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Pre-dispersal seed predation and seed limitation in an annual legume

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Abstract

In field experiments (1993–1998) using an annual self-pollinated vetch species, *Vicia angustifolia*, the effects of pre-dispersal seed predators (pre-DSPs) on plant performance and demography were studied. Insect exclusion by insecticides, vegetation perturbation at the start of the experiment by removing the top soil layer, and sowing extra seeds were applied at one site (Site 1) on 32 plots, and only insect exclusion at another site (Site 2) on 14 plots, respectively, in the plant's natural habitat. The number of seedlings was influenced most by perturbation at Site 1. There were 2–4 times more seedlings on bare plots in comparison with vegetation-covered plots, although significant difference was detected only in 1993, 1994 and 1997. Density of adult plants was lower on vegetation-covered plots. Dry matter, height, length of branches, and of pods were positively affected by perturbation on Site 1. The number of seeds infested by pre-DSPs was generally low, however, it was higher on unsprayed plots, regardless whether the plot was perturbed or not. Overall, seed loss to pre-DSPs was only ca. 10%. Sowing extra seeds among vegetation increased net plant recruitment demonstrating that microsites were available in the habitat. *V. angustifolia* is assumed to be a weak competitor and, in spite of the low percentage of seed loss to pre-DSPs, it is most likely limited by seed number. The deficit in using available microsites might be caused by post-dispersal mortality factors.

Der Einfluss von Vordispersions-Samenfresser (VDSF) auf Produktivität und Demographie der selbstbestäubenden Wickenart, *Vicia angustifolia,* wurde in Feldversuchen (1993–1998) untersucht. Am natürlichen Standort der Wicke wurden an 32 Quadraten folgende Varianten angelegt: Ausschluss der Insekten mit Insektiziden, Störung der Vegetation (Entfernen der oberen Bodenschicht) und Aussaat von extra Samen (Ort 1), sowie an 14 Quadraten bloß Ausschluss der Insekten (Ort 2). Die Anzahl der Keimlinge war am Ort 1 am meisten durch die Störung der Vegetation beeinflusst. In den Quadraten ohne Vegetation entwickelten sich 2 bis 4-mal mehr Keimlinge, als in den kahlen Quadraten, obzwar signifikante Unterschiede nur in 1993, 1994 und 1997 gefunden wurden. Die Populationsdichte der adulten Wickenpflanzen war kleiner in den Quadraten mit Vegetation. Trockensubstanz, Höhe, Länge der Seitentriebe und der Hülsen waren am Ort 1 durch die Störung positiv beeinflusst. Die Anzahl der durch VDSF beschädigten Samen war im allgemeinen klein, sie war jedoch an insektizidbehandelten Quadraten größer, unabhängig davon, ob die Vegetation gestört wurde oder nicht. Der durch VDSF verursachte Samenverlust war durchschnittlich bloß etwa 10%. Die Aussaat von Wickensamen in die natürliche Vegetation erhöhte die Anzahl der Keimlinge, d.h., es waren freie Mikroorte vorhanden. *V. angustifolia* ist wahrscheinlich ein schwacher Kompetitor und ihre Population ist durch die Samenzahl beschränkt, nämlich durch den Einfluss von Nachdispersions-Samenschädlingen.

Key words: Bruchid – *Bruchus luteicornis* – Hungary – Leguminosae – population dynamics – recruitment – vetch – *Vicia angustifolia*

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Introduction

Seed predation represents an important source of seed mortality. Its two main versions, pre- and post-dispersal seed predation, substantially differ in their net effect on plant recruitment. Pre-dispersal seed predation (pre-DSP), caused by specialist insects, can seriously affect plant performance (Janzen 1971, Crawley 1983, Louda 1989, Crawley 1989ab, 1992 and references therein) by reducing seed production, but has little impact on plant recruitment (Crawley 1992) for at least two reasons (Andersen 1989): (a) because of the limited availability of microsites (safe sites) suitable for germination, and (b) because of the nature of seed bank (Harper 1977).

Crawley (1990) hypothesized that most plant populations would be limited by the scarcity of safe sites, and that in woody habitats such a limitation can be more frequent than in grasslands. Eriksson & Ehrlén (1992) found that various limitations were equally probable in woody habitats. Although it seems that the number of plant species studied so far is not sufficient to generalize, microsite limitation does indeed seem to characterize grasslands (e.g., Maron & Simms 1997). Microsite limitation can properly be proved or disproved by seed augmentation experiments (Turnbull et al. 2000). If seedling recruitment increases following addition of seeds, then microsites are available and seed number limitation is the proximate cause (Crawley 1989ab). The type and level of seed limitation are, among others, functions of habitat and successional stage (Turnbull et al. 2000).

Pre-DSPs demonstrate great variability in impacts on plant performance. The value of seed destruction notoriously run between 0 and 100% on the parent plant (Janzen 1971, Crawley 1989ab, 1992, Szentesi et al. 1996, Crawley 1998, Szentesi 1999). Their effects on plant population dynamics, however, have been less often demonstrated experimentally (Louda 1982ab, 1983, Louda & Potvin 1995, Brown et al. 1987b). Exclusion experiments are a critical part of this type of work (Waloff 1968). However, as Crawley (1998) stresses, in such experiments it is impossible to separate the effects of herbivore release from that of plant competition. Furthermore, post-dispersal seed predators (Brown et al. 1986) and the characters of the seed bank (Leck et al. 1989) may crucially influence the number of seedlings. Annual plant species seem more prone to form persistent seed banks (Rice 1989) and leguminous annuals frequently produce short-term persistent seed banks.

In the present study, we add data to this relatively neglected area and attempt to clarify whether plant recruitment in populations of an annual vetch species, *Vicia angustifolia* L. (Leguminosae), is limited by mi-crosites or by seed numbers. We study the questions by sowing experiments, by insect exclusion and by assessment of plant recruitment rates on sites devoid of vegetation. The species has not yet been subjected to such investigations.

Specifically we aim to answer these questions: (1) is *V. angustifolia* population seed-limited due to the impact of pre-dispersal seed predators, or (2) is it microsite-limited, and (3) does the seed bank affect net recruitment?

Materials and methods

Experimental sites and design

The experiments were carried out in Hungary (47°32′N, 18°56′E), at ca. 360 m a.s.l. at two sites, where *Vicia angustifolia* was abundant. Precipitation is 600–800 mm per year falling in spring and autumn.

Site 1 was a seminatural grassy area. The experimental area was fenced (with holes sized to allow small mammals to move in and out, however, to inhibit large mammals trespassing and trampling). The 32 plots of 50 × 50 cm, separated by 50 cm wide walkways were set up in 1993 and were used until 1998.

Site 2 was located ca. 1 km from the first one and was not fenced. In addition to dominant graminaceous species, there were some individuals of *Trifolium* and *Coronilla* species. The site served as a simplified backup experiment, following a heavy rodent damage on Site 1 in 1995. On Site 2 only exclusion was applied. All 50 × 50 cm plots (7 sprayed and 7 control) plots were covered with vegetation and were scattered within the site. Experiments run here from 1996 to 1998.

Additional control plots. Near Site 1, there were eight control plots separated from the rest by a distance of 2 m. No treatments of any kind were applied here and they served as "null-treatments".

On Site 1 the following treatments were applied in a factorial design with 4 rows × 8 columns: (A) perturbation: *level 1* = no perturbation: 16 plots had the original vegetation, *level 2* = perturbation: vegetation removed from plots; (B) sowing: *level 1* = 50 seeds, *level 2* = 100 seeds; (C) exclusion of pre-DSPs: *level 1* = no exclusion, *level 2* = exclusion: sprayings with insecticides.

Perturbation was intended to answer the *microsite limitation hypothesis*. We removed the existing vegetation and the upper 1 cm layer of soil from 16 plots at the start of the experiment. Subsequently, all new or regenerating plants were continuously clipped off. The other 16 plots had their original vegetation.

Sowing was applied on both perturbed and intact plots. As the possible mortality factors affecting the test plants were not known, we applied two levels of

sowing with seeds harvested at different localities of Hungary in1993. The seeds were sown at a depth of 0.5–1 cm by a sowing template, on 15–16 Sept., 1993. Following a rodent (*Microtus* sp.) outbreak in 1995 there was no seed-production, therefore, each plot was re-sown with 25 seeds, as we expected some recruitments from the seed bank too (see Measurements, sampling and collections). In laboratory, *V. angustifolia* seeds had 55.6 ± 31.5% of germination (mean ± SD; 516 seeds from 8 samples, 1–3 replicates/sample) and a germination time range of 6–102 days.

Exclusion treatment was expected to answer the *seed number limitation* hypothesis. Sixteen plots received insecticide treatment, and the other 16 were sprayed with the same amount of water plus detergent (Tween 20 at 0.025% v/v). For the first three years Danadim® 40EC (Cheminova Agro A/S) in 0.2% (v/v), whereas in the second two years Enduro® (Bayer) was used in 0.25% (v/v) concentrations. The change of insecticides was necessary as the first one did not give a sufficiently high protection against pre-DSPs. Insecticide treatments can stimulate plants in several ways (Brown et al. 1987a). The N-content of insecticides is a possible nutrient source for treated plants (e.g., Root 1996). The amount of delivered N to a single plot by spraying insecticides amounted to 0.5–0.85 $gm^{-2}year^{-1}$ N that is insignificant, because a usual fertilizer treatment adds 32 $gm^{-2}year^{-1}$ N (Ganade & Brown 1997), i.e. a 37–64 times higher dose. Water applied by sprayings can also help damage compensation (Crawley 1983). We sprayed the plants weekly for at least 10 weeks between end of May and beginning of August. The amount of spray (150–170 ml/plot at a time) corresponded to 0.6–0.7 mm precipitation. We think that this amount of water was of negligible importance. During spraying we placed an 80 cm high frame around the plots. No phytotoxic effects were observed. Besides the regular sprayings, a granular rodenticide Redentin® (0.0075% chlorophacinone) had to be applied against rodents (*Microtus* sp.) from 1995 on.

The plant and its herbivores

V. angustifolia, named also as *V. sativa* ssp. *nigra* (Tutin et al. 1968), is a gap colonizer annual herb (Hanelt & Mettin 1989). It relies on the neighbouring plants for support. It is a selfer, thus netted flowering plants produce seeds. Flowering and pod ripening is continuous from the end of May until late September, however, most seeds are ripe by the end of June. Ripe pods are dark brown, dehiscent, 2–7 cm long, contain 6–12 spherical seeds of 1.5–2 mm size, and of 10–15 mg weight. *V. angustifolia* presumably forms a persistent seed bank of the short-term type (Thompson et al.

1993). Many seeds germinate still in the same year at the end of August and September. The young plantlets grow until November. The root overwinters and growth continues next March on. New seedlings appear in the next year, too (Á. Szentesi unpubl. obs. and see Results).

The pre-DSP guild of *V. angustifolia* consists of a single bruchid species, *Bruchus luteicornis* Ill. (Coleoptera, Bruchidae), four curculionids, *Apion cerdo* Gerst., *A. pomonae* (F.), *A. punctigerum* Payk. and *Tychius quinquepunctatus* (L.) (Coleoptera, Curculionidae), and a lepidopteran, *Cydia nigricana* Steph. (Lepidoptera, Tortricidae) (Szentesi & Jermy 1998). Based on 42 samples of *V. angustifolia* from Hungary, the relative frequency of the three main pre-DSP groups was roughly the same [percentage of infested seeds by the bruchid was 3.3 ± 5.6% (mean ± SD), by the curculionids 3.7 ± 5.0%, and by the tortricid 3.0 ± 5.6% (Á. Szentesi & T. Jermy unpubl. obs.).] Shrivelled seeds indicated that homopterous bugs also fed on seeds, however, neither the species nor the extents of damage have been determined. Damages of other types made to the plant included occasional slug-grazing, curculionid feeding on leaves, gall forming on apical parts by dipterous larvae, as well as damage done by rodents (*Apodemus* and *Microtus* spp., see Results).

Adults of the univoltine bruchid appeared on *V. angustifolia* in middle-May when the plant height was only 15.4 ± 8.0 cm mean ± SD, *n* = 81, 1996) and no flowers were present yet. The eggs are laid on the green pods from early June on. Females lay 1–10 or more eggs on the pods (Á. Szentesi & T. Jermy unpubl. obs.). L1 larvae bore in on site and enter a still developing seed. A single seed can support one bruchid larva only. Pupation takes place within the seed. The beetles of the new generation emerge in August.

The curculionid species are univoltine. They lay eggs at about the same time when the bruchids do. They chew holes on the pods and place the eggs, sometimes several in the vicinity of a seed within the pod's cavity. Curculionid larvae consume several seeds from outside in a sequence, but within the pod. For pupation they either leave the pod (*Apion* spp.) or remain inside (*Tychius* spp.).

C. nigricana lays eggs singly on pods that started ripening. The larva entering the pod consumes seeds one by one. The mature larva leaves the pod for pupation. This species is univoltine, however, sometimes a second generation is observed in Hungary (Reichart 1957).

Braconid (e.g., *Triaspis pallipes*) and chalcidoid (e.g., *Dinarmus acutus*, *Pteromalus sequester*) parasitoids have been found in the *V. angustifolia* samples collected over years.

Measurements, sampling and collections

In each year, at the end of May or in early June we estimated the density of adult *V. angustifolia* plants around the plots and in the surrounding in at least ten, 50 × 50 cm size, randomly placed quadrats. In addition, twice a year (in early May and in November) we counted the new *V. angustifolia* plants at seedling stage (4 leaf-storeyed and ca. 10 cm of height) on all plots.

We marked 5 randomly selected plants (or as many as were available if less than 5 were present) on each plot, at both experimental sites. Twice a year (at mid-May and at mid-June), we measured nine growth characters of the marked plants (number of stems, height of plant, number of leaf-nodes, number and length of branching shoots, number of flowers, number of pods, lengths of pods, amount of dry matter/plot) as well as the number of pre-DSPs present, that of the eggs laid on pods and damage done to plants.

Impact of pre-DSPs on plant performance was measured through seed production. Mature pods were continuously picked, until all pods of a plot were harvested. They were numbered and stored individually in vials. During August we opened the pods and removed all uninfested, intact seeds. These were pooled for each plot, and returned to it and spread by the first days of September. For the evaluation of the pods Andersen's note (1988) was considered. The number of seeds of various qualities (aborted = died in ovule stage; underdeveloped = larger than the former, but not reaching full seed size and frequently shrivelled; intact = fully developed without pre-DSP impact; infested = partly or totally consumed by a pre-DSP), the number of infertile, parasitized, and hatched eggs of the pre-DSPs, as well as number of boring holes of bruchid L1s on pods, were recorded. As a special impact, rodents' feeding activity was also noted. Most probably an *Apodemus* sp. functioned as a real pre-DSP.

We also collected *V. angustifolia* fruit samples at various parts of the country yearly and compared pre-DSPs infestation levels. A minimum of 30 pods per collection site were put into glass vials individually and evaluated as described above.

Bagged control plants. In the larger area surrounding the experimental sites, 14–54 individual *V. angustifolia* plants were covered yearly with 1 m long linen bags of <1 mm mesh attached to a long stick, at the start of flowering. Thus, these plants had no impact from pre-DSPs or from other herbivores. At the end of August, the aboveground parts were removed, dried at 105° C and weighed to the next 0.1 mg. Seeds were saved for other purposes, therefore, the weights of individual seeds were measured at room temperature only.

Microsite limitation experiments

Experiment "A" intended to answer the question whether additional seeding would increase recruitment rate ('seed augmentation' *sensu* Turnbull et al. 2000). It was set up in 3 replicates, two at Site 1, and one at Site 2. There were three 50 × 50 cm size plots arranged in a row with 100% graminaceous plant cover. We spread 10, 100 and 1000 seeds, respectively, over the plots on August 1, 1996. We counted the new recruits in the vegetation in October of the same year, then in spring of 1997 and 1998, respectively. The seedlings were left in place. Control density data of *V. angustifolia* from the same sites (spring of 1997 and 1998) were used for a comparison with "A"(see Results).

Experiment "B" answered the question whether the use of microsites was a function of germination rate at a time. For this we removed the vegetation and the upper 1 cm soil layer at 5 places of 25 × 25 cm size and 100 seeds were spread over trays of the same size made of plastic net, 1.5 mm mesh, on August 1, 1996 ('seed introduction' *sensu* Turnbull et al. 2000). The trays were covered with plant debris from the same site. Seedlings at 5 to 7 cm size were removed in October of the same year and for further two years (1997 and 1998).

Data and statistical treatments

Considering the effects of treatments, three years' data were used from both sites, because on Site 1, due to a rodent outbreak, plant performance data from 1994 and 1995 were lost with the exceptions of records on growth characters, mature seed weights and the numbers of seedling plants in spring and autumn. The data of the two sites were not pooled.

The experimental design (perturbation × sowing × exclusion) satisfied conditions for a 3-way ANOVA analysis. Most traits measured did not have homogeneous distribution of data, therefore, various transformations were applied. For data analysis general linear modelling (Visual GLM module of Statistica) was applied considering the treatments as categorical predictor variables. In order to avoid pseudoreplications (Crawley 1993) plot means were used. Means were compared by post-hoc Scheffé tests only if ANOVAs were significant. All tests were performed by the help of Statistica program package (ver. 5.5, Statsoft 1984–2000).

Results

Abundance of *V. angustifolia* in the neighbourhood of experimental plots

At Site 1 the mean (± SE) number of seedlings per 0.25 m²year⁻¹ in the plots' neighbourhood were: 1993

= 2.9 (±0.9), 1994 = 11.3 (±1.2), 1995 = 2.5 (±0.6), 1996 = 2.6 (±0.2), 1997 = 2.2 (±0.3), 1998 = 2.3 (±0.4). At Site 2 in 1996 = 11.2 (±10.8), in 1997 = 12.0 (±20.0), and in 1998 = 11.2 (±16.8) seedlings were counted. The constancy is remarkable with the exception of 1994, when the spring was very rainy.

The number of seedlings and adult plants on the experimental plots

At Site 1, it was perturbation that significantly influenced the number of seedlings in four out of six years (Table 1). Sowing had influence only in 1993 and 1994, as a main effect. There was no interaction effect. Accordingly, there were 2–4-times more *V. angustifolia* seedlings on perturbed (sprayed or unsprayed) plots in comparison with unperturbed ones (Fig. 1). Significant differences were detected in 1993, 1994 and 1997. On Site 2, where only plots with vegetation occurred, significantly more seedlings were present on sprayed plots than on unsprayed ones in 1997 ($F_{1,26}$ = 5.72, $P < 0.05$) and 1998 ($F_{1,12}$ = 8.55, $P < 0.05$), but not in 1996 ($F_{1,26}$ = 0.49, $P > 0.05$) (one-way factorial ANOVAs).

In 1996–1998 on Site 1, an average of 122.1 ± 55.5 (SE) of intact seeds were returned to a plot regardless of treatment. For the same period with the same conditions, an average of 175.8 ± 17.4 (SE) intact seeds were re-distributed per plot on Site 2. Remarkably, a yearly average of 10.5 ± 3.3% and 5.4 ± 0.6% (SE) of these seeds at the two sites, respectively, developed into seedlings. Thus, these seeds not only produced a new generation of adult plants, but also formed seed banks, which in turn considerably influenced variability of seedlings' numbers.

The number of adult plants per plot was affected by perturbation only at Site 1 in 1997. There were significantly fewer plants on vegetation-covered plots (6.9 ± 0.7, mean ± SE) than on bare ones (12.6 ± 1.8) ($F_{1,24}$ = 10.59, $P = 0.0034$; 3-way ANOVA). No such difference was detected in other years. At Site 2 the number of adult plants per plot was the same in 1996 and 1997 on vegetation-covered sprayed and unsprayed plots, and there was a significant difference only in 1998 (unsprayed: 6.0 ± 0.8, sprayed: 9.3 ± 1.2, t = 2.33, df = 12, $P = 0.038$ for independent samples).

Plant growth characters

No difference was found in plant growth characters attributable to treatments in the *first* surveys (details are not given). Plants were 10–30 cm high, pods were rarely present. At Site 2, however, plants of the exclusion treatment were significantly higher than those of the unsprayed ones in 1998 [sprayed: 26.2 ± 1.9 vs. unsprayed: 16.7 ± 2.7 cm (mean ± SE), $F_{1,12}$ = 8.86, $P = 0.0154$; one-way ANOVA].

The *second* survey (in June) showed that perturbation ultimately influenced plant growth characters (Tables 2a–c). Plants growing on bare plots showed significantly higher total dry weight and length of branches in 1996 (Fig. 2). At Site 2, sprayed plants generally grew larger and produced more and larger pods only in 1998.

Plant performance: seed quality

Insecticide treatment significantly determined the numbers of intact and infested seeds (Tables 2a–c), as well as that of the aborted seeds (see below).

As expected, the number of *infested* seeds per pod was 2–15–times higher on unsprayed than on sprayed plots, but it was independent of whether the plots were bare or covered by vegetation. On the other hand, in most cases the number of *intact* seeds per pod was higher on perturbed plots with pre-DSP exclusion than on plots covered with vegetation and unsprayed

Table 1. *F*-values of 3-way general linear model ANOVAs of mean numbers of *V. angustifolia* seedlings per plots at Site 1 treated with insecticide, sown with *V. angustifolia* seeds and perturbed in 1993.

Source of variation	df	1993	1994	1995	1996	1997	1998
Spraying (SR)	1	1.4908	12.2498***	2.9413	0.0406	1.8698	1.3020
Sowing (SO)	1	41.3514***	20.9558***	3.9288	0.1996	2.7177	0.4811
Perturbation (P)	1	5.5723*	13.0568***	1.0531	36.0750***	16.8277***	1.1058
SR × SO	1	3.7112	2.9933	0.4365	0.0258	2.9763	0.0606
SR × P	1	0.0361	0.6953	0.1441	0.2435	0.6482	1.6264
SO × P	1	0.0596	0.0541	0.4553	1.1234	1.4125	0.1447
SR × SO × P	1	0.3894	0.1277	0.0008	0.0802	1.4739	1.8627
Block	4						
Error	24–56						

*$P < = 0.05$, ***$P < = 0.001$
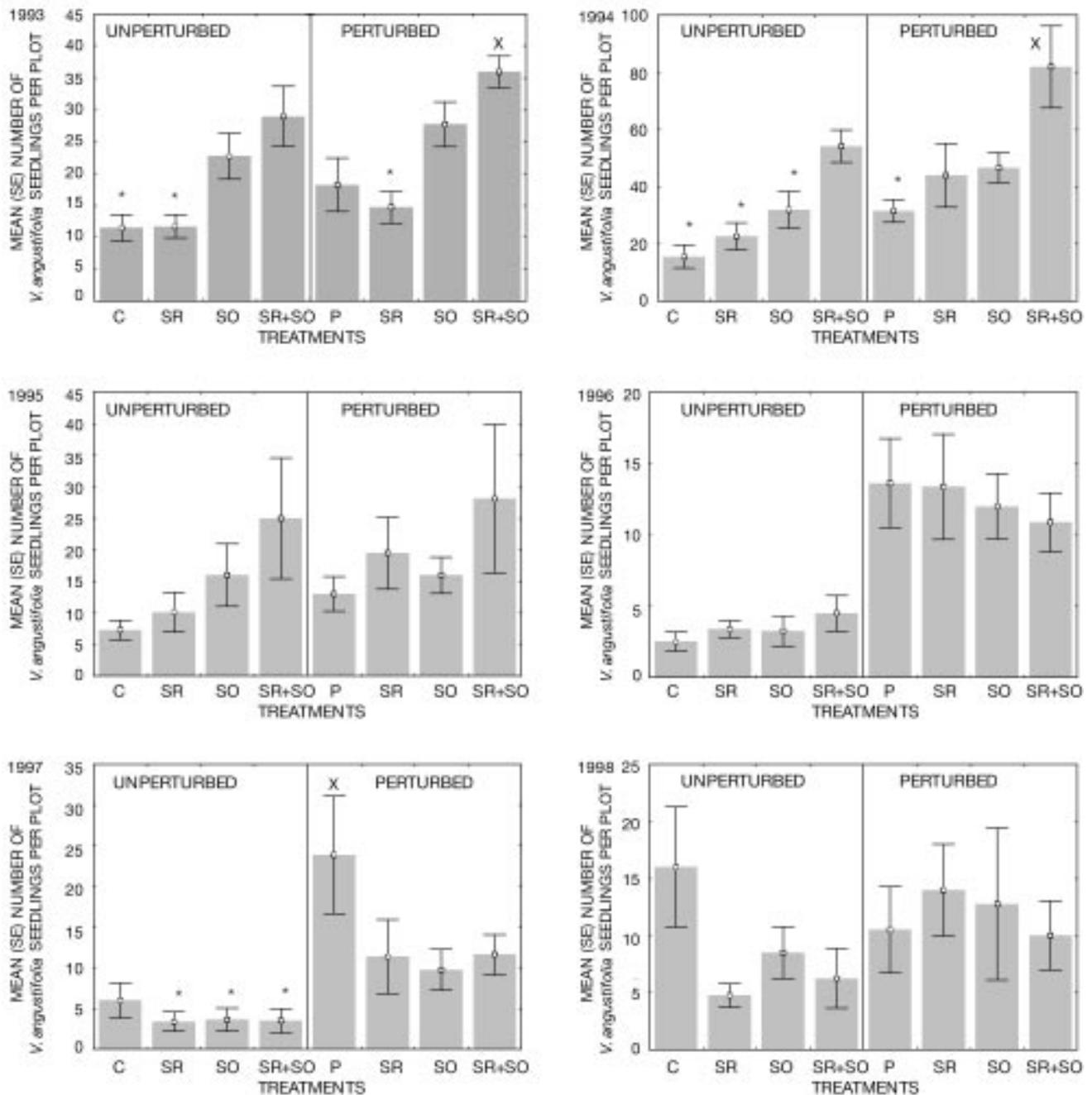
Fig. 1. Mean (± SE) number of established *V. angustifolia* seedlings on plots at Site 1 in years of 1993–1998, under three treatments: insecticide spraying, sowing and perturbation. Asterisks denote significant differences from X. Results of three-way ANOVAs. C = control (unsprayed, unsown, unperturbed), SR = sprayed, SO = sown, P = perturbed.

(Fig. 3). In all treatment combinations the number of seeds consumed by pre-DSPs was conspicuously low, about 1 seed per pod if there was no exclusion, and it was virtually nil on most sprayed plots. If no perturbation effect is considered and both sites' and all years' data are pooled, the mean (± SE) number of seeds per pod is 8.6 ± 0.2, including 3.4 ± 0.2 aborted, 0.5 ± 0.1

underdeveloped, 3.7 ± 0.3 intact, and 0.9 ± 0.1 pre-DSP-infested seeds. For seed infestation levels of Site 2 see Fig. 4. Density-dependence by pre-DMPs was not detected in any year or site.

The number of *aborted* seeds per pod was generally less on sprayed plots, regardless of the state of perturbation: at Site 1 in 1996, there were 2.78 mean abort-

Table 2a. *F*-values of 3-way general linear model ANOVAs of plant traits and infestation levels by pre-DSPs of *V. angustifolia*, for plots treated with insecticide, sown with *V. angustifolia* seeds and perturbed. Site 1, 1996.

Source of variation	df	Dry matter	Heigth	Branch length	Length of pods	Intact seeds	Infested seeds	All seeds
Spraying (SR)	1	0.0762	0.0581	3.3955	0.7634	5.8304*	12.0842**	0.2510
Sowing (SO)	1	5.8540	1.7283	0.0866	0.4045	2.4353	0.8568	0.3330
Perturbation (P)	1	22.9023***	0.1284	26.3136***	11.5002**	22.7147***	0.1371	28.5480***
SR × SO	1	1.2970	0.9050	0.1153	0.6372	0.2152	0.0068	7.2960*
SR × P	1	0.2671	2.4946	0.1345	0.3579	0.2152	0.0512	6.7810*
SO × P	1	7.3066*	6.3704**	4.7821*	0.0946	0.4697	0.0952	0.4540
SR × SO × P	1	0.1488	2.3646	0.1412	0.4693	0.3123	3.7385	14.2030**
Block	4							
Error	20–24							

*P < = 0.05, **P < = 0.01, ***P < = 0.001

Table 2b. *F*-values of 3-way general linear model ANOVAs of plant traits and infestation levels by pre-DSPs of *V. angustifolia*, for plots treated with insecticide, sown with *V. angustifolia* seeds and perturbed. Site 1, 1997

Source of variation	df	Dry matter	Heigth	Branch length	Length of pods	Intact seeds	Infested seeds	All seeds
Spraying (SR)	1	0.8136	1.4907	5.6298*	1.0541	13.1183**	12.9706**	0.0000
Sowing (SO)	1	0.1006	0.1271	0.0846	0.5527	0.3084	0.5819	0.2750
Perturbation (P)	1	4.0973	0.0202	10.2399**	3.9306	0.5603	0.5724	0.8590
SR × SO	1	0.2330	2.1673	0.1257	2.4266	0.4250	0.5444	0.7160
SR × P	1	0.9100	1.2571	0.3710	0.4525	0.8943	0.3832	1.1840
SO × P	1	0.0461	1.7736	0.2592	0.7834	0.1493	0.3678	1.0700
SR × SO × P	1	0.9960	1.4817	0.2150	3.1335	0.0093	0.1552	0.1130
Block	4							
Error	24							

*P < = 0.05, **P < = 0.01

Table 2c. *F*-values of 3-way general linear model ANOVAs of plant traits and infestation levels by pre-DSPs of *V. angustifolia*, for plots treated with insecticide, sown with *V. angustifolia* seeds and perturbed. Site 1, 1998.

Source of variation	df	Dry matter	Heigth	Branch length	Length of pods	Intact seeds	Infested seeds	All seeds
Spraying (SR)	1	0.3492	0.5771	0.5211	0.1053	15.5605***	5.7649*	8.3870**
Sowing (SO)	1	0.0656	0.0395	0.2859	0.0056	0.1613	0.8144	0.0000
Perturbation (P)	1	8.4976**	11.7357**	5.7262*	4.2940*	0.7656	0.0731	4.7250*
SR × SO	1	3.5012	0.2085	1.5960	1.1525	1.4175	0.4998	3.9440
SR × P	1	0.0993	1.9789	0.0022	0.0754	0.0403	0.3325	0.0710
SO × P	1	0.4225	4.8997*	0.0612	4.9373*	0.5780	0.0200	0.0010
SR × SO × P	1	2.1822	0.9863	0.1946	1.0478	0.0101	0.3554	0.0030
Block	4							
Error	23–24							

*P < = 0.05, **P < = 0.01, ***P < = 0.001

ed seeds per pod for sprayed and 3.67 for unsprayed plots ($F_{1,26}$ = 4.34, P = 0.0472); in 1997: 2.10 vs. 2.66 ($F_{1,30}$ = 2.89, P = 0.0994); in 1998: 2.27 vs. 2.98 ($F_{1,30}$ = 6.01, P = 0.0203). At Site 2, in 1996: 4.76 vs. 5.60 ($F_{1,12}$ = 3.08, P = 0.1049); in 1997: 1.72 vs. 2.86 ($F_{1,10}$ = 6.54, P = 0.0284); in 1998: 2.56 vs. 3.53 ($F_{1,11}$ = 5.38, P = 0.0406); one-way ANOVAs.

Plant performance: seed mass

At Site 1, in 1994, seed mass was significantly affected by the joint effects of sowing and perturbation (Table 3), but it did not differ by any other treatment combinations. In 1996 and 1998, there were also no differences in seed masses by any treatments and interactions. However, in 1997, seed mass was significantly
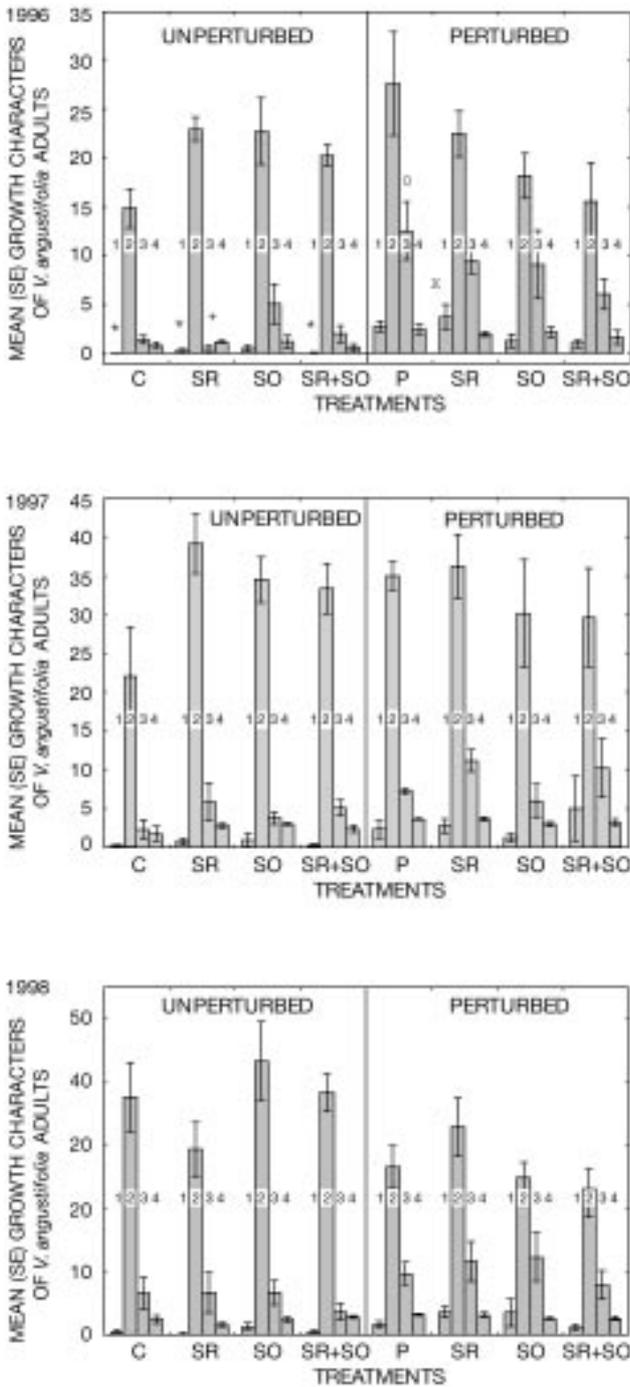
Fig. 2. Mean (± SE) values of plant traits of *V. angustifolia* recorded at Site 1 during the second phenological survey in June, under three different treatment-regimes: insecticide spraying, sowing and perturbation. 1 = Dry weight, 2= plant height, 3 = length of branches, 4 = length of pods. Symbol "+" denotes significant difference from O, and asterisks from X. Results of three-way ANOVAs. See caption of Fig. 1 for abbreviations.
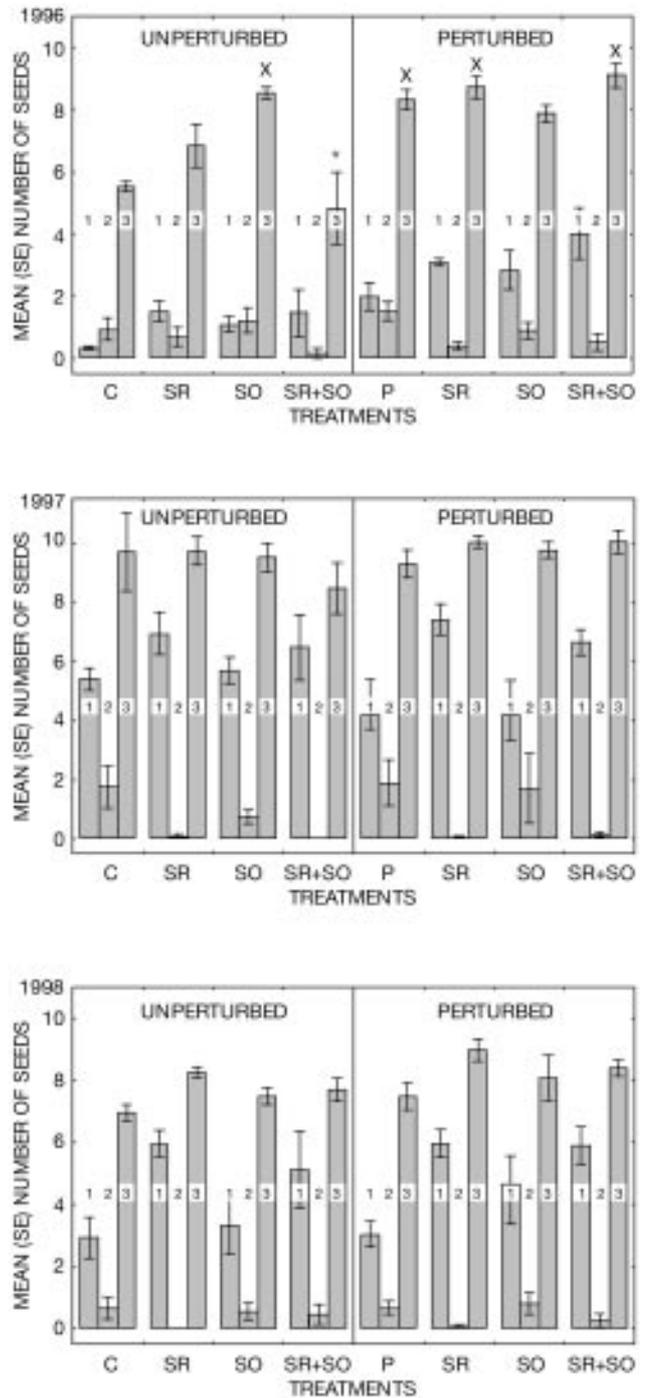
Fig. 3. Mean (± SE) seed infestation levels caused by pre-dispersal seed predators and all seeds produced by *V. angustifolia* plants on plots at Site 1, under three treatment-regimes: insecticide spraying, sowing and perturbation. 1 = Intact seeds, 2= infested seeds, 3 = all seeds. Asterisk denotes significant difference from Xs. Results of three-way ANOVAs. See caption of Fig. 1 for abbreviations.

Table 3. *F*-values of 3-way general linear model ANOVAs of mean masses of *V. angustifolia* seeds per plots at Site 1 treated with insecticide, sown with *V. angustifolia* seeds and perturbed in 1993.

Source of variation	df	1994	1996	1997	1998
Spraying (SR)	1	0.6838	0.0822	10.1840**	0.9460
Sowing (SO)	1	0.0084	0.0141	1.1070	0.1195
Perturbation (P)	1	0.8685	1.3452	1.1820	0.1999
SR × SO	1	0.0140	0.2135	0.0110	0.9640
SR × P	1	0.2110	0.2238	0.0010	2.5291
SO × P	1	5.0378*	3.3830	0.1230	2.5291
SR × SO v P	1	0.0622	0.0441	0.4440	1.6778
Block	4				
Error	20–24				

*$P <= 0.05$, **$P <= 0.01$

Table 4. Microsite use of *V. angustifolia* seeds sown in increasing density in 0.25 m² plots. Mean (± SE) number of seedlings among vegetation. $n = 45$, 62 and 32 for the control in the respective time periods.

Time of recording	Number of seedlings			
	from 10 seeds	from 100 seeds	from 1000 seeds	On control plots
October 1996	10.0 ± 2.1	36.7 ± 5.2	303.7 ± 38.9	1.9 ± 0.4
May 1997	1.3 ± 1.2	9.0 ± 4.0	48.0 ± 10.5	1.5 ± 0.3
May 1998	0	0	0	1.4 ± 0.5
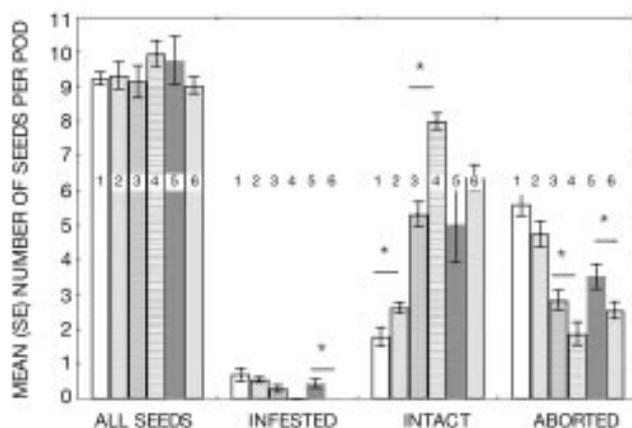
Fig. 4. Impact of pre-DSPs on seed quality on Site 2 by treatments on plots covered with vegetation. 1 = unsprayed 1996, 2 = sprayed 1996, 3 = unsprayed 1997, 4 = sprayed 1997, 5 = unsprayed 1998, 6 = sprayed 1998. *Intact seeds*, 1996: $F_{1,12} = 7.3664$, $P = 0.0000$; 1997: $F_{1,10} = 29.6413$, $P = 0.0000$; 1998: $F_{1,11} = 1.5177$, $P = 0.2437$; *infested seeds*, 1996: $F_{1,12} = 0.5282$, $P = 0.4813$; 1997: $F_{1,10} = 4.3308$, $P = 0.0641$; 1998: $F_{1,11} = 14.8393$, $P = 0.0027$ (one-way ANOVAs). Asterisks denote significant differences between adjacent means.

affected by exclusion; it was larger on sprayed plots regardless whether they were sown or perturbed: 14.0 vs. 11.6 mg ($P = 0.003$). At Site 2, there was no difference in seed mass by exclusion treatment in any year.

Microsite limitation

In experiment "A", indicating the amount of available microsites, the percentages of seedling emergence were 100% for 10 seeds, 45% for 100 seeds, and 35% for 1000 seeds, seemingly reaching a plateau (Table 4). This means 10, 45 and 350 seeds for the same area, a maximum of 35-times, or compared with the control (= 2 seedlings), a maximum of 175-times increase!

In experiment "B", seed germination percentage reached 60.0±5.3% (mean ± SE) in the year of sowing in autumn, and only a fraction (0.6 ± 0.2%) was observed next spring. No further germination was noted.

Infestation levels of samples collected elsewhere in the country

Of the samples collected during 1994–1998, we evaluated 372 pods of 7 randomly chosen samples originating from various parts of the country. From the mean (± SE) of 11.4 ± 0.1 seeds/pod, there were 6.8 ± 0.2 intact, 0.8 ± 0.1 infested seeds per pod and the rest were undeveloped seeds. The values are in the same range as those of the experimental plots. Thus, considering all seeds we found an infestation level of 6.7% (we round it up to 10%) caused by pre-DSPs. If, however, we relate it to fully-grown seeds only, it was 12.5%. In another group of 13 samples there was a bruchid infestation level of 5.5 ± 7.0% (mean ± SD) of seeds; 6.1 ± 9.6% of these seeds were parasitised by braconids and chalcids, and the number of bruchid eggs parasitised on the pods by *Trichogramma* spp. was 5.4 ± 8.1.

Discussion

The impact of pre-dispersal seed predators

Pre-DSPs cause a low level of damage on *V. angustifolia* (Tables 2a–c and Fig. 3). Several factors might contribute to it. (1) One could be a low efficiency of host-plant finding by the specialist seed predator, *Bruchus luteicornis*. However, the fact that we found it in host-plant stands devoid of any reproductive plant parts and intermingled with abundant graminaceous vegetation suggests the opposite. If host finding were only occasional it would result in overinfested pods, however, we did not find such cases. Furthermore, the overall pre-DSP infestation was the same on bare and vegetation-covered plots, suggesting the unimportance of surrounding vegetation in host finding. (2) Ants exploiting the vetch's extrafloral nectaries might provide defence against pre-DSPs. However, it is at least ambiguous (Koptur 1992) and largely ineffective as has been proven with *V. angustifolia* (O. Fegyveres unpubl. obs.). (3) Most pre-DSP species of *V. angustifolia*

are obligatorily univoltine and have a short egg-laying period, therefore, even if resources are available for an extended period of time, they are not used. (4) The pre-DSPs neither on *V. angustifolia*, nor on another species showed density-dependent effects (Á. Szentesi & T. Jermy unpubl. obs.), and in a third species, *Lathyrus vernus*, only on a very variable spatiotemporal scale (Ehrlén 1996). (5) There was roughly one seed per pod infested on the average (Figs. 3 & 4), meaning a low co-occurrence of pre-DSPs and making competitive interactions very improbable. (6) A high level of parasitisation might have caused a top-down control on pre-DSPs. However, the level of parasitisation in *V. angustifolia* samples was very low to be a likely population regulating factor. On a perennial relative, *V. tenuifolia*, we found that the effect of parasitoids was density independent (Á. Szentesi & T. Jermy unpubl. obs.).

Besides direct seed damage, indirect changes in plant or seed traits might be connected to the damage (Andersen 1988), like the increased number of aborted seeds in this study. In spraying treatments, regardless of perturbation, the number of aborted seeds was significantly less, in comparison with the control (see Plant performance: seed quality and Fig. 4), whereas there were no such differences between perturbed and vegetation-covered plots (regardless of spraying). Thus, the number of aborted seeds seemingly positively correlated with pre-DSP activity. Although the connection can be indirect and very complex, it may refer to an active plant response to the stress caused by the seed predators' impact. Induced and selective seed or fruit abortion due to seed predation has been described in several cases (Janzen 1971, Stephenson 1981), however, not in *V. angustifolia*. Louda (1982b) also observed less flower head abortion and more undamaged seeds reaching maturation in *Haplopappus squarrosus*, in the absence of pre-DSPs, due to insecticide treatment.

Microsite limitation

The number of seedlings (Table 1 and Fig. 1) and adult plants were higher on bare plots. This indicates that not only the number of microsites can be limiting, but the plant may be a weak competitor as well. On the other hand, on plots covered with vegetation (Site 2) spraying provided a higher level of recruitment, possibly by limiting post-dispersal seed predators or other herbivores. In a few traits adult *V. angustifolia* plants performed significantly better on bare than on vegetation-covered plots regardless of other treatments (Tables 2a–c and Fig. 2, Site 1, 1996) implying that *V. angustifolia* performs less successfully in dense vegetation.

Seed mass is generally considered an important fitness component the decrease of which due to herbivory affects germination rate (e.g., Crawley & Nachapong 1985). Seed size (virtually seed mass) is thought to be usually positively correlated with seedling size, which in turn can severely influence competitive ability. In reality, not only seed size is extremely variable (Szentesi & Jermy 1995), but seedling size is rather determined by relative growth rate which, however, is inversely correlated with seed size (Westoby et al. 1992). Apart from the interaction by sowing and perturbation in 1994 and insecticide treatment in 1997 when seed mass was significantly affected (Table 3), other treatments did not influence it.

Even if plant performance was weaker in dense vegetation, the sowing experiments (Table 4) demonstrated that *V. angustifolia* was able to recruit seedlings among full cover. The increase in recruitment proved that safe sites were available and a ca. 35% further increase in seedling number was still possible. Thus, *V. angustifolia* does not seem to be microsite limited.

Seed sowing on net-trays placed on bare soil surface has also proved that abundance of unoccupied sites enhances germination rate, although not all seeds germinate. This and the seed sowing experiment support the assumption that *V. angustifolia* has a short-term permanent seed bank. It would mean approximately 2 years of germination from the same seed crop (Table 4). Although plant and seed characters, e.g., annual life cycle, seed weight, etc. allow to place *V. angustifolia* among species having persistent seed banks (Garwood 1989, Pickett & McDonnell 1989, Rice 1989, Edwards & Crawley 1999), no sufficiently detailed studies have been performed yet.

Seed limitation

If *V. angustifolia* is not microsite-limited, why recruitment is not higher? In fact, the surplus of unoccupied microsites suggests that there must be a shortage of seeds in this vetch species. The exclusion experiments revealed that on unsprayed plots (whether perturbed or not) an average of 10% of seeds were infested by pre-DSPs. This is higher than the one detected in samples collected at several locations in the country (Szentesi et al. 1996). [Seed mortality caused by pre-DSPs is probably even less, as several studies (e.g., Ollerton & Lack 1996, Koptur 1998) prove that a large proportion of partially eaten seeds of *Vicia* and *Lotus* species by curculionid and lepidopteran pre-DSPs were still viable.] The data suggest that, seed limitation is not due to pre-DSPs and that theoretically, the portion (90%) of seeds untouched by pre-DSPs would form the seed bank even if ca. 40% represent aborted, 5% underdeveloped seeds, and only 42% have the ability

to germinate. From these, on the average, 8% (5.3 seeds) of the redistributed seeds per plot produced seedlings in a year at the two experimental sites combined (which is close to the naturally occurring seedling number). Unfortunately, we do not know what happens to the seeds after having left the mother plant. The values above and the data in Table 4 suggest that post-dispersal seed consumers and pathogens take a significant toll. From plots receiving 100 or 1000 seeds less than 50% of seeds, respectively, were recovered as seedlings. These plots also represented resource concentrations (*sensu* Root 1973, Kareiva 1983) for post-DSPs. Thus, it can be concluded that seed limitation occurs in *V. angustifolia* due to events taking place between seed dispersal and germination. This is also in agreement with the data of Edwards & Crawley (1999) who found that the impact of invertebrates on the species composition of grassland seed banks is negligible. From this, however, a proportional reduction in plant recruitment does not follow (Hendrix 1988).

Our data and the above conclusions raise doubts whether pre-DSPs are able to exert substantial impact on annual plants' population dynamics, and supports Crawley's conception (1992). Louda (1995) considers the consequences of reproductive herbivory substantial and specifically through seedling consumption. In our study, however, there was no positive relationship between seed production and the recruitment of seedlings. Although it is probable that *V. angustifolia* has a persistent seed bank, its buffering ability may diminish due to the effects acting post-dispersal. On the other hand, the results are also in agreement with the prediction (Crawley 1990) that seed limitation is a likely event in grassland communities. The best hypothesis that may explain the forces affecting net recruitment in *V. angustifolia*, is probably an asymmetric combination of seed and microsite limitation, similar to the one propounded by Eriksson & Ehrlén (1992). Accordingly, net recruitment in *V. angustifolia*, is affected by both the local and variable lack of safe sites for germination and, more importantly, by seed limitation, which can result from post-dispersal seed consumers. Indeed, studies where such organisms were also excluded demonstrated a higher level of microsite use (Alexander & Mihail 2000), weakening arguments based on plant competition (Hulme 1996).

References

Alexander HM, Mihail JD (2000) Seedling disease in an annual legume: consequences for seedling mortality, plant size, and population seed production. Oecologia (Berlin) 122: 346–353.

Andersen AN (1988) Insect seed predators may cause far greater losses than they appear to. Oikos 52: 337–340.

Andersen AN (1989) How important is seed predation to recruitment in stable populations of long-lived perennials? Oecologia (Berlin) 81: 310–315.

Brown JH, Davidson DW, Munger JC, Inouye RS (1986) Experimental community ecology: the desert granivore system. In: Diamond J, Case TJ (eds) Community ecology. Harper and Row, New York, NY, pp 41–61.

Brown VK, Lejn M, Stinson CSA (1987a) The experimental manipulation of insect herbivore load by the use of an insecticide (malathion): the effect of application on plant growth. Oecologia (Berlin) 72: 377–381.

Brown VK, Gange AC, Evans IM, Storr L (1987b) The effect of insect herbivory on the growth and reproduction of two annual Vicia species at different stages in plant succession. Journal of Ecology 75: 1173–1189.

Crawley MJ (1983) Herbivory. The dynamics of animal-plant interactions. Blackwell Scientific Publ., Oxford.

Crawley MJ (1989a) The relative importance of vertebrate and invertebrate herbivores in plant population dynamics. In: Bernays EA (ed) Insect-plant interactions. Vol. I, CRC Press, Inc., Boca Raton, pp 45–71.

Crawley MJ (1989b) Insect herbivores and plant population dynamics. Annual Review of Entomology 34: 531–564.

Crawley MJ (1990) The population dynamics of plants. Philosophical Transactions of the Royal Society, London B 330: 125–140.

Crawley MJ (1992) Seed predators and plant population dynamics. In: Fenner M (ed) Seeds: The ecology of regeneration in plant communities. CAB International, London, pp 157–191.

Crawley MJ (1993) GLIM for ecologists. Methods in ecology. Blackwell Science, Oxford.

Crawley MJ (1998) Plant-herbivore dynamics. In: Crawley MJ (ed) Plant ecology. 2nd edn. Blackwell Science, London, pp 401–474.

Crawley MJ, Nachapong M (1985) The establishment of seedlings from primary and regrowth seeds of ragwort (*Senecio jacobaea*). Journal of Ecology 73: 255–261.

Edwards GR, Crawley MJ (1999) Herbivores, seed banks and seedling recruitment in mesic grassland. Journal of Ecology 87: 423–435.

Ehrlén J (1996) Spatiotemporal variation in predispersal seed predation intensity. Oecologia (Berlin) 108: 708–713.

Eriksson O, Ehrlén J (1992) Seed and microsite limitation of recruitment in plant populations. Oecologia (Berlin) 91: 360–364.

Ganade G, Brown VK (1997) Effects of below-ground insects, mycorrhizal fungi and soil fertility on the establishment of *Vicia* in grassland communities. Oecologia (Berlin) 109: 374–381.

Garwood NC 1989. Tropical soil seed banks: a review. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. Academic Press, Inc., San Diego, pp 149–178.

Hanelt P, Mettin D (1989) Biosystematics of the genus *Vicia* L. (Leguminosae). Annual Review of Ecology and Systematics 20: 199–223.

Harper J (1977) Population biology of plants. Academic Press, London.

Hendrix SD (1988) Herbivory and its impact on plant reproduction. In: Doust JL, Doust LL (eds) Plant reproductive ecology. Patterns and strategies. Oxford University Press, New York, pp 246–263.

Hulme PE (1996) Herbivory, plant regeneration, and species coexistence. Journal of Ecology 84: 609–615.

Janzen D (1971) Seed predation by animals. Annual Review of Ecology and Systematics 2: 465–492.

Kareiva P (1983) Influence of vegetation structure on herbivore populations: resource concentration and herbivore movement. In: Denno RF, McClure MS (eds) Variable plants and herbivores in natural and managed systems. Academic Press, New York, pp 259–298.

Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays EA (ed) Insect-plant interactions. Vol. 4. CRC Press, Inc., Boca Raton, pp 81–130.

Koptur S (1998) Effect of seed damage on germination in the common vetch (*Vicia sativa* L.). American Midland Naturalist 140: 393–396.

Leck MA, Parker VT, Simpson RL (eds) (1989) Ecology of soil seed banks. Academic Press, Inc., San Diego.

Louda SM (1982a) Distribution ecology: variation in plant recruitment in relation to insect seed predation. Ecological Monographs 52: 25–41.

Louda SM (1982b) Limitation of the recruitment of the shrub *Haplopappus squarrosus* (Asteraceae) by flower- and seed-feeding insects. Journal of Ecology 70: 43–53.

Louda SM (1983) Seed predation and seedling mortality in the recruitment of a shrub, *Haplopappus venetus* (Asteraceae), along a climatic gradient. Ecology 64: 511–521.

Louda SM (1989) Predation in the dynamics of seed regeneration. In: Leck MA, Parker T, Simpson RL (eds) Ecology of soil seed banks. Academic Press, Inc., San Diego, pp 25–51.

Louda SM (1995) Effect of seed predation on plant regeneration: evidence from Pacific Basin Mediterranean scrub communities. In: Arroyo MTK, Zedler PH, Fox MD (eds) Ecology and biogeography of Mediterranean ecosystems in Chile, California and Australia. Springer Verlag, New York, pp 311–344.

Louda SM, Potvin MA (1995) Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. Ecology 76: 229–245.

Maron JL, Simms EL (1997) Effect of seed predation on seed bank size and seedling recruitment of bush lupine (*Lupinus arboreus*). Oecologia (Berlin) 111: 76–83.

Ollerton J, Lack A (1996) Partial predispersal seed predation in *Lotus corniculatus* L. (Fabaceae). Seed Science Research 6: 65–69.

Pickett STA, McDonnell MJ (1989) Seed bank dynamics in temperate deciduous forest. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. Academic Press, Inc., San Diego, pp 123–147.

Reichart G (1957) Neuere Angaben zur Verbreitung und Biologie des braunschwarzen Erbsenwicklers (*Laspeyresia nigricana* Steph.) in Ungarn. Annales Instituti Protectionis Plantarum Hungarici 7: 227–231.

Rice KJ (1989) Impacts of seed banks on grassland community structure and population dynamics. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. Academic Press, Inc., San Diego, pp 211–230.

Root RB (1973) Organization of plant arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). Ecological Monographs 43: 95–124.

Root RB (1996) Herbivore pressure on goldenrods (*Solidago altissima*): its variation and cumulative effects. Ecology 77: 1074–1087.

Statsoft (1984–2000) STATISTICA for Windows. ver. 5.5. Statsoft Inc., Tulsa.

Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12: 253–279.

Szentesi Á (1999) Predispersal seed predation of the introduced false indigo, *Amorpha fruticosa* L. in Hungary. Acta Zoologica Academiae Scientiarum Hungaricae 45: 125–141.

Szentesi Á, Jermy T (1995) Predispersal seed predation in leguminous species: seed morphology and bruchid distribution. Oikos 73: 23–32.

Szentesi Á, Jermy T (1998) Leguminosae-fajokon élő magfogyasztó rovar-guildek: közösségszerkezet és kölcsönhatások. [Insect guilds consuming seeds on leguminous species: community structure and interactions.] In: Fekete G (ed) A közösségi ökológia frontvonalai. [Frontiers of community ecology.] Scientia, Budapest, pp 105–113.

Szentesi Á, Jermy T, Takács V (1996) Niche relations in *Vicia*-inhabiting *Bruchus* spp. Entomologia experimentalis et applicata 80: 152–155.

Thompson K, Band SR, Hodgson JG (1993) Seed size and shape predict persistence in soil. Functional Ecology 7: 236–241.

Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. Oikos 88: 225–238.

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (1968) Flora Europaea. Vol. 2. Cambridge University Press, Cambridge.

Waloff N (1968) Studies on the insect fauna of Scotch broom *Sarothamnus scoparius* (L.) Wimmer. Advances in Ecological Research 5: 87–208.

Westoby M, Jurado E, Leishman M (1992) Comparative evolutionary ecology of seed size. Trends in Ecology and Evolution 7: 368–372.