Seed quality and germination in wild radish (*Raphanus raphanistrum* L.)

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Summary Seeds of wild radish (*Raphanus raphanistrum*) were assessed for germination in relation to segment position, seed size and silique size. Seeds from small segments had poor germination. Segments in proximal positions on the silique have larger seeds and tended to germinate earlier than seeds from distal segments on the silique. Seed size can be influenced by stress on the plant, for example stress caused by herbivory, which makes it important to include seed size in the assessment of the impact of potential biological control agents on wild radish.
Keywords Wild radish, seed, silique, germination.

INTRODUCTION

Wild radish (*Raphanus raphanistrum*) produces abundant siliques (pods), each consisting of one to twelve segments, with each segment containing a single seed. In late spring the mature silique falls from the plant and breaks into segments during summer. The segment provides a hard protective coating that also delays germination of the seed (Mekenian and Willemsen 1975), which usually occurs in autumn following rains and soil disturbance. Seeds are the sole means of regeneration in this annual species, but seed decline can be rapid (33% mortality per year) (Chancellor 1986) implying that seed quality is important.

The seed has six fold to twenty fold variation in seed mass (1.5 to 12 mg) (Stanton 1984a,b, Mazer 1987, Mazer *et al.* 1986). Large seeds (>6 mg) are more likely to emerge as seedlings than small seeds (<4 mg) (Stanton 1984a). Environmental sources of variation in seed weight include the maternal environment, the position within the silique, the number of seeds per silique, the age of the individual on which a flower is pollinated and the pollination history of an individual (Mazer 1987). Part of the maternal environment includes the stresses that might be caused to the plant by insects and pathogens such as biological control agents. Lehtilä and Strauss (1999) stressed plants of wild radish by simulating the effects of foliar herbivory. They found that plants were able to compensate in the total number of flowers and fruit they produced and that male fitness traits (petal size, pollen number and pollen size) were more strongly affected by herbivory than were female traits (ovule size and number, fruit and seed production).

In the study reported here we quantify the size differences between seeds within the silique and relate this to their germination success. The reason for this study was to determine if there are measurements that should be taken into account when assessing the impact of biological control agents, which must be on either seed number or quality.

MATERIALS AND METHODS

Number of seeds per silique Samples of siliques from eight sites in the Mediterranean region were measured to provide information on the number of seeds per silique.

Relationship between seed, segment and position Seeds were extracted from segments and 93 undamaged seeds and their corresponding segment were weighed separately. Seed size in relation to the position on the silique was measured by randomly selecting segments from x-rayed images of siliques from four sites until the selection included 30 seeds from positions one to eight.

Germination 1000 segments originating from Brittany, France, were sown into soil. Every three weeks, the soil was worked (to simulate cultivation) then watered. The seedling trays were not disturbed nor watered during summer, from June to September. One to three times a month from 21 September 1998 to 18 December 2000, seedlings were counted and removed from the tray.

Relationship between segment size and germination Segments from Brittany were classified into four categories according to their diameter measured with a vernier calliper: I <2.5 mm; II 2.5–3.5 mm; III 3.5–4.5 mm; IV >4.5 mm. Fifty segments per category were sown in seedling trays. The trays were watered and soil disturbed as above, seedlings were counted and removed up to three times a month. At the end of the trial the soil was sieved and the segments examined to determine mortality and dormancy.

Relationship between segment position and germination In this experiment, the siliques were collected from plants grown in the glasshouse from Brittany seeds. The siliques were classified according to their number of segments (from 1 to 8), and segments according to their position on the silique (segment 1 = first segment after the petiole, and so on from 1 to 8). Twenty-five segments per classification were sown in a seedling tray and the 36 seedling trays were organised at random on a table in the glasshouse. Results were divided into two periods; the first, from the 12th February 1999 to the 23rd August 1999 (first flush of germination), and the second, from the 12th February 1999 to the 2nd April 2001 (germination levelled out). At the end of the trial the soil was sieved and the segments examined to determine mortality and dormancy.

RESULTS

Wild radish has siliques with an average of 4 to 7 segments per silique. The number of segments per silique ranges from 1 to 12 (Table 1).

Larger segments contained larger seeds ($Y = 0.14x + 0.004$, $R^2 = 0.30$, $P < 0.05$). The weight of seeds ranged from 3 mg to 11 mg. The seeds in the four proximal positions were significantly larger and similar in size ($F = 15.16$, $P < 0.001$, $n = 240$) (Figure 1). In the preliminary observation of germination, a third of seeds germinated by the 18th of January 1999 and 50.2% by 18th December 2000.

The percentage germination was initially related to segment size (Figure 2). Small segments contained seeds that had poor germination (10%), due to high (84%) seed mortality or abortion. The second size category (II) showed a rapid germination when compared with the larger segments. However, after two years, the percentage germination of the three larger size categories of segments was similar (Figure 2). These three categories had similar seed mortality (II, 50%; III, 40%; IV, 52%).

There is considerable variation in the percentage germination according to the relationship between the number of segments and the position of the segment. An analysis of independence between germination below or above the median versus the segment position is shown in Table 2. This analysis shows that in the first flush of germination, the first two segments have a higher percentage germination. In contrast,

Table 1. Number of segments per silique from eight sites.

Location	N siliques	Segments per silique Mean ± se	Min–Max
San Romana (Spain)	100	6.1 ± 0.12	3–10
Vendres (France)	100	5.9 ± 0.14	2–9
Grassette (France)	100	7.0 ± 0.20	2–11
Sauvian (France)	100	6.4 ± 0.13	3–10
Tabarka (Tunisia)	84	4.0 ± 0.21	1–8
Bizerte (Tunisia)	100	7.4 ± 0.20	2–12
Bou Salem (Tunisia)	98	6.7 ± 0.25	1–11
Teboursuk (Tunisia)	99	6.9 ± 0.20	2–12

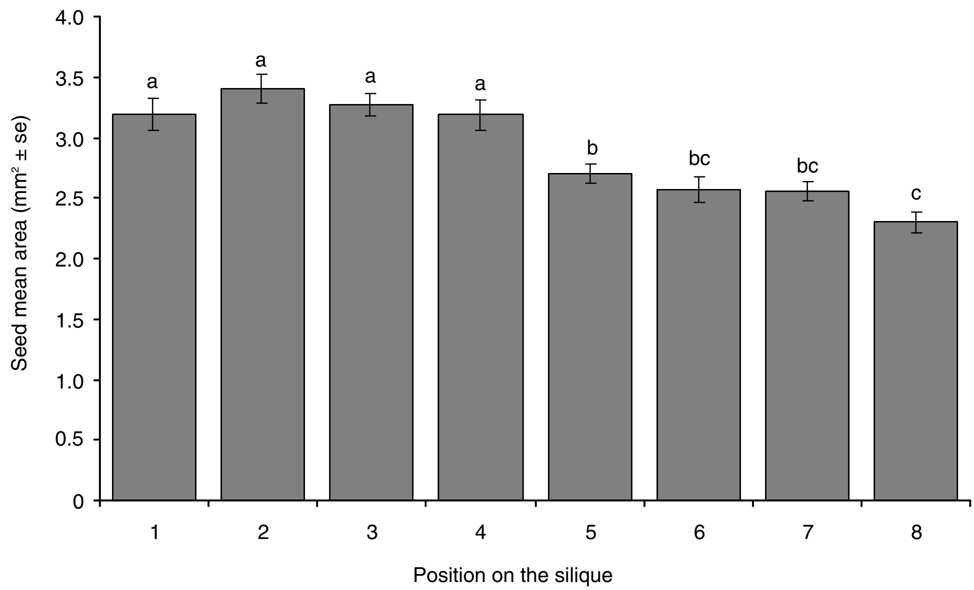

Figure 1. Mean seed area measurement according to the segment position on the silique (mean $mm^2 \pm se$). Similar letters indicates groups that are not significantly different by a LSD (t) comparison of means.

the relative positions of the segment, be it on a short silique or on a long silique, was not associated with increased germination.

DISCUSSION

Most of the information on the factors that affect seed size in wild radish are based on plants from North America and the study here is the first involving the plant from its native habitat.

Seed size is related to the position in the silique, with the larger seeds found on the basal and middle segments (Mazer *et al.* 1986, Stanton 1984b). Stanton (1984a) found that larger seeds (>6 mg) were more likely to emerge as seedlings, and develop into plants that produce more flowers than smaller seeds (<4 mg). Our results were similar which points to a minimum size for wild radish seed survival. Annual plants like wild radish have indeterminate growth and continue flowering and seed development until the plant dies, usually from the summer drought. This means that an increasing percentage of the seed will be immature as the plant increases in size and age. Cheam (1986) showed that early sown wild radish produce up to 93.4% dormant seed whereas plants sown three months

Table 2. Test for independence between the per cent germination and the segment position on the silique.

	% Germination	Number of trials: Segment position on silique 1 and 2	3 and 4	5 to 8	χ^2	P
First flush germination	<median	3	9	6	10.3	0.006
	>median	12	2	4		
Total germination	<median	3	7	5	5.37	0.068
	>median	12	4	5		
		Segment number on silique 1 to 4	5 and 6	7 and 8		
First flush germination	<median	4	7	7	1.28	0.526
	>median	6	4	5		
Total germination	<median	2	7	6	4.13	0.127
	>median	8	4	9		

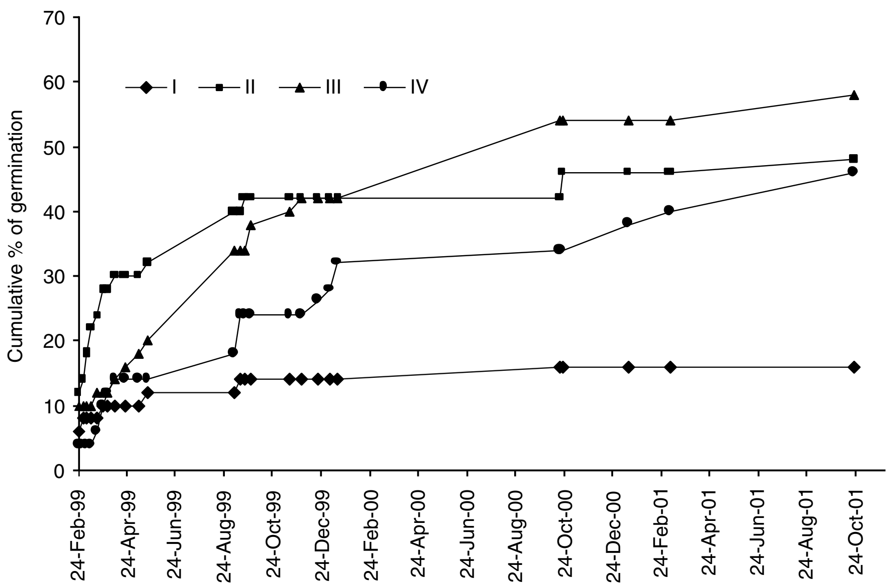

Figure 2. Cumulative percentage of germination according to the size (diameter) of the segment, grouped as follows I <2.5 mm; II 2.5–3.5 mm; III 3.5–4.5 mm; IV >4.5 mm.

later produced 68.4% dormant seed. It is possible that seeds with less development time would be smaller and this difference in dormancy could be explained by seed size.

Stanton (1984b) examined cross-sections of segments and found that as seed size increases, the seed coat thickness to volume ration decreases and concluded that there would be a decrease in structural strength of the seed coat. In other species, lower seed coat strength can result in premature breakdown of dormancy (references in Stanton 1984b). Our measurements of the weight of seed and segment without the seed found a positive relationship between the two, but with a slope less than one (0.14), which supports the idea that larger segments have thinner coats.

Stanton (1984a) concluded that time of emergence was not related to seed size. Our results indicate that smaller seeds (excluding the smallest group with limited germination) germinate earlier than larger seeds, but that this difference disappears with time. This may be more a question of timing, where larger seeds are not the first to germinate, but also do not have as high a level of dormancy as seeds of a smaller size. If this was the case then the larger, more readily germinating seeds may have an advantage initially, but it may be the smaller better protected seeds that become dormant. Young and Cousens (1998) using x-ray observation of seeds in intact segments and showed that seeds could imbibe moisture, but still not germinate. Both the seed coat and the segment play a role in dormancy, but the relative importance still needs to be elucidated.

Mazer (1987) concluded that seed weight in wild radish is not highly heritable. Subsequent studies point to the importance of environmental conditions and plant stresses playing an important role in determining the quality of reproduction in wild radish (Lehtilä and Strauss (1999). Indeed, Agrawal (1999) shows that in some situations it is possible to induce major changes in the plant defence against herbivores. A small amount of specialist herbivory early in a plant's development conferred resistance to generalist herbivores latter, allowing for a superior seed production in the 'induced' plants. The cost to seed quality has not been measured in these types of experiments and this needs to be taken into account in biological control programs, where only specialist herbivores would be used. However, Marshall and Ellstrand (1988) measured the effect of stress (water deprivation) on seed production in *Raphanus sativus* from a population naturalised in North America and concluded that *R. sativus* under stress selectively aborts seeds depending on the origin of the pollen, and position in the silique. Seed abortion occurs more frequently at the distal end of the silique.

It is important for future management of wild radish that the variation in seed quality be taken into account so that the result of control efforts, be it biological control or herbicides, leads to an overall reduction in seed survival.

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