of the cells of the collet may squeeze water from the wet cuticle of the inner integument to continue the germination process. Water enters through
the symplast of the coleorhiza, not through the cuticle covered hypocotyl. Safranin dye penetrates the former readily, but not the latter after the epidermis becomes rigid during the 'priming' period (Plate 7.1).

The role of the inner integument in germination, therefore, is to regulate the water relations of the embryo by restricting water intake during 'priming' and by releasing water to the hypocotyl for germination.

(ii) Outer integument

In this experiment the role of the outer integument in maintaining the water supply to the inner integument for germination was examined.

Materials and method

Ninety seeds were sown on the plates of covered Haines Apparatus established at -5, -10 and -15 kPa matric potential and allowed to imbibe for 24 h at -5 and -10 kPa and 72 h at -15 kPa, to reach 30% water content.

Thirty imbibed seeds were removed from each H.A. and placed on paper to air-dry at 70% R.H., 30 were removed and attached to cellotape suspended in a closed container above a free water surface and 30 were retained on the H.A. plates and dried by uncovering the H.A.

The water potential of embryos, removed from the integuments was determined using a Wescor Thermocouple Psychrometer at approximately hourly intervals over the following twelve hours. Four determinations were made each hour, selected from each treatment in turn, and the experiment was repeated twice.
Results and discussion

Embryos removed from the water source and suspended in a saturated atmosphere remained at a constant water potential of -4.5 MPa (Fig. 8.8a) and those not used to determine water potential germinated normally. The water potential of seeds air-dried on filter paper at 70% R.H. fell to -10 MPa within 20 m (Fig. 8.8e) whilst most retained on the plates but exposed to 70% R.H. fell to -10 MPa water potential within 1 h (Fig. 8.8b, c, d). A few seeds retained on the plates at all three matric potentials maintained the 'priming' potential of -4.6 MPa.

Had embryo water potential depended on a simple gradient between the embryo and its environment, seed on the -5 kPa matric potential plate might have dried more slowly than that on the -15 kPa plate. There was no evidence of this.

Some seeds removed from the tensiometer plate for weighing in experiment 8.2 lost weight while others regained it when replaced on the plate in low R.H. conditions, causing the large standard errors in seed weight means. This suggested that the outer integument absorbed water after replacement on the plate if the seed made a good contact with the surface. The few seeds remaining 'primed' (-4.6 MPa) in this experiment may have had sufficient contact with the plate to maintain the embryos in the same surroundings as embryos suspended in high R.H. conditions, which also remained 'primed'.
Fig. 8.8 Effect of seed/surface contact, matric water potential and R.H. on germination.
A mechanism for control of germination can be inferred from these results. The two integuments of *E. sieberi* are separated by an air gap, shown clearly in Plate 9.1. (When the seed lies on a wet surface the gap fills with water and the seed water potential becomes -0.5 MPa while the embryo water potential is -4.6 MPa). The wetness of the outer integument depends on the amount of water it gains from the substrate while maintenance of the 'priming' water potential depends on whether water is lost from the air gap between the integuments. Therefore, if the seed-substrate contact is sufficient for the outer integument to remain wet, the relative humidity in the air gap will remain high whatever the ambient R.H. of the seed, and the embryo will 'prime' and germinate. The role of the outer integument is to insulate the inner integument from water loss if the atmosphere is below saturation and thus maintain the 'priming' water potential of the embryo.

8.5 GENERAL DISCUSSION

Seeds vary in their ability to obtain sufficient water from the substrate to germinate. Those most able to germinate at relatively low soil water potentials have seed coats that allow free movement of water from soil to embryo because the surface is wetted readily and the path between it and the embryo is uninterrupted by impervious layers or air gaps which become dry at low water potentials (McWilliam and Phillips, 1971). The seed structure of *E. sieberi* combines ready wettability with barriers to
In many cases water enters seeds through the micropyle, but it is unlikely to be in contact with the wet substrate if the seed is lying on the surface. Moreover, Gauba and Pryor (loc.cit.) state that the exostome is occluded in Monocalyptus and that "it is, in any case, of secondary importance whether or not intake of water is effective through the hilum or the micropyle in view of the fact that moisture is readily absorbed by the whole of the cuticle free epidermis."

These authors consider that, in the mature Monocalyptus seed, the embryo has attained a far reaching physiological independence from its seed coat. That is, it is isolated within the coat, separated from the wet substrate, and therefore dependent on the water relationships of the coats for germination.
water absorption that allow control of germination by the 'stop-go' process.

The outer integument is readily wettable for it lacks a water repellant cuticle (Gauba and Pryor, 1958) and makes good seed-surface contact by means of a channelled or tubercular micro-relief that is not obliterated by copious mucilage which may dry into an impermeable coating (Bachelard, 1985). The outer integument is so wettable that the whole seed is able to absorb sufficient water from saturated air to allow germination, although it is slow - 14 days at 18°C to initial germination.*

When the outer integument is wet the relative humidity in the air gap between inner and outer integuments rises to saturation and water is adsorbed onto the cuticle on the inside of the inner integument and passed directly into the embryo. Imbibition continues and the water potential rises until further ingress of water is prevented by pressure of the inner integument against the swollen embryo and the water potential adjusts to the 'priming' level of -4.5 MPa.

If the relative humidity in the air gap remains high enough to maintain the 'priming' water potential (as it does in rain or when the outer integument is able to replace water lost to the atmosphere) the localized swelling of the collet at the end of 'priming' exerts sufficient pressure against the inner integument to squeeze water from it, allowing further expansion which breaks both integuments. This brings the water absorbing coleorhiza into contact with wet soil if the seed is buried, or with the saturated
atmosphere if the seed has germinated on the surface during rain. If the relative humidity in the air gap falls in response to a readjustment of the soil water potential/atmospheric relative humidity/seed-surface contact interaction germination does not occur, and the seed remains 'primed' until the relative humidity in the air gap again rises.

This elegant mechanism for the control of germination allows E. sieberi seeds to 'prime' in the intermittent wet periods characteristic of the habitat but confines their germination to periods in which the seedbed may remain wet sufficiently long for seedlings to become established.
INTRODUCTION

Previous chapters have shown that germination of *E. sieberi* occurs when the uppermost cells in a section of the hypocotyl immediately behind the collet expand, breaking the seed coat and directing the radicle downwards. Germination occurs only if water is readily available, suggesting that the upper cells expand more in response to turgor pressure than the lower cells. The site of the response is altered readily if the collet region is damaged or the seed reorientated with respect to gravity. A possible cause lies in the metabolism and translocation of food reserves within the seed to create osmotic potentials which will form appropriate water potential gradients for expansion of the upper cells of the hypocotyl.

The experiments described below were designed to examine this question.
9.1 DETERMINATION OF FOOD RESERVES

Eucalypt seeds lack specialized storage tissue. All reserves for differentiation and growth during 'priming' and germination are contained in the cells of the embryo axis and cotyledons. The chemical composition of these reserves and the mobilization of each component before germination was determined.

Materials and method

(i) Chemical

Duplicate samples of 0.25g of viable seeds (approx. 170 seeds) of E. sieberi were assayed for chemical composition before imbibition, after 40 h. of 'priming' and as germination began, 60 h. from imbibition. Lipid content was assayed gravimetrically (Te May Ching, 1977). The seed samples were ground in an aliquot of ice cold methanol and two volumes of chloroform were added to extract the lipids during 2 h. standing. The extraction was repeated twice after filtration, and the combined filtrates were washed with two volumes of distilled water in a separating funnel. The white emulsion at the interface and the organic phase were separated from the aqueous phase then dried, and the residue was weighed.

Soluble protein was assayed spectrophotometrically. The seed samples were ground in 0.05M phosphate buffer, pH 8.5, with 0.1% Tween20, and the mixture was stirred for 1 h. The mixture was centrifuged at 20 000 rpm for 20 minutes, then decanted and the supernatant was made up to 50ml.
Protein content was determined on 0.5ml aliquots by the Lowry Method (Lowry et al., 1951), using Folin reagent in an alkaline Cu solution. The E. sieberi extract was compared with serial samples of Standard Bovine Serum Albumin (B.S.A.) at 550nm against a blank.

Soluble sugar and amino acids were assayed spectrophotometrically after grinding and extraction in 80% warm methanol. The extract was decanted into a 100ml flask, boiled for 1 minute, filtered, and made up to 50ml volume. Soluble sugar was determined on 2ml aliquots by Bell's Method (Bell, 1955) using 80% aqueous phenol and conc. H₂SO₄. The E. sieberi extracts were compared with serial samples of glucose at 490nm against a blank. Amino acid was determined on 1ml aliquots of the extract using Ninhydrin in methyl cellosalve in cyanide-acetate buffer, and compared with serial samples of leucine at 570nm against a blank (Rosen, 1957).

Starch was determined on embryos from which the seed coats had been removed, after extraction of soluble sugar and amino acids as described above. The residue from that extraction was dried, then placed in a 100ml flask with 50ml water and 1ml acetate buffer, boiled for 3 minutes, then cooled. One ml 5% (w/v) diastase was added and the mixture was digested for 3h. at 30°C, then filtered and made up to 100ml. The quantity of glucose was determined by Bell's Method, as above, and converted to starch equivalents by multiplying by 0.9 (Pucher et al., 1948). The E. sieberi extracts were compared with serial samples
of glucose against a blank and against a diastase only control. Whole seeds were not used for this determination as seed coats give a positive test for sugar due to the presence of mucilage in the coat.

The results of the chemical analysis of reserve mobilization during 'priming' were compared with data obtained from microscopic examinations.

(ii) Cytological

Ten seeds of *E. sieberi* were removed from the Petri dishes on which they had been placed to germinate at 18°C, after 0, 24, 48, 72 and 96 h. The non-micropylar end of each seed was cut off to facilitate entry of the fixative, 3% gluteraldehyde, and the seed was passed, after fixing for 12 h, through the dehydration series 2-methoxyethanol (three times), ethanol, propanol, and butanol at 12 h. intervals, all at 0°C. Then it was infiltrated with monomer mix' (94.5% glycol methacrylate, 0.5% 2,2 Azobis (2 methol)propionitrile, 5.0% polyethylene glycol 400) and embedded in it, in a gelatine capsule (Feder and O'Brien, 1968). The capsules were dried at 40°C for 48 h. and then at 60°C for 12 h. Then 5 micron thick sections were cut through each seed, parallel to the embryo axis.

Sections were mounted and stained with toluidine blue for detailed histological examination. Representative sections were stained with 0.1% aqueous bromo-phenol-blue for 10 minutes and washed in 0.5% acetic acid to detect basic proteins. Further sections were stained with 0.05% bromo-phenol-blue in 2% acetic acid, in 1% HgCl₂ for 2 h.
to detect total protein (Pearse, 1960).

Results and discussion

Lipid and protein are the principle reserves in E. sieberi seed, as is general for eucalypts (Gauba and Pryor, 1958), Table 9.1.

Table 9.1 Mobilization of food reserves during 'priming'

<table>
<thead>
<tr>
<th>Food reserve</th>
<th>% composition of 'priming' seed - duplicate samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hours</td>
</tr>
<tr>
<td>Lipid</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Starch</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Sol. sugar</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Sol. protein</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Amino acid</td>
<td>0.15</td>
</tr>
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The quantity of lipid in E. sieberi seed fell from about 30% to about 20% by weight of air dry seed during 'priming' but no other reserves fell significantly. The free amino acid content increased.

Protein mobilization

Most of the cells of the fresh embryo contain large storage bodies (Plate 9.1) which stain black with bromo-phenol-blue, indicating that they are protein bodies (Plate 9.2).
Plate 9.1 Structure of the fresh seed of *E. sieberi*

(a) Longitudinal section showing reserves in the micropylar end of the seed. a, outer integument; b, inner integument; c, collet of hypocotyl; d, radicle; e, coleorhiza; f, cotyledon.

(b) Detail of cells in the hypocotyl and cotyledons
Plate 9.2 Fresh seed of *E. sieberi* stained with bromo-phenol-blue to show protein bodies

(a) Longitudinal section showing protein reserves in the micropylar end of the seed

(b) Protein bodies in the epidermis of the hypocotyl
Plate 9.3 Distribution of protein bodies in hypocotyl and cotyledon of imbibed seed. Note loss of reserves from the epidermis.

Plate 9.4 Distribution of protein bodies in the collet of a fully 'primed' seed. Note loss of reserves from the hypodermal cells on top of the collet.
Plate 9.5 Distribution of protein bodies in the collet cells below those enlarging to break the seed coat.

Plate 9.6 Distribution of protein bodies in the micropylar end of a germinating seed of *E. sieberi*. Note loss of protein bodies from the collet, coleorhiza and stele and note broken integument above the uppermost portion of the collet.
Plate 9.7 Distribution of protein bodies in the hypocotyl as it makes a positive geotropic growth curvature. Note that reserves are still present in cells below the hook.

Plate 9.8 Distribution of protein bodies in the collect end of the hypocotyl after the 'clinging disc' and the hook straightens. Note peripheral hairs on the disc and note that reserves under the hook have been mobilized.
Soon after imbibition the protein bodies in the cells which will form the stele of the hypocotyl disappear and those in the epidermis of the hypocotyl and cotyledons become less prominent (Plate 9.3). As germination approaches the bodies disappear from the hypodermal cells that will expand to break the seed coat and become less prominent in the cells below and around them (Plates 9.4, 9.5) and in the coleorhiza which will form the 'clinging disc' (Plate 9.6).

Mobilization of the protein bodies spreads into the cortex of the hypocotyl as it hooks downwards (Plate 9.7) but it does not occur in the cells under the hook until the 'clinging disc' is in contact with the water supply for raising the cotyledons (Plate 9.8). The protein reserves in the apical end of the hypocotyl are not mobilized until that end elongates.

These results suggest that mobilization of the protein bodies in individual cells creates osmotic potentials which may form appropriate water potential gradients for expansion of the cells. The observed increase in amino acids is consistent with this explanation for the expansion of the cells, since they may lower osmotic potentials in plant cells (Harborne and Van Sumere, 1975). Failure to record an overall drop in soluble protein during 'priming' and germination appears to be due to the extremely localized nature of its mobilization.

**Lipid mobilization**

The decline in lipid content appears to be associated with a change in the cuticle of the embryo. The cuticle of
fresh and recently imbibed embryos is a 'semi-solid envelope' at this early stage (Gauba and Pryor, loc.cit.). Later in the 'priming' period the cuticle solidifies, adheres to the cell wall and becomes strong enough to resist collapsing when the hypocotyl is freeze-dried (Plate 7.1). The fall in lipid content may represent a change in the ease with which lipids are extracted from the 'semi-solid' and the 'solid' cuticles, rather than a drop in the seed lipid reserves.

9.2 GENERAL DISCUSSION

Physiological processes that control germination may be inferred from these results.

After the solid cuticle has formed on the hypocotyl and the cotyledons the protein reserves in the hypodermal cells on the uppermost segment of the collet are mobilized rapidly and the cells enlarge radially, breaking the seed coat. Mobilization of the protein reserves may lower the osmotic potential of the cells sufficiently to set up a water potential gradient between the cells that have mobilized all their reserves and those around them, still in the early stages of mobilization. Water moving into the former cells, increasing turgor pressure, may cause the cells to expand sufficiently to press against the inner integument and obtain a further supply of water as described in Chapter 8. This further supply of water would allow the cells around those that broke the coat to elongate as their protein reserves are progressively mobilized, lengthening the hypocotyl.
Since the reserves in the cells in the lowest segment of the hypocotyl are not mobilized at the time of germination, or immediately after, their osmotic potential does not change and they remain small. Enlargement of the cells above them causes the hypocotyl to hook downwards.

Mobilization of the reserves in the cells underneath the hook coincides with the development of the peripheral hairs on the expanded coleorhiza. This ensures that the hook will be straightened as soon as water is freely available from the substrate through the 'clinging disc' hairs.

Gravity determines which group of cells in the hypocotyl will expand to break the seed coat of the eucalypt. In wheat the pericarp is broken by the rapid growth of a specialized 'beak' of cells so thin walled that they collapse on sectioning, suggesting that they expand in response to a decrease in wall pressure (Symons et al., 1984). After the cuticle 'solidifies' the epidermal cells of *E. sieberi* are relatively rigid but the cortical cells beneath are thin walled. Expansion of such thin walled cells beneath a rigid epidermis causes geotropic growth curvature in sunflower hypocotyls (Firn and Digby, 1977). The mechanism postulated is that increased respiration in the cortical cells in response to the gravity stimulus lowers the pH of the cytoplasm and alters the structure of the cell wall, making it less resistant to pressure. The wall stretches and the respiring cells expand, stretching the epidermal cells above them. Thus the sunflower hypocotyl is under tension from the epidermis, as the 'primed' hypocotyl of
E. sieberi is under tension from the inner integument, and the tension is overcome when the metabolism of a small group of cells alters sufficiently for them to exert more growth pressure. Data presented for E. sieberi does not distinguish between decrease in osmotic potential and lowering of pH as the physiological basis for the partitioning of water into the cells that swell to cause germination. Both may stem from the mobilization of protein, and both may operate, change in wall structure allowing rapid and extensive expansion in response to increased water potential gradients.

Sequential mobilization of protein reserves in the hypocotyl during germination and in the post-germinative stages facilitates the establishment of E. sieberi on or in seedbeds subject to intermittent drying. Confinement of mobilization to a small group of cells on top of the collet causes the hypocotyl to hook downwards, protecting the radicle and placing it as quickly as possible in the soil. It also ensures that the bulk of the embryo remains undamaged, in its pre-germinative state, should there be insufficient water for post-germinative development to proceed. Even if post-germinative development proceeds to the point of expanding the coleorhiza the reserves in the axis are largely unmobilized and if the radicle dies they may be mobilized to establish the seedling by atypical germination. The embryo's resources are conserved for successive attempts to become established. The significance of this mode of germination is discussed further in the concluding chapter.
A survival strategy is a 'grouping of similar or analogous genetic characteristics which recur widely among species or populations and cause them to exhibit similar ecology' (Grime, 1981). One regenerative strategy which occurs widely among forest tree species is profligate seeding which takes advantage of any site where local disturbance has reduced competition for light from established plants (Grubb, 1977). In the dry sclerophyll forests of Australia the site disturbance is generally due to fire which is spatially and temporally unpredictable, but in the context of this study disturbance is due to logging, which produces much the same exposed conditions for seedling establishment as does fire.

Individual seeds of species which employ the profligate seeding strategy are often small and their resources are so limited that photosynthesis must begin soon after germination. Germination of seeds with the longest dimension less than 3 mm is generally epigeal, with leafy cotyledons expanding soon after emergence from the seed, which lies on, or close to, the surface (Ng, 1978). The consequence for _E. sieberi_, germinating in the dry sclerophyll forest, is that its germination strategy must include characteristics which enable the small seedling to avoid or survive intermittent dry periods during germination and establishment.
Thus the germination strategy should include stress tolerance mechanisms, the general strategy for which is that the plant has persistent, stress tolerant structures capable of making effective use of resources during periods in which there is a relaxation of stress (Grime loc. cit.). The strategy for tolerance of water stress includes small xeromorphic leaves and a slow growth rate with strongly developed acclimation (Smith, 1982) to changes in conditions. Post-germinative and cotyledonary seedlings of _E. sieberi_ show such characteristics (Chapters 5 and 6).

An effective germinative strategy for stress tolerance might include recovery after stress in addition to making effective use of resources during periods when stress is relaxed. The production of adventitious roots from the hypocotyl after loss of the radicle is an example of such a mechanism (Chapter 5).

Stress avoidance contributes to survival and establishment of the seedling, as does stress tolerance. The morphology of the embryo and of the post-germinative seedling is a stress avoidance mechanism, as the desiccation prone radicle is protected by the coleorhiza during germination and placed vertically downwards by growth of the hypocotyl in the most effective position for soil penetration and subsequent rapid growth (Chapters 3 and 4).

A requirement for low water stress at germination may be interpreted as an adaptation, or a strategy, for drought avoidance as it ensures that seeds on the surface will germinate only following rainfall of sufficient
intensity to maintain high moisture levels for a considerable period of time (Mott and Groves, 1981). In *E. sieberi* drought avoidance is achieved by 'priming' the seed in intermittent wet periods (Chapter 7) and restricting germination to rainy periods by means of an interaction between the integuments and the relative humidity of the air gap between them (Chapter 8). Germination of karri *E. diversicolor* occurs after 50-75 mm of rainfall over a 3 day period (Christensen and Schuster, 1979); it may have a similar drought avoidance mechanism to that of *E. sieberi*.

Stress avoidance and stress tolerance during 'priming', germination and soil penetration in *E. sieberi* are associated with the 'precocious' development of the hypocotyl relative to the radicle (Carr and Carr, 1970) and with the 'clinging disc' around the collet. In 1885 Klebs classified seed plants according to the mode of germination, making a sub class (Type 5) for those which are epigeal and 'have a main root little or not elongating during germination; at the collet a whorl of hairs' (de Vogel, 1980). Klebs referred to the hairs as a seedling fastening apparatus but he did not speculate on their significance for establishment in each of the diverse families in which radicle development is suppressed or delayed: Chenopodiaceae, Palmae, Bromeliaceae, Typhaceae, Guttiferae, Dipterocarpaceae, Saxifragaceae, Balsaminaceae, Onagraceae, Lythraceae, Myrtaceae. All germinate on or in anaerobic, dry, saline or unstable conditions which are unfavourable for radicle penetration and extension. 'Type 5' germination
is, therefore, a survival strategy for establishment in inhospitable seedbeds.

Baranov (1957) traced the evolution of Type 5 germination in the Family Myrtaceae. The most ancient genus to exhibit it is Myrtus, which has sticky papillae on the collet. In Melaleuca there is a brush like arrangement of sticky hairs. In Leptospermum ‡ Callistemon the collet is modified into a coleorhiza which develops sticky hairs as in Eucalyptus, but in the modern genus Angophora there are no hairs and the coleorhiza grows into a 'bell' which protects the radicle as it penetrates the soil. Baranov concluded: "it is possible that in the course of adaptive evolution such a pronounced vagina (the coleorhiza) was developed because of the necessity to protect the tender embryonic radicle in its emergence through the seed coat" and that "the sticky hairs might have arisen and developed in the process of evolution as a device for better attachment of the embryo to the soil as well as for the rapid supply of water". These functions are confirmed in Chapter 3 and 4 and related to establishment of E. sieberi in modern Australian dry sclerophyll seedbeds where the 'clinging disc' stabilizes the seedling and provides water for raising the cotyledons before the radicle develops, rather than merely supplementing it.

The Family Rhizophoraceae is closely related to the Family Myrtaceae, the seeds of both families having a sclerotic mesotesta and no exotegumen (Corner, 1976). Genera with hard seed coats in both families have the
radicle protected by a cap, referred to as the coleorhiza in Myrtaceae, although it is derived late in embryogenesis from the collet not formed from the ground meristem as in Gramineae. In view of the demonstrably close relationship between the two families studies on the embryogenesis and germination of the mangrove are relevant to this study of the eucalypt. Embryogenesis in the estuarine mangrove *Rhizophora mangle* L. is unusual in that the axis grows from a diffuse intercalary meristem below the cotyledonary node (Juncosa, 1982). The meristem produces a hollow procambial cylinder separated from the cotyledonary provascular strands and connected to the late-developing radicle through tangential and random metaxylem vessels in the collet. Juncosa considers this unusual 'compartmentalized' development critical for establishment of mangroves in saline, anaerobic, unstable mud as it favours shoot growth over radicle growth, facilitating the evolution of a large viviporous seedling that is stable in the mud and quickly established by surface spreading lateral roots initiated during embryogenesis behind the suppressed radicle tip. In the more primitive dry land mangrove, *Cassipourea elliptica* (S.W.) Poir, the compartmentalized or phasic development of the embryo is also present, although less pronounced (Juncosa, 1984a), but in the wetland species *Bruguiera exaristata* Ding Hou, the intercalary growth of the hypocotyl is marked but the radicle is incompletely suppressed and no lateral roots are formed during embryogenesis. The seedling must use the positively geotropic
radicle, which does not tolerate anaerobic conditions well, for establishment (Juncosa, 1984b). The evolution of the tidal species appears to have come about through strong environmental pressure against seedlings with vulnerable apices. The functional constraint applied to the radicle caused changes in the timing of meristematic activity during embryogenesis; changes made possible by the unusual phasic development of the embryo.

The eucalypt, as exemplified by *E. sieberi*, appears to have evolved as specific a germination and establishment strategy as the mangrove. The strong environmental pressure against vulnerable apices in the eucalypt is not saline, anaerobic mud but rapidly drying seedbeds which resist penetration and cause water stress, but the delay in radicle development relative to hypocotyl development in order to protect the radicle tip from damage during germination is the same as in the mangrove, and it is associated with sequential mobilization of reserves in 'priming' and germination (Chapters 8 and 9). This mobilization enables the hypocotyl to make several attempts to grow without drawing on the cotyledonary reserves or committing the radicle to meristematic growth.

Compartmentalization of reserve mobilization during germination (Chapter 9) conserves resources for atypical germination. It also maintains the cotyledons in the low water potential 'priming' phase (Chapter 8). In that phase they are not vulnerable to damage if desiccated (Chapter 5), possibly because water is withdrawn slowly in contrast to
the sudden withdrawal of water from vacuolated cells, which causes rapid volume change and damages the membrane physically (Hegarty, 1978).

A further consequence of using expansion of the cells of the hypocotyl for germination and for raising the cotyledons, rather than meristematic growth, is that the limited energy reserves are conserved. Small seeds must use reserves to convert the cotyledons from storage and water absorbing organs in the seed to assimilating organs in the seedling, or to produce true seedling leaves rapidly (Lovell and Moore, 1970). The first alternative applies in *E. sieberi* but the only conversion necessary is that of the upper epidermis from the water absorbing to the foliage form (Chapter 6). Reserves remain, after the cotyledons expand, to supply the radicle or the two replacement adventitious roots, to establish the seedling. The growth of the plumule begins only after the cotyledons are able to supply assimilate in low water stress conditions (Chapter 6).

Germination and establishment are, arguably, the most critical phases in the life of a plant. This study shows how well adapted *E. sieberi* seeds and seedlings are for survival in the intermittently dry seedbeds of the habitat, which includes a series of harsh, sandy ridges 50 km north west of Melbourne, on the edge of the mallee (Carolan, 1966). The general strategy includes stress avoidance, stress tolerance, and recovery from damage during germination and establishment. The mechanisms for the strategy are the water relations of the 'priming' seed, the
precocious development of the hypocotyl with the coleorhiza, and the sequential mobilization of food reserves during germination. Thus the morphology and physiology of the seed and seedling fit *E. sieberi* for regeneration from seed on logged sites as described in Chapter 2.

It is likely that most seeds germinated at the end of September in the Field Trial but most seedlings died before the first inspection in mid-October. The first seedling leaves had not expanded at that inspection (Fig 2.7) indicating that the seedlings had experienced a long dry period since germination. Nevertheless, 80 of a potential 300 seedlings were still alive in October on a low disturbance plot of sedimentary soil C (Table 2.4). Under ideal conditions in the Clod trial 34 of a potential 50 survived on the same soil (Table 2.4). Seeds on this site lay directly on the pale coloured mineral soil and the seedlings may have had time to establish the radicle in the soil before it dried. Seeds on the other low disturbance sites lay amongst fine débris and ash which may have dried before the seedlings could become established in the mineral soil. Some seedlings on the more disturbed sites may have died before reaching the mineral soil under the ash. Others may have died because the drying soil became too strong for them to penetrate (Table 2.3).

Thus it appears that newly germinated cotyledonary seedlings will survive a dry period after germination if they have penetrated the mineral soil. If the soil is compacted, or if the seed is not in contact with the mineral soil the radicle takes longer to penetrate, and the wet
period that caused germination must last longer than is necessary if the seed germinated directly on low strength mineral soil.

Once established, the survival of the cotyledonary seedling will depend on its ability to obtain sufficient water from the soil and through the cotyledons to prevent desiccation, and its chance of avoiding the heat shock that may occur on exposed sites.

Possibly, the unexpectedly low establishment in the 1 m² plots of the Field Trial was due to the onset of dry weather so soon after germination that the only seedlings to become established effectively were those from seeds lying on the pale coloured, lightly disturbed sites, and those in sheltered microsites on plots with less hospitable surfaces. The disturbed, exposed seedbeds were subject, in the severe Spring of 1982, to a long continuous dry period rather than to the intermittent dry periods. Had the plots been watered soon after germination sufficient seedlings probably would have become established for assessment of the effect of soil disturbance on seedling growth.

The original aim of this project was to use *E. sieberi* to test the effect of logging disturbance on regeneration of the forest from seed. The outcome of the project is a description of the germination strategy of the species in relation to its establishment on the difficult seedbeds of its habitat. Further research might include an examination of the germination strategy of species occurring in association with *E. sieberi* and an examination of species occurring in more mesic habitats, germinating on less difficult seedbeds.
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APPENDIX A

ANOVA FOR EFFECT OF SOIL DISTURBANCE ON
SEEDLING GROWTH, EXPERIMENT 2.2

I. DRY ROOTS

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*Means linked --- are not significantly different at 5% level.

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