Pod and seed coat factors in seed dormancy of wild radish
(Raphanus raphanistrum L.)

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Summary There has been some confusion in the literature about the relative contribution of the pod and seed coat in the expression of seed dormancy in wild radish. Studies were undertaken with two wild radish biotypes (SE and RO). Pods of the SE biotype were collected from plants grown under a rain shelter erected after the flowering stage to impose water stress as well as from plants growing outside the shelter. Collected pods were placed on the soil surface under a transparent rain shelter as well as in the open. Germinability was tested for all the treatments monthly for six months after ripening. All seeds enclosed in pods were highly dormant at maturity and lost their dormancy gradually during winter. There was a higher level of germination in the pods stored in the open compared to those under the shelter, which could be due to the effect of rain on pod breakdown. Seeds produced under water stress conditions in the SE biotype were less dormant than those produced outside the shelter under normal seasonal rainfall. At six months after maturity, removal of pods enclosing the seed increased germination by 1.3-fold in the SE biotype whereas the increase in the RO biotype was 5-fold. Greater response to pod removal in the RO biotype is likely to be due to its thicker pods. Partial removal of seed coat also increased the germination of SE and RO biotypes by 20 and 50%, respectively. The results indicate that both pod and seed coat play an important role in the dormancy of wild radish seeds.

Keywords Dormancy, pod, seed coat.

INTRODUCTION

Wild radish (Raphanus raphanistrum L.), a winter annual plant, is one of the most common and competitive broad-leaved weed species on Australian farms. The success of wild radish as a primary invader during secondary succession and as a weed in cultivated crops may be largely attributed to its specific germination and seed dormancy ecology (Bhatti 2004, Blackshaw et al. 1999, Code and Donaldson 1996). Wild radish seeds are dormant at the time of production. By the start of the cropping season, up to 70% of the seeds are still dormant (Cheam 1986). The exact mechanism of seed dormancy in wild radish has not been described and there has been some confusion in the literature about the relative contribution of the pod and seed coat in the expression of dormancy in wild radish seeds. According to some researchers, wild radish dormancy is largely due to the pod surrounding the seed (Cheam 1986), while others believe that the seed coat is the major contributor to dormancy (Young 2001).

Seed dormancy can be affected by the environment experienced by the parent plant (Baskin and Baskin 1998). There is a lack of information about the climatic effects on wild radish dormancy. Water deficit is a prevalent issue in spring in southern Australia, but the consequences of this stress on wild radish seed dormancy has not been investigated. There is also a need to investigate the effect of biotic factors, such as plant competition, on wild radish seed dormancy.

This paper aims to identify the relative contribution of pod and seed coat in wild radish seed dormancy. Results are also presented on the pattern of dormancy release of wild radish seeds in the field.

MATERIALS AND METHODS

Studies were undertaken with two wild radish biotypes: (a) SE biotype collected from the south-east of South Australia in 2002 and (b) RO biotype, which has naturalised at Roseworthy. Pods of the SE biotype were collected from the plants grown under a rain shelter erected after the flowering stage to impose water stress (SE_Stressed) as well as from plants growing outside the shelter. The detailed descriptions of experiments are as follows:

General protocol for germination tests Seeds or pod segments were placed in 9 cm Petri dishes lined with two discs of filter paper, moistened with 5 mL deionised water or GA3 (1mM) when required. Petri dishes were sealed with Parafilm to minimise evaporation. Germination tests were conducted for 14 days in germinators maintained at a day/night temperature of 25°C/15°C in continuous darkness, dishes wrapped in two layers of aluminium foil. Number of germinated seeds was counted at the end of germination test and germinability of each treatment was recorded as a percentage of viable seeds.
**Experiment 1: pattern of seed dormancy release in the field**  
Wild radish pods of the SE biotype were collected in November 2004 from a field study at Roseworthy research farm prior to pod dispersal. The pods were collected from the monoculture plots of wild radish growing at a density of 15 plants m\(^{-2}\) (SE_C0W1) and also from the mixture plots of wheat and wild radish with 400 wheat m\(^{-2}\) and 60 wild radish m\(^{-2}\) (SE_C3W3). These pods were placed on the surface of soil contained in pots buried in the field so that the soil surface in the pots was level with the field soil. These pots were placed under a transparent plastic rain shelter. Germinability of pods was tested at the start of the experiment and thereafter at monthly intervals until July 2005. There were five replicates for each treatment (10 seeds per replicate).

The effect of rainfall during summer and autumn on dormancy release of pod-enclosed seeds was also investigated. The pods from Roseworthy (RO) and South-east (SE) biotypes were used for this study. Plants of SE biotype had grown under a rain shelter after flowering commenced in spring (SE_Stressed). These pods were placed in pots as described earlier and the pots were either placed under a transparent shelter or in the open in the same field. Tiny Tag® temperature data loggers (Hastings Data Loggers Pty Ltd) were installed inside and outside the shelters for recording the temperature. Germinability of pods was tested at the start of the study and then at monthly intervals until July 2005. There were five replicates of each treatment (10 seeds per replicate).

**Experiment 2: Effect of pod removal, partial seed coat removal and GA\(_3\) on dormancy release**  
Four accessions of wild radish (SE_C0W1, SE_C3W3, SE_Stressed and RO) were used to study the relative contribution of pod and seed coat to seed dormancy in wild radish at six months after maturity in 2005. Pods were selected at random and only the middle segments from each pod were used. After assessment of germination of intact pods after seven days, seeds from non-germinated pods were carefully extracted and placed in the incubator for another seven days. Seed coat of naked seeds that failed to germinate at the end of this period was partially removed and placed back into the incubator and germination assessed after seven days. Finally seeds that did not germinate after partial seed coat removal were treated by adding 5 mL GA\(_3\) (1mM) to each Petri dish and germination assessed at the end of the final seven day period.

**Data analysis**  
Microsoft Excel was used to calculate means and standard error of means of dormant and germinable seeds from each observation.

**RESULTS**

**Experiment 1: pattern of seed dormancy release in the field**  
Temperature outside and inside the shelters was nearly identical. Although 2005 was drier than the long-term average, there was some rainfall received during each month of field storage (Table 1). At maturity fresh seeds of all accessions were strongly dormant (Figure 1). After 30 days storage in the field, SE_Stressed_Exposed pods which were stored in the open showed 45% germination while all other treatments were still highly dormant. At 60 days storage, the germination of SE_Stressed_Exposed pods reached 60% and SE_C0W1 germination dramatically increased to 45%. All other accessions treatments did not show any germination at this stage. After 90 days of field storage (March), germination of exposed pods of Roseworthy and SE biotypes increased to 40% and 55% respectively. Further increase in storage duration in 2005 did not increase germinability of wild radish pods. Rain had a substantial effect on pod degradation in both SE and RO pods stored in the open. Visual evidence indicated that pod degradation was much greater in SE_Stressed_Exposed than RO_Exposed.

**Experiment 2: Effect of pod removal, partial seed coat removal and GA\(_3\) on dormancy release**  
Presence of pod was an important obstacle to germinability particularly in the RO biotype. In SE_Stressed 55% of intact pods germinated, while only 5% of the intact pods of RO biotype germinated (Figure 2). Removal of the pod enclosing the seed increased germination by 5-fold in the RO biotype whereas this increase in the SE biotype was between 1.3 to 3-fold depending on the treatment (Figure 2).

Although pod removal increased germination percentage in all treatments, it had biggest impact on the RO biotype. This biotype was found to have the heaviest and thickest pods (data not shown). On the

<table>
<thead>
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<th>Months</th>
<th>2005 (% of long-term mean)</th>
<th>100 year average rainfall (mm)</th>
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<tr>
<td>Jan</td>
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<td>21.4</td>
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<td>Mar</td>
<td>46.3</td>
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<td>37.0</td>
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<td>May</td>
<td>6.0</td>
<td>46.8</td>
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<tr>
<td>June</td>
<td>192.8</td>
<td>52.5</td>
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<tr>
<td>July</td>
<td>68.1</td>
<td>49.9</td>
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other hand, SE_Stressed had thin and weak pod walls. This large difference in pod characteristics between these two biotypes appears to be responsible for contrasting germination of their intact pods. Even within the SE biotype, there were large differences between the stressed and non-stressed samples in terms of their germinability of intact pods (Figure 2). Partial removal of seed coat increased the germination of seeds of RO biotype by 50%, whereas this increase was lower for all treatments of SE biotype. Interestingly, addition of GA3 increased the germination of all treatments to 100%. Stimulation of seed germination by GA3 varied from about 20% in SE_Stressed and SE_C0W1 to around 40% in RO and SE_C3W3. These results provide strong evidence for the existence of physiological dormancy in wild radish seeds.

**DISCUSSION**

Seeds were strongly dormant at maturity and dormancy became alleviated only slowly in the field in 2005. The high level of dormancy in early summer is an important ecological factor as the period of dormancy coincides well with the average length of unfavourable conditions for seedling establishment in Southern Australia. Dormancy is further complicated by variations among the accessions of wild radish.

Seeds from the south-eastern agricultural districts of South Australia had a lower dormancy level than those from Roseworthy. This order was consistent when collected seeds from these locations were grown and tested under identical conditions. This suggests that dormancy is under genetic control. This has been reported in previous studies with wild radish (Cheam 1986) and also other weed species (Gill and Blacklow 1985).

Wild radish shows dormancy cycling in the field, behaving as a facultative winter species. It appears that a dormancy cycle in wild radish helps to regulate the germination and increase the probability of germination under favourable field conditions.

The low germination of seeds with intact fruits (pods) may be the result of mechanical resistance of the
indehiscent fruit. As Roseworthy biotype seeds with heavier and thicker pod walls showed lower germination percentage, our results confirm this mechanical resistance.

The marked effect of seed coat removal on germinability in all experiments indicated that some form of seed coat-imposed dormancy was involved in germination regulation. The germination in response to removal of part of the seed coat might be explained by a germination-inhibiting substance being present. As seeds from all populations did germinate without any mechanical treatment of the seeds in response to gibberellic acid, there should not be a mechanical barrier. This research has shown that the seed coat is responsible for dormancy but it is not the only cause of dormancy, since its removal permitted germination in only a proportion of the seeds. In contrast, when the seed coat was partly removed, gibberellic acid 3 (GA3) increased the germinability to 100%, suggesting a component of dormancy is present in the embryo.

In conclusion, this work shows that three dormancy mechanisms appear to control the germination of wild radish seeds. This study has identified dormancy mechanisms in pod, seed coat and embryo as well. These mechanisms will enable wild radish to provide a persistent dormancy in a large proportion of the seed pool.

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REFERENCES


