

Direct and Indirect Effects of a Shoot-Base Boring Weevil and Plant Competition on the Performance of Creeping Thistle, *Cirsium arvense*

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Received August 21, 2000; accepted June 5, 2001; published online August 13, 2001

Creeping thistle or Canada thistle, *Cirsium arvense* (L.) Scop., is considered one of the world's worst weeds and the third most important weed in Europe. Biological control of this indigenous weed in Europe by use of native agents may provide a low-cost alternative to use of chemical or mechanical control measures and contribute to a more sustainable weed management. We investigated the potential of a shoot-base boring weevil, *Apion onopordi* Kirby (Coleoptera: Apionidae), for biological weed control, in the presence or absence of plant competition by three grass species. Infestation of thistle shoots by *A. onopordi* at natural infestation levels reduced above- and belowground plant performance after 2 years. Plant competition at natural levels had an overall greater effect than that of herbivory, significantly reducing both above- and belowground thistle performance in both years, thereby slowing the propagation of the weed. Weevil infestation and grass competition had a synergistic effect on *C. arvense* growth; the combined effects of the two factors was greater than the sum of both single-factor effects. The experiment revealed that *A. onopordi* promotes systemic infections of the rust fungus *Puccinia punctiformis* (Str.) Röhl in the year following weevil infestation. Systemically infected thistle shoots died before the end of the growing season. Although the direct effect of *A. onopordi* may not be sufficient to control creeping thistle, the synergistic interaction with plant competition and the indirect effect via promotion of systemic rust infections makes *A. onopordi* a promising agent for the biological control of this weed.

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Key Words: biological weed control; herbivory; plant competition; plant pathogen; rust fungus; *Cirsium arvense*; *Apion onopordi*; *Puccinia punctiformis*.

INTRODUCTION

Creeping thistle or Canada thistle, *Cirsium arvense* (L.) Scop., is considered one of the world's worst weeds (Holm *et al.*, 1977) and the third most important weed in Europe (Schröder *et al.*, 1993). In an inquiry among

Swiss farmers, *C. arvense* was considered the most important weed on ecological compensation areas in agricultural land, but also problematic in adjacent fields and after recultivation of the compensation area in the course of crop rotation (Bacher *et al.*, 1997). Ecological compensation areas are part of integrated and organic farming systems in Europe, designed to increase the species diversity in agricultural areas. In Switzerland, for example, certified integrated farming demands that at least 5% of the farmland be assigned for ecological compensation (Häni *et al.*, 1998). Typical compensation areas are sown wildflower strips, fallow strips, or grass strips (Pfiffner and Schaffner, 2000). The plant communities in ecological compensation areas are also expected to suppress problematic weeds, thus restricting spread of the latter into adjacent fields and facilitating recultivation of the compensation area in the course of crop rotation (Bacher and Schwab, 2000). Since the use of chemical or mechanical control measures is restricted in ecological compensation areas, biological control of *C. arvense* with native insects as agents may provide a low-cost alternative and a contribution to a more sustainable weed management.

C. arvense is a deep-rooted, dioecious perennial that reproduces vegetatively and from seeds, but under most circumstances seed production does not contribute significantly to its weediness (Donald, 1994; but see Oesau, 1998). The weediness of *C. arvense* can be attributed largely to its capacity for vegetative reproduction and regenerative growth from the numerous buds produced on the roots (Donald, 1994).

Because *C. arvense* is indigenous to Europe, a bio-control approach would aim at increasing the densities of native control agents by augmentative and conservation techniques. The latter technique involves the identification and manipulation of factors that limit or enhance the abundance and effectiveness of control agents (Newman *et al.*, 1998). To date, augmentation and conservation of native agents has received little attention compared to other approaches (Julien, 1998; McFadyen, 1998), but interest in the use of native agents is growing (e.g., De Bach and Rosen, 1991;

Campbell and Wykes, 1992; Sheldon and Creed, 1995; Müller-Schärer *et al.*, 2000). In a number of cases, manipulation of native biocontrol agents resulted in successful control of their target weeds (summarized in Julien (1998)). Unfortunately, successes are poorly documented; therefore, experienced strategies for biocontrol attempts involving native agents are lacking. Recently, a number of workers have attempted to develop a theoretical approach for exploiting the potential of native agents for biological weed control (Newman *et al.*, 1998; Frantzen and Müller-Schärer, 1998; Valenti *et al.*, 1999; Bacher, 2000). For a successful approach, knowledge must be gained on agent densities required to obtain the desired control level, likely interactions with other control measures and agents, and factors preventing the agent from attaining such high population levels (Bacher, 2000). Information on these considerations may lead to the development of strategies to increase population densities of the agent.

Ang *et al.* (1995) and Bacher and Schwab (2000) showed that a combination of plant competition and defoliating insect herbivores can lead to mortality of *C. arvense* plants during the growing season and thus may aid in controlling this weed. In this study, we evaluated the combined effects of a shoot-base boring weevil, *Apion onopordi* Kirby (Coleoptera: Apionidae), and plant competition on the performance of *C. arvense* clones in a 2-year experiment. Adults of this oligophagous weevil feed on leaves of their host plants, and the larvae develop in the shoot-base of *Cirsium* and *Carduus* spp. (Freese, 1997).

MATERIALS AND METHODS

Experimental Setup and Procedure

The study was conducted in the garden of the Zoological Institute, University of Bern. Two hundred and twenty-four large tubs of 20-liter volume were filled with organic loamy soil. Roots of *C. arvense* were collected on 16 April 1998 from a *C. arvense* population near Bern. The roots were dug from an area smaller than 100 m² to obtain plant material with low genetic variability. They were stored at 6°C in the dark and in the original soil for 1 week. On 24 April a root cutting of 10- to 13-cm length and 3- to 6-mm diameter was planted at a depth of 10 to 12 cm in the center of each tub. Root pieces of similar size were often found when digging for *C. arvense* roots on freshly tilled agricultural fields (personal observation). The nitrogen content of each root piece planted was determined with an elemental analyzer (Carlo Erba Instruments, Italy, Model NA2000) from an extra 2-cm sample of the planted root piece.

The study was conducted in a two-factorial randomized complete block design. The two factors were plant competition (at two levels: no vegetation vs a sown

mixture of three grass species) and herbivore infestation (at four levels: no, low, moderate, and high levels). On 25 April, seeds of three grass species (perennial ryegrass, *Lolium perenne* L.; Italian ryegrass, *L. multiflorum* Lam.; and orchardgrass or cock's foot, *Dactylis glomerata* L.; in equal proportions) were added to half of the tubs chosen at random at a density of 0.1 g/tub, corresponding to 12 kg/ha. The grass mixture and seed density are commonly used for establishing grass strips in Switzerland. Thistles in tubs were infested with *A. onopordi* when the largest shoot of the clone was 10- to 20-cm high. This corresponds to the most common plant stage in the field infested by the weevil (personal observation). However, due to differences in *C. arvense* shoot emergence in tubs in the first year we were not able to infest all the clones at the same time. Instead, *A. onopordi* infestation was set up at seven different dates within a period of 4 weeks. The seven dates were treated as blocks. At each infestation date four replicates of each treatment were set up. Thus, there were 2 (plant competition) × 4 (herbivore infestation levels) = 8 treatments replicated four times within each block. Within each block, the four infestation levels of *A. onopordi* were assigned randomly to the tubs of the two plant competition treatments. In 1998, the four herbivore infestation levels no, low, moderate, and high were attempted by allowing one female to oviposit for 0, 24, 48, and 72 h, respectively, on a single shoot of the clone developing from the planted root pieces. Females were confined to the largest (primary) shoot of *C. arvense* in each tub by means of a transparent plastic tube (15-cm diameter and height depending on the shoot height) sealed on top with gauze mesh. *A. onopordi* used in the experiment in 1998 were field-collected from a site in the Swiss Valais. In 1999, weevils were partly collected from the experimental plants in autumn 1998 and were supplemented by field-collected adults from the Swiss Valais. Only females that laid eggs in the laboratory prior to the experiment were used. In 1999, the experiment was conducted with only one infestation level, 72 h.

Data Collection

Three harvest dates were planned: after the first growing season in autumn 1998, after overwintering in early spring 1999, and after the second growing season in autumn 1999. Because of the three harvest dates and the four replicates in each block, the number of tubs harvested from each block was not always equal at each harvest date. In autumn 1998, 90 ± 4 days after infestation by *A. onopordi*, 72 tubs were harvested (one replicate of each treatment from five blocks and two replicates of each treatment from two blocks chosen at random). The number of *A. onopordi* larvae that developed in the primary shoot was counted. Plants were then oven-dried at 50°C for 48 h to deter-

mine dry weights. The following parameters were measured: the biomass of leaves, the shoot weight of the primary shoot, the total aboveground biomass, the total belowground biomass, the nitrogen content of the root system, and the weight of the flower heads and seeds. Weevil infestation levels for the tubs to be harvested in 1999 were verified by dissection of all shoots treated with *A. onopordi* females at the time when the shoots started to die back before winter. Only tubs in which we found weevil mines were considered further. The second batch of 72 tubs (one replicate of each treatment from five blocks and two replicates of each treatment from two blocks chosen at random) was harvested before shoots emerged aboveground between 1 and 5 March 1999. At this harvest date, only the belowground dry weight and the nitrogen content of the root system were measured. The last 80 tubs (one replicate of each treatment from four blocks and two replicates of each treatment from the remaining three blocks) were harvested and dissected in autumn 1999, between 2 and 9 September, and the total aboveground biomass, the total belowground biomass, and the nitrogen content of the root system were determined. Again, only herbivore-infested tubs in which we found weevil mines were considered for analysis. In addition, we recorded whether a thistle clone in a tub was systemically infected by the rust fungus *Puccinia punctiformis* (Str.) Röhl (Uredinales).

Larval Densities in the Field

The number of *C. arvensis* shoots infested with *A. onopordi* and the number of individuals per shoot were recorded from dissections of shoots collected three times between March (eggs and early instar larvae) and August (late instar larvae) 1998 in the field at four sites with natural populations of the weevil. Two of the sites (Köniz, Bern) were located in the vicinity of Bern; the other two were located in the Swiss Valais (Sierre, Pfywald). At each site, between 12 and 40 *C. arvensis* shoots representative of the thistle population with respect to size were collected arbitrarily along a transect at each visit and dissected under a stereomicroscope.

Oviposition Rate

The number of eggs laid by 11 female weevils, kept singly in transparent plastic boxes (10.5 cm diameter, 15 cm height), on cut *C. arvensis* shoots was recorded daily from 23 to 26 June 1998.

Statistical Analysis

Data of the tub experiment were analyzed with a general linear model (Systat, 1997), with "weevil infestation" and "plant competition" as main factors, an

interaction between them, and a blocking factor. To meet assumptions of normality and homogeneity of variances implicit in the parametric analysis, the logarithmic transformation [$\ln(y + 1)$] was applied to weights, the square root transformation [$\sqrt{y + 0.5}$] was applied to the counts of flower heads per tub, and the arcsine transformation [$\sin^{-1}(\sqrt{y})$] was applied to percentages (Sokal and Rohlf, 1995). Data on number of seeds per flower head were left untransformed, because transformation did not improve the homogeneity of variances and normal distribution. Tub containing thistle shoots systemically infested by the rust fungus *P. punctiformis* in 1999 were excluded from statistical analysis for estimation of the effect of *A. onopordi*. Comparisons of frequency data were made with the χ^2 test (Zar, 1996). Comparisons of root biomass between harvest dates were made with analysis of variance (ANOVA). Overall comparisons of the number of weevil individuals in thistle shoots between more than two treatment groups were made by Kruskal-Wallis ANOVA and multiple comparisons with the Dunn test (Zar, 1996).

RESULTS

Larval Densities

Female weevils kept singly in transparent boxes on cut thistle shoots laid on average one egg per day (Table 1). We found up to five eggs in the same shoot cutting after 72 h. In the garden experiment, however, weevil infestation periods of 2 or 3 days did not result in higher larval infestation levels than an infestation period of 1 day. In the experimental plots in autumn 1998, 75% of the *A. onopordi*-infested shoots bore one larva only, and 25% bore two larvae. In autumn 1999, one larva was found in 72%, whereas two larvae were found in 28% of the infested shoots. The larval infestation levels from the tub experiment corresponded well with the natural infestation levels found at the four field sites (Table 1). The number of eggs and first instar larvae in field-collected thistle shoots in spring 1998 did not differ from the number of older larvae found later in the year (88 shoots contained no, 17 shoots one, and 4 shoots two or more weevils early, and 57, 9, and 3, respectively, late in the season; $\chi^2 = 0.27$, $df = 2$, $P = 0.87$). Thus, mortality during larval development was assumed to be negligible in *A. onopordi*. Because our method of letting the female *A. onopordi* oviposit for different lengths of time failed to produce different larval densities per shoot, tubs were classified as either infested or uninfested by *A. onopordi* for further analysis.

Harvest

Of 30 creeping thistle clones in which we found indications of larval mining in 1998, 18 developed shoots

TABLE 1

Percentage of Shoots Infested by *A. onopordi* and Number of Eggs/Larvae per Infested Shoot, from the Laboratory Experiments, the Tub Experiments in 1998 and 1999, and the Field

Groups	N ^a	% Shoots infested ^b	Number of eggs/larvae per infested shoot ^c
Laboratory (eggs)			
24 h	11	81.8	0.91 ± 0.54a
48 h	11	90.9	1.73 ± 0.79a
72 h	11	100	3.00 ± 1.18b
Experiment 1998/plant competition (larvae)			
24 h	28	46.4	1.40 ± 0.55a
48 h	28	46.4	1.20 ± 0.45a
72 h	28	71.4	1.38 ± 0.52a
Experiment 1998/no plant competition (larvae)			
24 h	28	50.0	1.40 ± 0.55a
48 h	28	75.0	1.38 ± 0.52a
72 h	28	64.2	1.29 ± 0.49a
Experiment 1999 (larvae)			
Plant competition	30	66.7	1.38 ± 0.50a
No plant competition	30	73.3	1.30 ± 0.47a
Field sites (eggs/larvae)			
Bern	56	28.5	1.00 ± 0.00a
Köniz	59	8.5	1.33 ± 0.52a
Sierre	87	20.7	1.60 ± 1.34a
Pfynwald	53	18.9	1.43 ± 1.13a

^a N, number of shoots dissected.

^b % of shoots infested by *A. onopordi*.

^c Mean ± SD. Different letters indicate significant differences between the number of eggs/larvae per infested shoot of the treatments within the five groups (Dunn test; $P < 0.05$).

systemically infected by the rust fungus, *P. punctiformis*, in 1999, whereas none of the 20 controls without weevil infestation grew rusted shoots ($\chi^2 = 13.04$; $P < 0.001$). Of all the thistle clones infested by *A. onopordi* in 1998, significantly fewer clones grown with plant competition (22.2%, $N = 9$) developed shoots systemically infected by the rust in 1999 than clones grown without plant competition (76.2%, $N = 21$; $\chi^2 = 5.56$; $P = 0.02$). Systemically infected shoots died before flowering.

At the first harvest date in autumn 1998, almost all above- and belowground parameters of *C. arvensis* measured, except the number of seeds per flower head and the weight of the seeds, were significantly affected by plant competition, whereas herbivore infestation showed no measurable effect on any of these parameters (Table 2). There was a significant block effect on aboveground thistle performance in 1998, which can be attributed to the different emergence dates of thistles in different blocks. In autumn 1999, both the total above- and the total belowground thistle biomasses were significantly affected by plant competition. *A. onopordi* infestation reduced the total aboveground biomass of *C. arvensis* in the second year (Tables 2 and 3). There was a significant interaction between plant competition and herbivory for the aboveground biomass (Table 2).

In spring 1998, the root pieces planted in the tubs showed no differences in weight or nitrogen content. The biomass of the root system did not change significantly between harvest dates within each treatment (ANOVA; $P > 0.6$; Table 3). Root systems of clones grown without plant competition were about three times heavier than root systems of clones grown with plant competition. The nitrogen content of the root systems was significantly reduced in the plant competition treatments at all harvest dates (Table 2).

DISCUSSION

Oviposition by *A. onopordi*

Although females of *A. onopordi* laid on average one egg per day in the laboratory, we found a second larva in 25% of the shoots of the tub experiment only, indicating that females avoided laying additional eggs in previously infested shoots over a 3-day period. This may be achieved, for example, by means of an oviposition-marking pheromone as known from other weevils (Kozłowski *et al.*, 1983; Kozłowski, 1989). The results from the laboratory oviposition experiment showed that on cut shoots such a mechanism did not affect egg densities. At the field sites, egg and larval densities in *A. onopordi*-infested shoots were almost identical to

TABLE 2

Two-Way ANOVA *F* Statistics (*df* = 1) for Effects of the Block, Plant Competition (Plant), and Infestation by *A. onopordi* (Weevil) on Thistle Plant Performance

Parameter	Block		Plant		Weevil		Plant × Weevil	
	<i>F</i> Ratio	<i>P</i>	<i>F</i> Ratio	<i>P</i>	<i>F</i> Ratio	<i>P</i>	<i>F</i> Ratio	<i>P</i>
Spring 1998								
Nitrogen content of root system (%)	3.635	0.067	1.042	0.316	0.882	0.316	3.434	0.074
Autumn 1998								
Biomass of leaves (g)	19.582	0.000	109.308	0.000	0.228	0.638	0.063	0.804
Primary shoot biomass (g)	28.508	0.000	113.874	0.000	0.647	0.430	0.033	0.858
Total aboveground biomass (g)	11.909	0.002	132.495	0.000	0.340	0.566	0.194	0.664
Number of flowerheads per pot	23.556	0.000	82.922	0.000	1.881	0.184	0.014	0.908
Number of seeds per flowerhead	0.088	0.770	1.087	0.310	1.175	0.291	2.619	0.121
Seed weight (mg)	0.899	0.377	0.033	0.864	0.145	0.727	0.055	0.730
Biomass of root system (g)	0.516	0.480	31.403	0.000	0.112	0.741	0.728	0.402
Nitrogen content of root system (%)	0.092	0.764	28.262	0.000	0.204	0.655	0.001	0.973
Spring 1999								
Biomass of root system (g)	0.009	0.866	11.120	0.000	0.331	0.570	0.166	0.687
Nitrogen content of root system (%)	2.234	0.149	13.550	0.001	0.053	0.820	0.030	0.864
Autumn 1999								
Total aboveground biomass (g)	1.438	0.322	121.550	0.000	7.121	0.016	4.647	0.045
Biomass of root system (g)	0.571	0.655	21.733	0.000	5.385	0.032	0.830	0.374
Nitrogen content of root system (%)	0.499	0.798	12.357	0.002	1.558	0.228	1.698	0.209

the densities found in the garden experiment. However, if there is self-limitation in the number of larvae per shoot in *A. onopordi*, this may be an obstacle to the

increase of weevil densities and thus the impact on the target plant in the field, which could make the species less suitable for biocontrol.

TABLE 3

Effects of Plant Competition and Infestation by *A. onopordi* on Thistle Performance

Parameter	Treatment			
	No plant competition/ no <i>A. onopordi</i> ^a	No plant competition/ <i>A. onopordi</i> ^a	Plant competition/ no <i>A. onopordi</i> ^a	Plant competition/ <i>A. onopordi</i> ^a
Spring 1998				
<i>N</i> ^b	28	84	28	84
Nitrogen content of root system (%)	1.87 ± 0.47	2.06 ± 0.61	2.48 ± 0.61	1.97 ± 0.57
Autumn 1998				
<i>N</i>	9	19	9	16
Biomass of leaves (g)	7.44 ± 1.98	7.42 ± 1.03	2.61 ± 1.25	2.56 ± 1.22
Primary shoot biomass (g)	12.23 ± 2.47	11.72 ± 3.42	4.01 ± 2.44	3.66 ± 1.75
Total aboveground biomass (g)	20.16 ± 3.47	19.77 ± 3.03	5.13 ± 2.94	4.52 ± 2.26
Number of flowerheads per pot	38.17 ± 13.83	34.32 ± 17.12	7.43 ± 9.46	3.86 ± 2.19
Number of seeds per flowerhead	74.58 ± 6.12	75.56 ± 7.48	71.09 ± 8.64	75.92 ± 3.30
Seed weight (mg)	1.19 ± 0.37	1.24 ± 0.17	1.14 ± 0.19	1.13 ± 0.48
Biomass of root system (g)	15.21 ± 6.48	16.49 ± 6.54	5.83 ± 2.66	4.70 ± 2.34
Nitrogen content of root system (%)	0.73 ± 0.27	0.69 ± 0.11	0.44 ± 0.11	0.43 ± 0.14
Spring 1999				
<i>N</i>	9	12	9	10
Biomass of root system (g)	18.73 ± 10.11	17.80 ± 8.81	4.95 ± 2.52	3.95 ± 2.26
Nitrogen content of root system (%)	1.36 ± 0.46	1.43 ± 0.59	0.89 ± 0.19	0.89 ± 0.14
Autumn 1999				
<i>N</i>	10	5	10	7
Total aboveground biomass (g)	28.31 ± 4.84	27.71 ± 5.91	5.87 ± 3.05	2.10 ± 1.54
Biomass of root system (g)	17.11 ± 7.19	11.51 ± 4.61	6.44 ± 3.38	4.41 ± 3.85
Nitrogen content of root system (%)	0.87 ± 0.18	0.86 ± 0.08	0.65 ± 0.05	0.72 ± 0.02

^a Values (untransformed data; mean ± SD) of the four treatments for the different parameters measured during the experiment.

^b *N*, number of replicates.

Aboveground Thistle Performance

Plant competition had a strong negative effect on aboveground plant performance in both study years. This is consistent with previous work on the combined effect of herbivory and plant competition on *C. arvensis* (Ang *et al.*, 1995; Bacher and Schwab, 2000). Not only vegetative growth but also sexual reproduction, as measured by the number of flower heads produced per clone, was significantly affected by plant competition. It is likely that this effect is even more pronounced in field populations because plant competition inhibits germination of seeds and seedling establishment of *C. arvensis* (Donald, 1994). However, the importance of seeds for the spread and population dynamics of *C. arvensis* is controversial (e.g., Donald, 1994; Oesau, 1998).

In the first year, infestation by *A. onopordi* did not result in significant differences in any of the aboveground parameters measured. The impact of the weevil was only starting to show in the second year, emphasizing the importance of longer-term testing for effects of herbivory on perennials such as *C. arvensis*.

In our experiment, *A. onopordi* and grass competition showed a classical two-factor synergy in which both factors had an impact on the weed in isolation (at least in 1999), but the combined effects of the two factors was more than the effect of each single factor. Synergism between biocontrol agents and plant competition was found only in a minority of interactions (Sheppard, 1996). However, synergistic interactions are suspected to be the basis of some cases of successful weed biocontrol, and are therefore highly desirable characteristics of biocontrol agents, yet their existence and contribution to biocontrol is poorly documented (Sheppard, 1996). In our experiment, plant competition seems to have strengthened the effect of *A. onopordi* infestation on thistle plant performance in a measurable way. Rees and Brown (1992) used a simple plant growth model to explain interactions of competition and herbivory on plant performance,

$$\frac{dB}{dt} = B[r(t) - d(t)],$$

where rate of change in plant biomass B is determined by the biomass multiplied by the difference of the plant growth rate $r(t)$ and the loss rate of biomass $d(t)$. Competition affects only the growth rate, whereas herbivores may affect the loss rate or both the loss rate and the growth rate. Sheppard (1996) argues that herbivory must affect the weed growth rate and not just reduce plant size for a synergistic outcome of the interaction between herbivores and plant competition. This may well be the case in *A. onopordi*, because its feeding site at the interface between root and shoot

may represent a serious intervention with resource allocation of the plant. Alternatively, a synergistic interaction may have been observed because plant competition reduced the number of shoots developed by a *C. arvensis* clone and therefore the number of ramets available to compensate for the herbivore impact.

Belowground Thistle Performance

The observed reduction of root growth in the presence of plant competitors leads to a reduced propagation of the weed locally. Even though tub size chosen in our experiment was fairly large, the growth of the root system of plants growing without plant competition was limited by the space of the tubs, so that the biomass of the roots was the same at all harvest dates measured. The biomass of the root system in tubs with plant competition also had the same weight at all harvest dates. Pairwise comparison revealed that plant competition suppressed growth to about a third to a fourth of the space-limited weight. In addition, the higher nitrogen content of thistles grown without plant competition may result in an increased potential to grow shoots as demonstrated in other clonal plants (De Kroon and Bobbink, 1997). Thus, plant competition significantly reduces the spread of *C. arvensis*.

Crawley (1983, 1997) suggests that, in general, stress on the shoots should reduce root growth. The effect of *A. onopordi* on belowground parameters of *C. arvensis* was significant in autumn 1999. Even at a low infestation level, *A. onopordi* showed a measurable effect on root growth of *C. arvensis* and therefore may reduce the spread of *C. arvensis* locally. However, the reduction in root biomass by *A. onopordi* was much smaller than the reduction by plant competition.

A. onopordi and the Systemic Rust Fungus *P. punctiformis*

Our experiment established that *A. onopordi* promotes systemic infections of the rust fungus *P. punctiformis* in the year following weevil infestation. Infection of thistles by *P. punctiformis* in 1999 occurred only in clones infested with *A. onopordi* the year before. *P. punctiformis* was absent from the institute's garden or its vicinity before the experiment and the root pieces used in the experiment originated from a rust-free *C. arvensis* population which was monitored since 1996. Weevils used in the experiment were collected at sites with rust-infected *C. arvensis* plants in spring 1998. Therefore, it is likely that the female weevils introduced the rust to the experimental plants. However, our study does not reveal the mechanism by which the rust is transmitted or the spore type involved. Alternatively, it may be that the field-collected root pieces were already infected by the fungus and that infestation by *A. onopordi* suppressed the defense system of *C. arvensis* to a point at which systemic infections of the

rust could develop. Despite numerous studies, the life cycle of *P. punctiformis* is still not fully understood (French and Lightfield, 1990). The only way known of inducing systemic infections of *P. punctiformis* in adult plants is by inoculation of *C. arvensis* root buds or seedlings with teliospores of the rust in the laboratory (Van den Ende *et al.*, 1987; French and Lightfield, 1990). However, it is unlikely that the weevil transmitted spores to the root buds directly. More studies are needed to understand the mechanics of the natural infection process.

To date, attempts to increase the number of systemically infected shoots in the field by the spraying of teliospores have failed (Frantzen and Scheepens, 1993). Our study is the first to show a method of promoting systemic infections of the thistle rust *P. punctiformis* under seminatural conditions. It remains to be investigated, however, whether systemic infections promoted by an insect are the rule in this weed pathosystem.

Thistle clones grown without plant competition were in a higher number of cases rust-infected than clones grown with grass competition. It is yet unknown whether this difference is due to the fact that promotion of rust infection is more effective in thistles growing without plant competition or whether infected thistle shoots have a greater mortality in a competitive environment and die before they reach the soil surface. Because of the premature death of rust-infected thistle shoots (see also Frantzen, 1994; Watson and Keogh, 1980) *P. punctiformis* was considered a highly effective and host-specific biocontrol agent against creeping thistle (Frantzen, 1994, and references therein). To date, however, techniques for successful field application are lacking.

Suitability of A. onopordi for Biocontrol

An effect of *A. onopordi* on the performance of the perennial *C. arvensis* became apparent only after the second year, for both above- and belowground parts of *C. arvensis*. However, the herbivore impact was small compared to the impact of plant competition. In our study, *A. onopordi* attack was restricted to a single shoot of *C. arvensis* clones. Thus, uninfested shoots may have compensated for losses imposed by herbivory, as is expected in clonal plants (Hutchings and Wijesinghe, 1997). Whereas it may be feasible to increase the impact of *A. onopordi* by the infesting of more shoots, it may be difficult to increase the number of *A. onopordi* larvae in single *C. arvensis* shoots in the field because of the weevils' tendency to avoid multiple infestations of the same shoot. *A. onopordi* seems to be best suited for biocontrol of creeping thistle in combination with plant competition, because of the synergistic interaction between both, and because of other insect species feeding on *C. arvensis*. One advantage of *A. onopordi* is its

feeding niche in the interface of shoot and root. Because most herbivores of *C. arvensis* feed and develop on the leaves or in the stems (Zwölfer, 1965), direct competition between *A. onopordi* and other biocontrol agents of creeping thistle is unlikely. A promising aspect of *A. onopordi* as biocontrol agent of *C. arvensis* is its ability to promote systemic infections of the rust fungus *P. punctiformis*. The avoidance of *A. onopordi* females of ovipositing in thistle shoots occupied by conspecifics, which is responsible for the limited direct effect of the weevil on its host, may even help to spread the rust at a higher rate by forcing the weevils to encounter more plants during their search for suitable oviposition sites. The potential of *P. punctiformis* for biocontrol of *C. arvensis* was already recognized early in this century (Ferdinandsen, 1923), but because of the failure to increase the incidence of systemic infections in the field, so far the rust has remained unsuccessful as a biocontrol agent (Julien, 1998). The discovery of the potential of *A. onopordi* to promote systemic *P. punctiformis* infections may help in spreading the rust in the field, for example by augmentative releases of the weevil.

ACKNOWLEDGMENTS

We are grateful to Jos Frantzen, Wolfgang Nentwig, and the anonymous reviewer for helpful comments on earlier versions of the manuscript.

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