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Impact of root mining by *Eteobalea* spp. on clonal growth and sexual reproduction of common toadflax, *Linaria vulgaris* Mill.

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Summary: Résumé: Zusammenfassung

Experiments with potted plants of the weed *Linaria vulgaris* Mill. (Scrophulariaceae) were carried out to evaluate the effects of root mining by *Eteobalea* (syn. *Stagmatophora*) spp. (Lep. Cosmopterigidae). Attacked plants had a shorter flowering season and produced seeds of lower weight. Continuous mining during winter resulted in a doubling of the number of stems in spring, but the total plant biomass remained unaffected. This may have been caused by disrupted apical dominance due to root-crown damage. The observed effects are expected to complement those of other agents used in the biological control of *L. vulgaris* in North America.

Keywords: impact, root feeding, reproduction, biological control, *Linaria vulgaris*, toadflax, *Eteobalea*, Cosmopterigidae.

L'incidence du minage des racines par Eteobalea *spp. sur la croissance végétative et la reproduction sexuée de la linaire commune* Linaria vulgaris *Mill.*

Des expériences en pot sur *Linaria vulgaris* Mill. (Scrophulariacées) ont été conduites pour évaluer l'effet du minage des racines par *Eteobalea* (syn *Stagmatophora*) spp. (Lep. Cosmopterigidae). La période florale des plantes attaquées était plus courte et les graines produites avaient un plus faible poids. Un minage continu pendant l'hiver se traduisait par un doublement du nombre de tiges au printemps mais la biomasse totale de la plante n'était pas affectée. Cela pourrait être dû à l'arrêt de la dominance apicale par suite de dommages au collet. On attend des effets observés qu'il complètent ceux d'autres agents utilisés dans la lutte biologique contre *L. vulgaris* en Amérique du Nord.

Einfluß der Befalls der Wurzelbohrer Eteobalea spp. *auf Wachstum und sexuelle Fortpflanzung des Gemeinen Leinkrauts* (Linaria vulgaris *Mill.*)

Bei Topfversuchen über die Wirkung des Fraßes von *Eteobalea* (=*Stagmatophora*) spp. in den Wurzeln von *Linaria vulgaris* Mill. hatten die befallenen Pflanzen eine kürzere Blühperiode, und die Samen waren leichter. Über den Winter anhaltender Befall führte zur Verdopplung der Sproßdichte, doch die Gesamt-Biomasse blieb unverändert. Dies könnte auf die Unterbrechung der Apikaldominanz infolge der Schädigung der Wurzelkrone zurückzuführen sein. Die beobachteten Wirkungen könnten die anderer biologischer Agentien zur biologischen Bekämpfung von *Linaria vulgaris* in Nordamerika ergänzen.

Introduction

More than half the net primary production in ecosystems is commonly allocated to belowground parts (Andersen, 1987). Further, plant species with substantial amounts of below-

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ground reserves are commonly attacked by below ground herbivores, with devastating effects in some cases (e.g. corn rootworm). Nevertheless, most studies on the effects of herbivore damage concentrate on the above-ground plant parts (Brown & Gange, 1990). The extent and direction of such effects on plant performance have been discussed extensively in recent years (Belsky, 1986; Crawley, 1987; Maschinski & Whitham, 1989). The ability of many plant species to regenerate, and compensate for damage, is the reason that reactions to attack may lie anywhere on a continuum between 'overcompensation' (Belsky, 1986; Müller, 1991a; Steinger & Müller-Schärer, 1992) and death.

The impact of herbivory on plant performance not only depends on the amount and type of plant tissues removed, but also on time of attack and its relation to the life cycle of the host plant (Maschinski & Whitham, 1989). In temperate regions it is difficult to evaluate the effects of herbivory on plant performance during the winter, or very early in the spring, if only the above-ground biomass is taken into account. Testing the consequences of natural root damage (rather than root clipping experiments [Schmid *et al.*, 1990]) provides a chance to estimate these effects more completely. Insight into clonal integration may also be gained, if resource allocation is being examined. Clearly, a general theory of plant reactions to herbivore attack can emerge only if below-ground plant parts are taken into account.

The experiments described below tested the effects of two species of small root-mining moths, *Eteobalea* (syn. *Stagmatophora*) *serratella* Treitschke and *E. intermediella* Riedl (Lepidoptera: Cosmopterigidae), on their natural host *Linaria vulgaris* Miller (Scrophulariaceae). *L. vulgaris* was introduced to North America from Europe. It is now an increasingly serious weed in some parts of the United States and Canada, and is thought to be related to the adoption of reduced-tillage methods (Holm *et al.*, 1979; McClay, 1992). As a perennial with vigorous vegetative growth and high seed production (Kraus, 1909) it provides a suitable system for investigating the potentially diverse effects of root mining. *E. serratella* and *E. intermediella* were identified as the most important root feeders based on a comprehensive literature review and on field collections in Central Europe (Saner, 1991). The two species are very similar in size and mining behaviour, but differ in the time of their main feeding period (Saner, 1991). Impact experiments can help to determine which plant parts are most susceptible to damage, and to facilitate the choice of biological control agents (Müller, 1989). Finding the most efficient agents early in a programme may decrease both the cost and the residual risks of biological control. Both species of *Eteobalea* are now being released in North America for the control of *Linaria* spp. (Saner *et al.*, 1990; P. Harris, personal communication).

The following assumptions are tested in this paper: (1) *Eteobalea* spp. decrease sexual reproduction of *L. vulgaris*, (2) vegetative growth is enhanced because of the reallocation of resources by *L. vulgaris* (*Eteobalea* spp. usually do not damage buds in the lateral root system).

Materials and methods

The species

Linaria vulgaris ('common toadflax') is a perennial weed with circumpolar distribution. It occupies dry, open habitats such as roadsides, railway tracks, clear cuts and old fields (Hartl, 1974). Germination may take place shortly after seed set but usually occurs in spring. The main stem dies off after germination, but up to 100 secondary shoots, all of which flower, are produced in the first year (Hartl, 1974). Flowering stems range in height from 25 to 120 cm. Estimates for seed production of German populations vary from 800 up to 33 000 per individual (Kraus, 1909; Kock, 1966). Vegetative growth can start as early as 14 days after germination (Zilke, 1954). Growth is highly plastic owing to the production of additional shoots from all parts of the stem and by vegetative propagation from adventitious root buds (Bakshi & Coupland, 1960). The species has the ability to regenerate from root fragments. The root system forms a widespread network of runners close to the surface as well as a main root which may penetrate more than 1 m into the soil (Kutschera, 1960).

E. serratella and *E. intermediella* are root-mining moths specialized on hosts within the tribe Antirrhineae (Scrophulariaceae) (Saner, 1991). *E. serratella* has an Eurasiatic distribution that widely overlaps with the Mediterranean distribution of *E. intermediella*. The two species have very similar ecological requirements and life cycles, but the former has one while the latter has two generations per year (Saner, 1991). The insects used in this experiment were collected in a population of *L. vulgaris* in the environs of Rome, Italy, where both insect species co-occur. Larvae feed specifically on *Linaria* spp. and complete development takes place on, or within the host plant. Eggs are placed on the lower parts of the shoot. Larvae penetrate the epidermis of the stem immediately after they hatch from the eggs and mine down to the main root. Most of the development takes place in the main root, where the larvae tunnel in the cortex and central parts. At the end of the larval development they tunnel back to the lower parts of the stem (larvae are approximately 1 cm long and 1 mm wide at this stage). Pupation takes place in the lower parts of the stems or in the upper part of the main root. Both species overwinter as larvae (Saner, 1991).

Effects of Eteobalea serratella *on plants of* Linaria vulgaris *collected in the field*

Ten individual uninfested plants, each with at least four root shoots ('clones') were collected at the end of June 1988 in the area of Delémont, Switzerland. Four clones of similar size were chosen from every plant and planted into clay pots of 15 cm diameter (19 cm height) in a mixture of half sand and half commercial garden soil (peat soil). These 40 plants were kept in a small glasshouse to protect the plants from rain. Temperature in the glasshouse was kept within natural range by ventilation. Three freshly emerged laboratory-reared larvae of *E. serratella* were randomly assigned to each of two clones of each plant and transferred with a small paint brush to the lower part of the stems on 20 July 1988. Larvae have to penetrate the host plant quickly in order to survive; a migration from the assigned plant to another pot can be excluded. The remaining two clones from each plant were used as controls. The position of the pots in the greenhouse was changed bi-weekly at random.

Vegetative growth and fruit production was recorded on six occasions, 20 and 27 July, 3, 11, and 18 August, and 15 September. All ripe fruits were recorded and removed weekly to collect seed samples, from 2 September until 3 November, when the remaining above-ground biomass was harvested. Pots were then transferred outside (dug into the ground) and the entire plants (roots and new shoots) were harvested on 6 June 1989. Attack by *Eteobalea* was verified by root dissection. Dry weight was assessed after drying at 65°C for 2 days. The effects of *Eteobalea* spp. on *L. vulgaris* were determined with analysis of variance procedures (Sokal & Rohlf, 1981) using the GLM procedure of SAS (SAS Institute, 1988). Clones from the same plant individual were grouped as blocks. Shoot weight (June 1989) and shoot/root ratio were log transformed to satisfy assumptions of the statistical test.

Effects of Eteobalea intermediella *(autumn generation) on* Linaria vulgaris

Seeds of *Linaria vulgaris* collected in British Columbia, Canada, were sown 10 May 1988. Six first instar larvae were transferred onto each of 20 plants at the end of September 1988 and another 20 plants were used as controls. Potting of plants, method of larval transfer, date and method for overwintering, harvest of shoots, dissection, and weight determination were done as described above. Only six of the 20 treated plants were attacked in June 1989 and two plants from the control group had also to be excluded because of experimental errors. *t*-Tests were used on log transformations of results for number and weight of stems, shoot/root ratio and total weight.

Results

Survival and development of Eteobalea *spp.*

Most larvae of *E. serratella* were in the final larval stage at the end of the first experiment, but one individual had already completed its pupal stage. One root contained all three transferred larvae, but in all other roots only a single larva had survived. The former clone performed close to the average in the attacked group except for the number of new shoots (June 1989), which was exceptionally high (61, see below). The variability in development of the transferred larvae was higher in the second experiment with *E. intermediella*. Most larvae were in the second to last instar but also two exuviae, one of them in the same root as two very small larvae, were recorded. All other plants were attacked by one larva only. No attack by other root feeding insects occurred in the two experiments.

Survival and clonal growth of Linaria vulgaris

Root mining had no effect on plant survival. In

Table 1. Effect of *Eteobalea serratella* on *Linaria vulgaris*: comparison of plant traits of uninfested (control) and infested clones (means±SE)

Plant trait	Control	*E. serratella*	*P*
Autumn 1988			
Fruit number	9·3±2·9	6·6±1·9	NS
Fruit weight (g)	0·11±0·4	0·10±0·4	NS
Individual seed weight (mg)	0·221±0·025	0·167±0·019	0·0024
Shoot weight (g)	0·99±0·11	0·99±0·10	NS
June 1989			
Shoot number	12·8±1·8	27·1±4·8	0·0035
Shoot weight (g)	1·32±0·29	1·17±0·19	NS
Individual shoot weight (g)	0·111±0·021	0·052±0·010	0·015
Root weight (g)	2·54±0·33	2·33±0·22	NS
Shoot/root ratio	0·51±0·07	0·49±0·06	NS
Total biomass (g)	4·96±0·64	4·59±0·49	NS

P-values are derived from two-way ANOVA for block (plant individual) and treatment ($d.f._{error}=8$ (6 for individual seed weight), $d.f._{block}=8$, $d.f._{treatment}=1$). Block effects are discussed in the text. Total biomass is the sum of the dry weights of fruits, both shoot harvests, and roots. NS=not significant at $P<0·05$.

the experiment with *E. serratella* two plants died in each of the treatments, and in the control group. In the second experiment on the effects of *E. intermediella* all plants survived.

Clones attacked by *E. serratella* had an increased production of vegetative shoots in the first month after attack, but at the end of the season this trend was reversed. The shoot weight at the end of the season was identical for both groups (Table 1). However, after the winter, attacked clones produced, on average, more than double the number of new shoots than unattacked clones and this trait was also more variable among attacked plants (Table 1). The total weight of these shoots did not differ between the groups, although the individual weight of stems from attacked plants was halved. The slight reduction of the various weights was not significant at the 5% probability level. No block effects (plant individuals) on traits related to clonal growth were detected.

For *E. intermediella* only the effects after the winter were measured. The mean number of shoots was higher in attacked plants (19·2±3·8 [±SE of means]) than in unattacked plants (16·9±1·3) but this difference was not statistically significant ($d.f._{error}=22$, $P=0·55$). Changes in shoot, root and total weight due to attack were not statistically significant at the 5% probability level.

Sexual reproduction of Linaria vulgaris

The influence of mining on sexual reproduction in *L. vulgaris* was evaluated only for *E. serratella*. Fruit number was on average almost one-third lower in attacked clones, however this difference was not statistically significant ($P=0·13$, see Table 1). Block effects (plant individual) were present for number of fruits (d.f.=8, $P=0·0045$) and weight of individual seeds (d.f.=8, $P=0·0005$). Comparison of fruit phenologies (Fig. 1) shows that attacked clones produced fruits early in the season only and lacked the second peak of production of unattacked clones late in the season. The dry weight of individual seeds was decreased, on average, by more than a quarter in attacked clones and this difference was statistically highly significant (Table 1).

Shoot/root ratio

At the end of both experiments the shoot/root ratios were calculated as a crude measure of

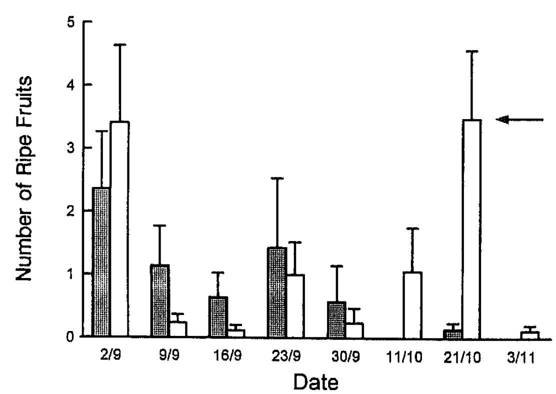

Fig. 1. Effect of *E. serratella* on fruit production of *L. vulgaris* (mean number of ripe fruits per clone ±SE of means). The arrow indicates the second peak of fruit production in unattacked clones (open bars) which did not occur in attacked clones (shaded bars). The graph represents all fruits produced during the season.

resource allocation. In the experiment with *E. serratella* differences between control and infested plants were not statistically significant (Table 1). Plants attacked by *E. intermediella*, however, had lower shoot:root ratios ($0{\cdot}45 \pm 0{\cdot}05$ versus $0{\cdot}58 \pm 0{\cdot}04$, d.f.$_{error}$=22, P=0·043).

Discussion

The data presented show that root feeding affects parameters related to sexual reproduction of *L. vulgaris*. Plants attacked by *E. serratella* lacked the second peak of fruit production that occurred in the control group. This can be explained by the increased damage to the plants through the season, owing to the increased food consumption by the developing larvae. McClay (1992) found that shoot herbivory by the sap beetle *Brachypterolus pulicarius* L. (Coleoptera: Nitidulidae) depressed early-season flowering in *L. vulgaris*. This constitutes a similar coincidence of time of herbivory and time of depressed flowering. McClay (1992) also reported that plants attacked by *B. pulicarius* produced seeds of reduced individual weight. However, he did not find a correlation between seed viability and individual seed weight. For the related *L. dalmatica* Alex (1959) found that reduced seed weight may yield lower germination rates. It may be of some interest to further investigate the relationship of seed weight and seedling performance in *L. vulgaris*.

The observed block effects for number of fruits and individual seed weight indicate an effect of plant size or of genotype. Initial fresh weight had a statistically significant effect on seed weight in a covariance analysis of the same experimental data (Saner, 1990), but plant size and genotype cannot be separated in these field-collected plants.

The increase in shoot number of plants attacked by *E. serratella* only partly agrees with the second assumption that the host plants reallocate resources to lateral parts, since the biomass of these parts did not differ between infested and uninfested plants. Therefore, it may simply indicate that apical dominance was broken because of root-crown feeding. Disturbance of the functional integrity of a host plant by root mining has also been shown for knapweed, *Centaurea maculosa* Lam. (Asteraceae) (Müller, 1991a; Steinger & Müller-Schärer, 1992). The absence of an increase in shoot number in the second experiment discussed here may be explained by the fact that two-thirds of the larvae of *E. intermediella* had not reached their final instar at the time of evaluation, owing the late larval transfer.

The differences in shoot/root ratio are probably conservative estimates in both experiments, since the growth of the root systems was restricted by the pots. It was observed that roots were densely packed at the end of the experiments and that the root weights varied little between attacked and unattacked plants and between experiments. This may explain the absence of large effects in the first experiment with *E. serratella*. The observed decrease in the shoot/root ratio in plants attacked by *E. intermediella* agrees with the hypothesis that *L. vulgaris* reallocates resources during root attack.

The absence of treatment-specific mortality in our experimental plants does not indicate that the insect cannot kill the plant host. In nature, *L. vulgaris* is often attacked by several larvae of *Eteobalea* spp. simultaneously. In populations of *L. vulgaris* at very dry sites in Italy dead plants were often found with roots heavily attacked by *Eteobalea* spp. (M. Saner, personal observation). A comparison with living plants nearby suggested that the combined effect of drought and high attack rate may kill individual plants. In other investigations it was shown that root herbivory and interspecific competition may affect host plants synergistically (Müller, 1991b), hence, the absence of natural competition in the experiments may have resulted in an underestimation of the potential effects of *Eteobalea* spp.

For the purpose of classical biological control it is of interest that both clonal growth and sexual reproduction of *L. vulgaris* can be negatively affected by *Eteobalea* spp. Monitoring the distribution and effect of released insects may be facilitated by the easily visible plant reactions documented here. This is valuable since adult *Eteobalea* spp. are active during the night and the larvae invisible owing to their root-mining habits. The effects of *Eteobalea* spp. reported here are expected to be complementary to the effects of biological control agents already established in North America, namely weevils of the genus *Gymnetron* and the sap beetle *B. pulicarius*. Shoot feeding by *B. pulicarius* (and possibly by *Gymnetron* spp.) suppresses early season flowering (McClay, 1992), root feeding by *Eteobalea* spp. suppresses late season flowering, and fruit feeding by the larvae of *Gymnetron* destroys a large proportion of the seeds that are produced despite the former two effects (Harris & Carder, 1971).

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