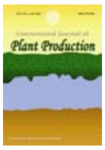

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Effect of water stress during seed development on morphometric characteristics and dormancy of wild radish (*Raphanus raphanistrum* L.) seeds

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Abstract

The effects of water stress on morphometric characteristics of wild radish seeds as well as the expression of its seed dormancy were investigated in a pot trial at Roseworthy, South Australia in 2005. This experiment was carried out in a completely randomised design with five water regimes (control, S4, S5, S6 and S8) and four replicates. The water stress was exerted by withholding irrigation and placing the pots under a rain shelter at different growth stages starting with the onset of flowering (S4) and ending with the completion of flowering (S8). Wild radish pods from all the treatments were collected fortnightly from November 11 to December 23, 2005 (four collection dates). Seed dormancy was tested with and without pod for all treatments in January and May 2005. All morphometric characteristics of pods including pod wall weight and seed weight were negatively affected by water stress. Pods sampled earlier had thicker pod walls and greater pod segment weight. The germinability of all treatments increased with pod removal. The dormancy level tended to be lower in later collected pods. This Study showed that expression of seed dormancy was influenced by water stress during reproductive development. Therefore, variability in spring rainfall could have a major influence on the level of dormancy in seeds of this weed species produced in different growing seasons.

Keywords: Mother plant; Seed weight; Seed germination; Drought; Leaf relative water content.

Introduction

Wild radish (*Raphanus raphanistrum* L.), a winter annual plant, is one of the most common and competitive broad-leaved weed species in 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 germination behavior and the level of seed dormancy (Bhatti, 2004; Blackshaw et al., 1999; Code and Donaldson, 1996). Dormancy characteristics of a species, or of a particular population, are often assumed to be the result of adaptation to the particular habitat where the species occurs (Esmaeili et al., 2009). Seed dormancy is essential for the survival of annual weed species in areas with a Mediterranean climate. Winter annual species adapted to Mediterranean climate survive the summer as dormant seed in the soil. An appropriate seed dormancy strategy will enable these species to germinate in the favorable conditions of late autumn and winter and prevent their germination after summer rains, which are invariably followed by a period of drought (Dunbabin and Cocks, 1999).

Water deficit, the major factor limiting plant growth and productivity worldwide, is expected to increase with the spread of arid lands. Global climatic trends may accentuate this problem (Alishah and Ahmadikhah, 2009). Seed dormancy in many species can be affected by the environment experienced by the parent plants during seed development (Baskin and Baskin, 1998). Anches et al. (1981) found that withholding water during seed development of *Datura ferox* L. caused a decrease in the degree of its seed dormancy. Peters (1982) similarly found that wild oat (*Avena fatua* L.) seeds produced in hot dry summers are less dormant than those produced in cool moist ones. Lampei Sr and Tielboerger (2008) subjected two plant species (*Biscutella didyma* L. (Brassicaceae), and *Bromus fasciculatus* L. (Poaceae)), to 12 levels of increasing water stress and evaluated the germination of their seeds in the following year. They found that water stress reduced the level of seed dormancy of either species and concluded that maternal environmental effects play an important role in the evolution of seed dormancy strategies. In contrast, Sharif-Zadeh and Murdoch (2000) found that seed dormancy of *Cenchrus ciliaris* L. increased when water stress was imposed during seed maturation. There is a lack of information on the effects of climatic factors on wild radish seed dormancy. Spring rainfall in South Australia can be quite variable. It is widely accepted that such climatic variation has a major impact on grain yield, grain size and quality of crops such as wheat (Saastamoinen, 1998; Wrigley et al., 1994). Low spring rainfall could also have an impact on the quality of weed seeds in particular their dormancy status. Although the long tap root of wild radish enables it to withstand drought (Cheam and Code, 1995), water stress because of weather conditions might influence the seed dormancy of this weed species. Therefore, for planning an effective wild radish management program, it is important to understand the effect of water stress on the expression of seed dormancy. The aim of this study was to investigate the effect of water stress at different phenological stages on morphometric characteristics of wild radish seeds as well as the expression of its seed dormancy.

Material and Methods

This experiment was carried out at Roseworthy Research Farm in a completely randomised design with five water regimes and four replicates in June 2005. Spring rainfall is fairly erratic at the experimental site (Figure 1).

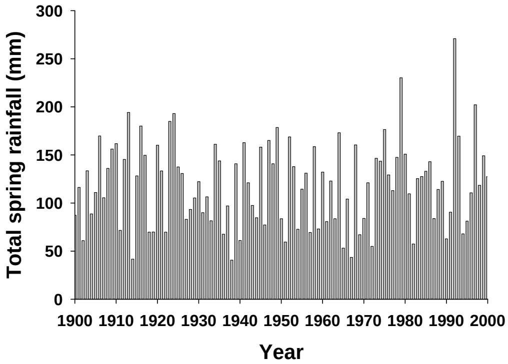

Figure 1. Total spring rainfall during 1900-2000 at Roseworthy.

Wild radish seeds were extracted out of pods collected from Roseworthy biotype. These pods had been collected in December 2004 at Roseworthy and stored in paper bags in the laboratory at a temperature of ~20°C until they were used in the experiment. Seeds (n=100) were initially planted into small pots filled with silt loam soil in the glasshouse and after seedling emergence, 20 seedlings of similar size were transplanted to pots in the field. Each pot was 60cm in depth and 15cm in diameter, containing one wild radish plant. Measurement of pot water holding capacity (PWHC) was made using four additional pots of same size and amount of soil. The pots were watered gradually until the first sign of water drainage from the bottom of the pots. This was considered as the saturation point of these pots and after this stage, pots were left overnight to drain. The pots were weighed after 24 hours and the volume of the water required to bring the soil to field capacity (FC) was calculated.

A slow release fertilizer (100:100:80 of N:P:K) was applied at the rate of 2 g pot^{-1} at sowing and 4 leaf stage. All pots were watered regularly and maintained near FC. Plants were subjected to water stress by withholding irrigation and placing the pots under a rain shelter at different growth stages starting with the onset of flowering stage, as follow:

1) Stage 4.0 (S4), first bud opened (based on Madafiglio decimal codes, (Madafiglio et al., 1999).
2) Stage 5.0 (S5), continued flowering, largest pods less than 1 mm diameter.
3) Stage 6.0 (S6), continued flowering, largest pods 1-2 mm diameter, slight constrictions between pod segments.
4) Stage 8.0 (S8), flowering completed, leaf senescence.
5) Control or nonstressed treatment which was watered twice a week until pod maturity.

In order to estimate plant water potential, the LRWC (leaf relative water content) was measured at midday at weekly intervals over three weeks starting on 15th October. Three leaf disks were taken from the youngest fully expanded leaf of each plant and leaf disks were then immediately weighed (fresh mass, FM) (Smart and Bingham, 1974). In order to obtain turgid mass (TM), leaf disks were floated in distilled water inside a Petri dish for 24h under fluorescent light. Leaf samples were weighed after gently wiping free water from the leaf surface with tissue paper. In order to obtain the dry mass (DM), the samples were placed in an oven at 80 °C for 48h and weighed. Values of FM, TM, and DM were used to calculate LRWC, using the equation 1:

$$LRWC = \frac{FM - DM}{TM - DM} \times 100 \quad (1)$$

Wild radish pods from all the treatments were collected fortnightly from November 11 to December 23, 2005 (four collection dates). Pods from each collection date were stored separately in paper bags at a room temperature of ~20 °C until they were used for germination studies.

All morphometric characteristics of pods including pod length, pod width, segment number per pod, pod weight were measured for 30 pods per treatment. In addition, pod wall thickness and seed weight were measured for the middle segment of each pod-enclosed seed. The pod wall weight to seed weight and also seed coat weight to seed weight ratios were used as indictors of pod wall and seed coat mechanical restriction against seed germination, respectively. There were five replications for each treatment. Seed dormancy was tested with and without pod for all treatments in January and May 2005.

ANOVA was used in this experiment to assess treatment effects (completely randomised design).

Results

The leaf relative water content (LRWC) showed clear differences between the control and different water stress regimes (Figure 2). Water stress reduced the leaf relative water content in stressed plants relative to the control. At the time of last measurement, treatment S4 (the highest stress level) had almost completely senesced.

Water stress caused a significant reduction in plant biomass and seed production of wild radish (Table 1). There was no significant difference between the dry matter and seed number per plant for S4, S5 and S6, while S8 and control produced significantly larger plants with more seeds.

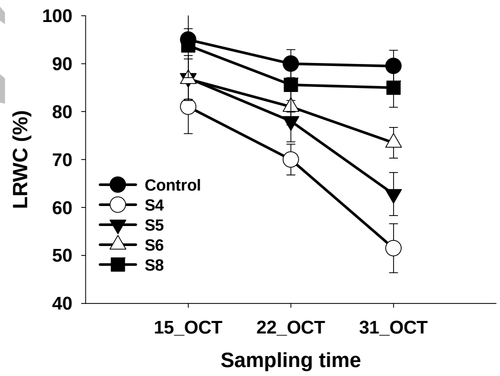

Figure 2. Leaf relative water content (LRWC) of wild radish for non-stressed and stressed plants. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

Table 1. Dry matter, seed number per plant and seed mass of wild radish in different water stress treatments in 2005. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

Treatment	Dry matter (g plant^{-1})	Seeds plant^{-1}	Seed mass (mg seed^{-1})
S4	25.5	337	3.9
S5	35.2	351	4.8
S6	36.7	739	5.1
S8	89.8	2182	5.5
Control	99.4	2389	6.0
SED	9.72	376.4	0.46

The stressed plants matured sooner and produced significantly smaller seeds compared to the control (10-35%). The S4 treatment which received water stress earlier than the other treatments finished its growth two weeks sooner than the other treatments (on December 9).

The dry matter, seed production and seed mass of the S8 which faced with water stress only after natural leaf senescence was not reduced as much (only 10% below the nonstressed plants) as the other stressed treatments (S4, S5 and S6).

Water stress had a greater impact on seed number per plant (9-85% reduction compare to nonstressed plants) relative to the other traits measured in this study (Table 1). Irrespective of the water stress treatments, earlier sampled pods had thicker pod walls and greater pod segment weight (Table 2). The pod wall weight/seed weight ratio was considerably higher in the control and S8 treatments compared to other water stress regimes, indicating the higher pod wall strength in these treatments. Although seed coat weight tended to be slightly lower in later collecting times, the seed coat weight/seed weight ratio did not show a substantial difference between different collection dates (Table 2).

Table 2. Pod wall, seed, and seed coat characteristics (±SEM) in different collection dates for different water stress treatments in 2005. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

Treatment	Collection Date	Pod wall thickness (µ)	Pod wall weight (mg)	Seed weight (mg)	Pod wall weight/seed weight	Seed coat weight (mg)	Seed coat weight/seed weight
S4	Nov_11	514 ± 27.5	15.0 ± 0.63	4.6 ± 0.26	3.3 ± 0.22	0.6 ± 0.07	0.13 ± 0.008
	Nov_25	428 ± 21.9	14.8 ± 0.81	4.0 ± 0.37	3.8 ± 0.54	0.6 ± 0.03	0.15 ± 0.006
	Dec_9	341 ± 19.2	9.3 ± 0.44	3.5 ± 0.22	2.7 ± 0.34	0.4 ± 0.09	0.11 ± 0.010
	Dec_23	-	-	-	-	-	-
S5	Nov_11	775 ± 51.2	19.8 ± 0.83	5.1 ± 0.57	3.8 ± 0.53	0.6 ± 0.07	0.12 ± 0.007
	Nov_25	622 ± 48.2	18.8 ± 0.69	5.1 ± 0.34	3.7 ± 0.50	0.6 ± 0.03	0.12 ± 0.014
	Dec_9	524 ± 31.1	18.0 ± 0.78	4.8 ± 0.24	3.7 ± 0.10	0.5 ± 0.04	0.10 ± 0.010
	Dec_23	377 ± 28.1	11.6 ± 0.58	3.6 ± 0.18	3.2 ± 0.20	0.5 ± 0.05	0.14 ± 0.011
S6	Nov_11	763 ± 33.3	20.8 ± 0.65	5.3 ± 0.61	3.9 ± 0.20	0.6 ± 0.06	0.11 ± 0.008
	Nov_25	685 ± 27.8	20.6 ± 0.98	5.3 ± 0.32	3.9 ± 0.33	0.6 ± 0.04	0.11 ± 0.006
	Dec_9	612 ± 22.1	19.6 ± 0.51	5.0 ± 0.33	3.9 ± 0.27	0.5 ± 0.07	0.10 ± 0.010
	Dec_23	508 ± 34.2	12.3 ± 0.52	4.3 ± 0.47	2.8 ± 0.51	0.5 ± 0.03	0.11 ± 0.009
S8	Nov_11	873 ± 46.3	23.8 ± 0.63	4.8 ± 0.34	5.0 ± 0.73	0.7 ± 0.02	0.15 ± 0.012
	Nov_25	754 ± 41.2	24.5 ± 0.58	5.4 ± 0.45	4.5 ± 0.54	0.6 ± 0.05	0.11 ± 0.005
	Dec_9	682 ± 53.2	20.8 ± 0.91	6.2 ± 0.19	3.3 ± 0.51	0.6 ± 0.03	0.09 ± 0.004
	Dec_23	614 ± 36.8	18.7 ± 0.51	4.6 ± 0.22	4.0 ± 0.33	0.5 ± 0.04	0.10 ± 0.008
Control	Nov_11	982 ± 52.2	30.2 ± 0.85	5.7 ± 0.27	5.3 ± 0.83	0.7 ± 0.02	0.12 ± 0.010
	Nov_25	790 ± 42.4	27.5 ± 0.13	5.8 ± 0.38	4.7 ± 0.18	0.7 ± 0.06	0.12 ± 0.009
	Dec_9	787 ± 24.6	23.7 ± 0.79	6.3 ± 0.27	3.8 ± 0.30	0.6 ± 0.09	0.09 ±0.010
	Dec_23	674 ± 20.9	20.5 ± 0.66	4.8 ± 0.16	4.3 ± 0.20	0.5 ± 0.04	0.10 ± 0.007

There were clear differences between S4-S6 and the control in pod wall weight and seed weight. Although, all treatments produced smaller seeds in later collection dates, differences between S4-S6 and the control were quite large.

Application of water stress to maternal environment caused an increase in total germination percentage in both January and May germination tests (Figures 3, 4, 5 and 6). The germinability of all treatments increased with pod removal. The dormancy level tended to be lower in later collected seeds relative to the earlier collected ones. There was a significant negative relationship between seed weight and germinability of naked seeds (Figure 7). A similar relationship was also found between pod wall weight and germinability of pod-enclosed seeds (Figure 7).

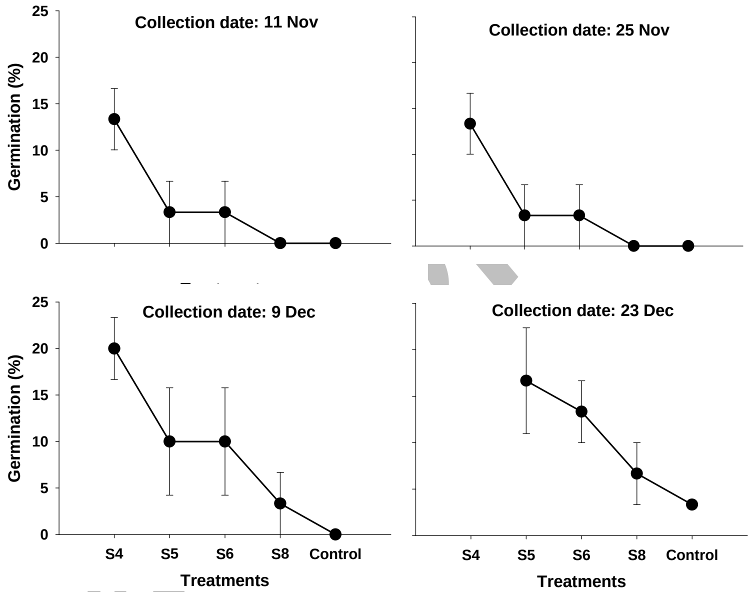

Figure 3. Germinability of pod-enclosed seeds of wild radish in different collection dates for different water stress treatments in January 2005. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

Discussion

Leaf senescence was initiated earlier in the water-stressed plants than in the control (nonstressed). The LRWC clearly showed that the water stress became more severe at later stages of reproductive development (Figure 2). However nonstressed plants also showed a reduction in pod weight, pod wall thickness and seed weight in pods formed later in the season which could be due to other limiting factors such as supply of photosyntates. Not surprisingly, seed weight was negatively correlated with the severity of water stress. Water stress showed strong adverse effect on pod wall weight and seed weight in all collection dates. The results showed that even in early collection dates water stress caused clear differences in pod wall weight and seed weight between S4-S6 and the control.

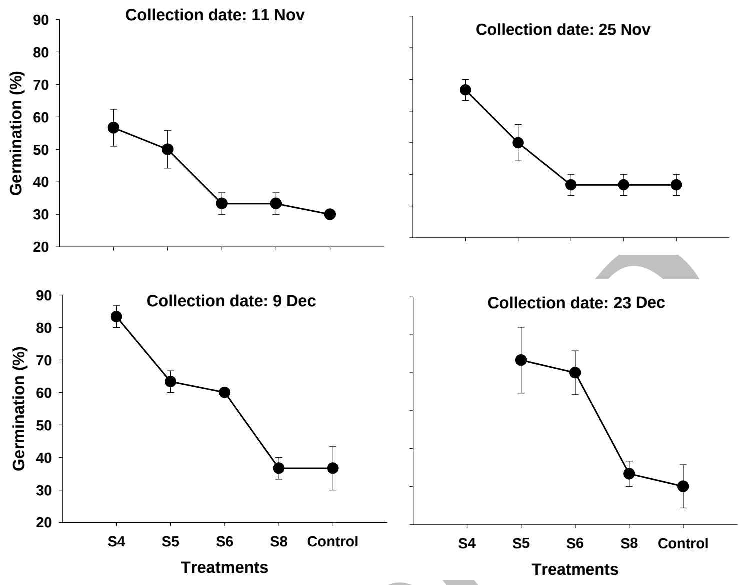

Figure 4. Germinability of wild radish seeds without pod in different collection dates for different water stress treatments in January 2005. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

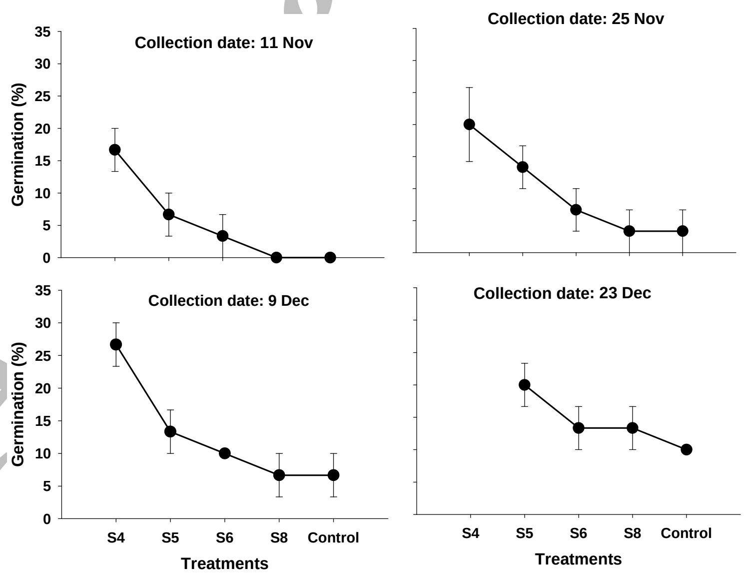

Figure 5. Germinability of pod-enclosed seeds of wild radish in different collection dates for different water stress treatments in May 2005. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

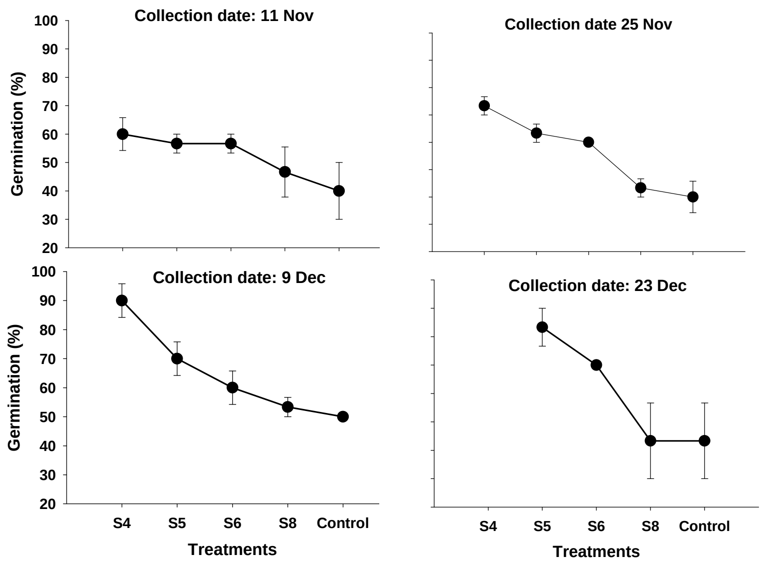

Figure 6. Germinability of wild radish seeds without pod in different collection dates for different water stress treatments in May 2005. Water stress was imposed at different developmental stages (S4: first bud opened, S5: largest pods less than 1 mm diameter, S6: largest pods 1-2 mm diameter, and S8: leaf senescence).

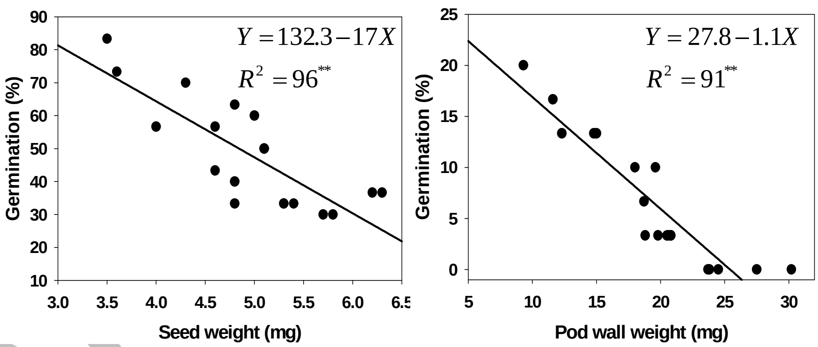

Figure 7. Relationships between seed weight and germinability of naked seeds (left) and between pod wall weight and germinability of pod-enclosed seeds (right).

At first collection date, the pod wall weight from the S4 (imposed to water stress at flowering) was almost half of that in the control. The seed mass of S4 was about 80% of that in the control and decreased to around 60% at the third sampling date, indicating that pod wall was more sensitive to water stress than the seed. It also shows that plant allocates less assimilates to the pod wall in favor of seed filling under water stress conditions. It is also possible that some of the late-formed seeds may have aborted thereby affecting the level of reduction in mean seed weight observed in this study. It was interesting to observe faster dormancy release in wild radish seeds produced under water stress conditions (Figures 3-6). These seeds were enclosed by thinner pod walls and were also smaller in size. It is noteworthy that 50-60% of naked seeds from the control and S8 were still dormant in May (start of the growing season). In contrast, at this time only 10% of the S4 treatment showed dormancy in December collected seeds. There were strong negative relationships between seed weight or pod wall weight and germinability of extracted or pod-enclosed seeds of wild radish (Figure 7). Seeds produced earlier were heavier and also had a higher dormancy level, indicating the existence of more favourable condition at early stages. Moreover, early collected pods showed heavier and thicker pod wall which could partly explain lower germinability of the early collected seeds when subjected to germination test with the pod. The negative relationship between seed weight and also pod wall weight with germinability provided some evidence for the existence of physical dormancy in this species. However, this result does not preclude some contribution by physiological dormancy as reported earlier by Eslami et al. (2006).

These results point to the role of maternal environment in regulating seed dormancy in this weed species. There are several previous studies where it has been shown that water stress during fruit or seed development leads to reduced dormancy (Benech Arnold et al., 1991; Benech Arnold et al., 1992; Peters, 1982; Sanchez et al., 1981; Sawhney and Naylor, 1982; Sharif-Zadeh and Murdoch, 2000; Wright et al., 1999).

The climatic conditions experienced in agricultural areas of South Australia can be quite variable; it might have a wet or dry spring in different years. The ecological implication of these differences is that seeds produced in a wet spring are likely to have slower rate of dormancy release than those produced in a dry spring. Thus, seeds from a wet year may be more likely to become part of the persistent soil seedbank than those produced in a dry year. In contrast, in a dry year wild radish plants will produce fewer seeds and the potential for these seeds to persist in the soil seedbank will be reduced. Most seeds produced under water stress conditions are likely to germinate in the first winter after shedding. In conclusion, these results showed that wild radish has a seed dormancy strategy which, through a combination of elements, makes it superbly adapted to the Mediterranean environment in which it thrives. This weed species showed several mechanisms which could be responsible for long-term seed dormancy but also provide sufficient germinable seeds for infestation when conditions become favourable.

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