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Interaction among Two Biological Control Agents and the Developmental Stage of their Target Weed, Dalmatian Toadflax, *Linaria dalmatica* (L.) Mill. (Scrophulariaceae)

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An experiment with potted Linaria dalmatica *(L.) Mill. was set up to test the effects of root-feeding by* Eteobalea *(syn.* Stagmatophora) intermediella *Riedl (Lep. Cosmopterigidae) and stem-mining by* Mecinus janthinus *Germar (Col. Curculionidae), as well as the interaction among the insects and the developmental stage of the host plant. During the summer season, mining by* M. janthinus *decreased the stem biomass substantially, while* E. intermediella *did not produce statistically significant effects on the performance of host plant individuals (the experimental conditions represented the effect of the univoltine* M. janthinus *adequately, but the potentially important effect of mining by the second generation of* E. intermediella, *at the beginning of the growing season, was not assessed).* M. janthinus *survived almost exclusively on plants in the flowering stage, although adult females readily used vegetative (younger) plants for oviposition.* E. intermediella *had a higher chance of survival on vegetative plants. Both species have recently been released as biological control agents on* L. dalmatica *in North America. For this purpose the complementary survival rates on the two developmental stages of the host plant is advantageous.*

Keywords: *impact, miners, weed, interaction,* Linaria dalmatica, *Cosmopterigidae, Mecinus*

INTRODUCTION

Pre-release assessments of agents for the classical biological control of weeds commonly focus on safety aspects, i.e. on the potential host-range of the agents. Efficacy, and compatibility with other control methods are important features of all pest control products or methods, but these data are rarely generated. The major reason, besides cost, is the low accuracy of predictions of efficacy in classical biological control. Nevertheless, we argue, that research in this area has valuable applications. Firstly, information on efficacy facilitates the understanding of post-release

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monitoring, even if the data does not allow the formulation of accurate predictions. Secondly, "introductions of animals and plants into new habitats are some of the most important field experiments ever carried out in ecology" (Crawley, 1986), i.e. there is an important spin-off of information from biological control to ecological theory, provided that projects are well planned and documented. Thirdly, predictions have to be attempted in order to be tested. There is no clear reason why appropriate test methods and models could not be developed to predict efficacy with sufficient accuracy to be useful in application.

This study is part of a project on the biological control of *Linaria dalmatica* (L.) Miller (Scrophulariaceae), a weed in western North America. The root-mining moth *Eteobalea* (syn. *Stagmatophora*) *intermediella* Riedl (Lep. Cosmopterigidae) and the stem-mining weevil *Mecinus janthinus* Germar (Col. Curculionidae) are two of its major natural enemies in Europe. Both insect species occur sympatrically in southern Europe (Saner *et al.*, 1990; Jeanneret & Schroeder, 1992) and are now released as biological control agents on *L. dalmatica* in Canada (R. DeClerck-Floate, personal communication). *L. dalmatica* was introduced from Europe into North America at the end of the last century. Since then it has spread across the continent, and has become a weed in dry rangelands in western USA and Canada (Alex, 1962). *L. dalmatica* is considered a weed because of its ability to displace native plants in natural areas and because it is not consumed by livestock, in contrast to some of the plant species it replaces (Jeanneret & Schroeder, 1992). Previous biological control programme using seed-feeding beetles of European origin were locally successful for common toadflax, *L. vulgaris* Mill. (Harris, 1984; McClay, 1992), but control of *L. dalmatica* has not yet been achieved using biological means (Julien, 1987).

The experiment described here examines the effects of the two insect species on plant growth and on survival of insects under different conditions. Since the interactions between host plant and herbivores are dependent on environmental conditions (e.g. Müller-Schärer, 1992), and since *L. dalmatica* (as a weed) occurs over a large environmental gradient, the environmental factors presumed to be the most important have been included. These are water supply (as a limiting factor for plant growth) and developmental stage of the host plant (as an element of the insect environment). The following are explored in this paper: (1) the effects of single and joint attack by *E. intermediella* and *M. janthinus* on performance of *L. dalmatica* individuals, and (2) the effect of plant developmental stage and inter-specific interaction on survival rates of *E. intermediella* and *M. janthinus*. These questions address two issues of basic ecological research that have received recent attention. Recent studies have shown that plant responses to damage are often more complicated than initially assumed, i.e. plant performance may vary on a continuum from 'overcompensation' to death (Belsky, 1986; Maschinski & Whitham, 1989; Müller, 1991a). Most of the detailed studies on this phenomenon are based on external damage of above-ground parts (Andersen, 1987; Wilson, 1988). The measurement of the effects of 'cryptic' damage, e.g. damage by miners or root feeders, is more difficult, but nevertheless essential to complete the understanding of the effects of herbivory on individual plants. Ecological research on plant-mediated interactions between two insect species has been mostly based on the interactions between folivorous insects (but see Gange & Brown, 1989). Again, the inclusion of 'cryptic' herbivory in the discussion on the prevalence and importance of this interaction is essential, since internal feeding is common among herbivores of the largest insects orders (Borror *et al.*, 1989).

MATERIALS AND METHODS

The Species

L. dalmatica is a perennial herb native to southern Europe and south-western Asia, that was introduced to Britain and North America, probably as an ornamental (Alex, 1962; Sutton, 1988). It is adapted to very dry sites with little competition (Alex, 1962). The principle areas of infestation are south central British Columbia to Washington, Oregon and Montana (Jeanneret & Schroeder, 1992). Two different kinds of stems, tall flowering stems and shorter prostrate stems are produced. The latter form a loose rosette, are produced by seedlings as non-flowering stems (early in the season) and by mature plants in autumn as a basal mat that persists over winter (Robocker, 1974). In addition vegetative reproduction occurs in the form of adventitious shoots produced from the root system. Only a few flowering stems are produced in the first year, but up to 25 in each of the following seasons. However, under our experimental conditions first year plants did not flower at all (see below). Large individuals produce up to half a million seeds per year. Seeds may germinate in autumn but usually germinate in spring. The lifespan of a main root is typically 3 to 5 years, but plant individuals may theoretically persist forever due to vegetative reproduction (Lange & Wolfe, 1954; Robocker, 1974).

E. intermediella is a nocturnal moth with a wing span of about 17 mm, specific to hosts within the Antirrhineae (Scrophulariaceae) (Riedl, 1969). Eggs are laid in clusters on the lower parts of the stems from whence the larvae mine downwards to the central root. Most of the development takes place in the root. No particular area of the main root could be defined to be the typical niche of *E. intermediella* larvae and considerable amounts of vascular root tissue can be destroyed. The final instar larvae bore back into the lower parts of the stem, where they may damage the base of several stems. Pupation takes place in the stem base or in the upper part of the root. The number of larvae per root under field conditions is highly variable and varies with root diameter, but three to seven larvae per plant individual are most commonly found. Two generations a year are produced, and overlapping generations are possible. Adults of *Eteobalea* spp. do not feed and live for about 2 weeks (Saner, 1991).

M. janthinus is a weevil with a body length of 3.0–4.2 mm, specific to hosts within the Antirrhineae (Scrophulariaceae) (Scherf, 1964; Lohse, 1983). Eggs are laid inside a cavity gnawed by the females in prostrate and flowering stems, and are covered with a lid. The entire stem may be used for oviposition. Larval development takes about 40 days. Several weevils may develop in a single stem in separate mines of 1–3 cm in length. The species is univoltine and adults remain in their pupal cases for hibernation. The active period of adults outside the plants in early summer is about 1 month, during which time they feed on the leaves and stems of the host plants (Scherf, 1964; Jeanneret & Schroeder, 1992).

The Experiment

Plants were grown in clay pots from seeds collected in western Canada. Sixty plants were divided into 10 blocks; each block contained six plants of similar size and architecture. Four blocks were comprised of 6-month-old plants, which did not flower during the experiment ('vegetative plants'), and six blocks were comprised of 18-month plants that all produced flowers ('flowering plants'). The following experimental treatments were assigned at random to the plants within each block: (1) root mining by *E. intermediella*, (2) stem mining (larvae) and feeding (adults) by *M. janthinus*, (3) combination of the two, (4) combination of the two plus water stress (watered half as often), (5) control (no insect feeding) and (6) control plus water stress.

Eggs or first-instar larvae of *M. janthinus* cannot be transferred easily. Therefore, the plants were kept for 4 weeks in a gauze covered cage of 13 × 150 × 130 cm into which one copulating pair of weevils (collected in Macedonia) was transferred on to each plant on 28 June 1989. The plants without *Mecinus* treatment were kept under the same conditions in an adjacent cage of the same kind. Root mining was initiated by transferring three freshly hatched laboratory reared *E. intermediella* larvae on to the lower plant parts on 14 July. The adults used for breeding had been collected near Belgrade, Yugoslavia, from *L. genistifolia* (L.) Mill. which is closely related to *L. dalmatica* (Alex, 1962). All plants were transferred into a small greenhouse on 25 July that served as a protection from rain. On 3 October the proportion of dead stems among the prostrate stems of vegetative plants was recorded. Biomass yield, and, by dissection, oviposition rate of *M. janthinus* (number of eggs) and survival rates of both species were determined on 15 November. Shoot, roots and seeds were collected separately, and dried for 2 days at 65°C. A sample of 30 seeds was collected from each of the flowering plants, weighed and analyzed in a C–H–N-analyzer for the measurement of nitrogen content (as an indirect measure of seed quality).

The effects of the treatments on plant traits were examined by analysis of variance (Sokal &

Rohlf, 1981) using the generalized linear models procedure of SAS (SAS Institute, 1988). Block effect was included as a random effect (nested in the variable developmental stage). Log transformations were performed on weights of shoots and roots, and the nitrogen content of seeds. A two-way ANOVA for treatment and block was used to determine whether differences in oviposition rates of *M. janthinus* occurred.

The effects of plant developmental stage and the presence of the other insect species on survival of *E. intermediella* and *M. janthinus* were examined with a log linear model (Sokal & Rohlf, 1981) in the CATMOD procedure of SAS (SAS Institute, 1988). The model was based on the three-way interaction between developmental stage of the host plant, survival of larvae of one species, and presence of the other species (2*2*2 table). Data from the low water treatment were not included and again only data from plants with verified attack were used. The survival rate of *M. janthinus* on vegetative plants with *E. intermediella* had to be changed from 0 to 1 (from 0 to 6% survival) in order to calculate the three-way interaction. This is a conservative manipulation since it results in a slight underestimation of the effects of developmental stage and the presence of *E. intermediella*.

RESULTS

Effects of Insect Damage and Water Stress on Plant Performance

A two-way ANOVA for treatment and block revealed no statistically significant differences between oviposition rates of *M. janthinus*. Therefore, it can be assumed that the weevils made no active choice between plants of the different blocks (this covers potential differences between the two developmental stages) and that the subsequent assignment of treatments did not result in treatment groups with significantly different attack rates. Attack by *M. janthinus* resulted in a decreased shoot biomass from 5.50 to 3.86 g ($F_{1,33}$ = 6.58, $P = 0.015$) and consequently in a decreased shoot/root ratio. In this analysis, no interaction between *M. janthinus* attack and developmental stage of the host plant occurred ($F_{1,33} = 0.008$, $P = 0.85$). A substantial increase in dead prostrate stem due to both *M. janthinus* attack (from 51 to 77%, $F_{1,15} = 3.50$, $P = 0.081$) and water stress (from 47 to 81%, $F_{1,15} = 3.96$, $P = 0.065$) was detected in autumn. Shoot biomass was not affected by *E. intermediella* nor water stress and root biomass was not affected by any of the treatments. No statistically significant differences in the measured seed quality parameters between the different treatment groups or blocks, nor any interactions between any of the treatments, could be detected.

Interaction among the Insects and the Developmental Stage of *L. dalmatica*

Survival to the adult stage of *M. janthinus* was drastically greater in flowering plants than in vegetative plants (Figure 1). The mean oviposition rates per plant shown in Figure 1 appear to indicate a preference for vegetative plants; however, these differences were statistically not significant (as reported above). No effect of the presence of *E. intermediella* on survival of *M. janthinus* could be detected and no indication of a three-way interaction of developmental stage, presence of *E. intermediella* and survival was found (Table 1).

The corresponding analysis for survival of larvae of *E. intermediella* is given in Figure 2 and Table 2. *E. intermediella* survived on both developmental stages but with better success on vegetative plants rather than on flowering plants. Again, no effect of the presence of the other species or a three-way interaction could be detected.

DISCUSSION

Effects of Insect Damage and Water Stress on Plant Performance

Feeding by the weevil *M. janthinus* resulted in reduced stem biomass of *L. dalmatica* individuals and in a higher mortality of prostrate stems in vegetative plants. The latter effect was also observed as a result of the water stress treatment. Probably, the physical damage of oviposition by females and feeding by larvae also caused water stress that resulted in the higher stem

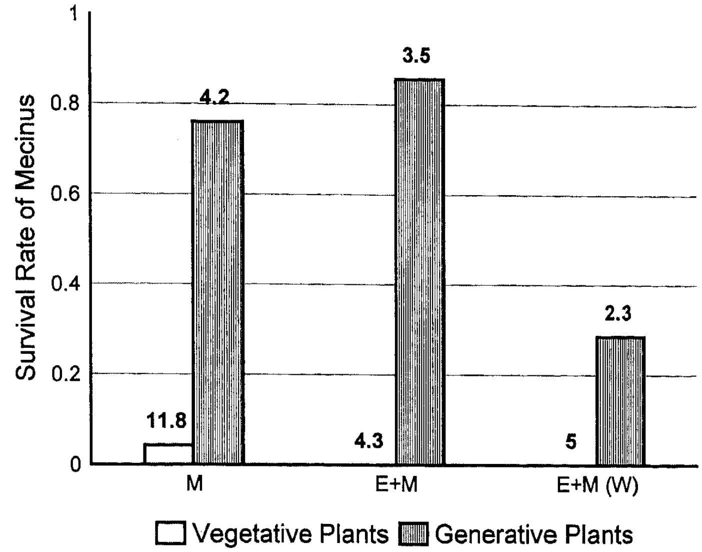

FIGURE 1. Effect of treatment and plant developmental stage on survival of *M. janthinus* (number that developed to the adult stage/number of eggs oviposited). The numbers on top of the bars represent the mean number of eggs per plant. Abbreviations: M = *M. janthinus*; E + M(W) = *E. intermediella* and *M. janthinus*, and low water treatment.

mortality. Robocker (1974) reports that prostrate stems contribute to the carbohydrate content of roots and that clipping of prostrate stems is most damaging to young plants, and late in the season. Therefore, attack by *M. janthinus* can act as an energy sink. *M. janthinus* is a univoltine species, its impact on the host in one season was simulated adequately by a single application of the insects. The following testable hypotheses can be made: attack rates of (at least) one pair of weevils per individual of *L. dalmatica* will result in (1) decreased vigour of regrowth of plants early in the growth season and (2) occasional mortality of stems or plants after the flowering season (the summer drought season).

Root-mining and water stress by *E. intermediella* were expected to have similar effects on the plants (Gange & Brown, 1989). Additional water stress killed prostrate stems of vegetative plants, but did not affect biomass production or the shoot/root ratio. Similarly, mining by *E. intermediella* had no statistically significant effects on plant performance. A previous experiment on the impact of *E. intermediella* and *E. serratella* Tr. on *Linaria vulgaris* (Saner & Müller-Schärer, 1994) showed that the weight of individual seeds was decreased as a result of root-feeding during the summer season. However, the growth pattern of the two weed species differ in that *L. vulgaris* produces flowering stems continuously from summer to autumn, while *L. dalmatica* produces its flowering stems in a single flush in early summer. Therefore, effects of summer root-mining on seeds are probably more apparent in *L. vulgaris* than in *L. dalmatica.* The experiment with *L. vulgaris* (Saner & Müller-Schärer, 1994) also showed that overwintering

TABLE 1. Survival of *M. janthinus*: results from a maximum likelihood analysis of the 2*2*2 table of developmental stage (vegetative/flowering), *E. intermediella* (presence/absence) and survival of *M. janthinus* (survival to adult/non-survival to adult). Relevant two-way interactions and the three-way interaction are presented

Source	χ^2	P
Developmental stage * survival of *M. janthinus*	33.92	< 0.0001
Presence of *E. intermediella* * survival of *M. janthinus*	0.32	0.57
Developmental stage * presence of *E. intermediella* *survival of *M. janthinus*	0.04	0.83

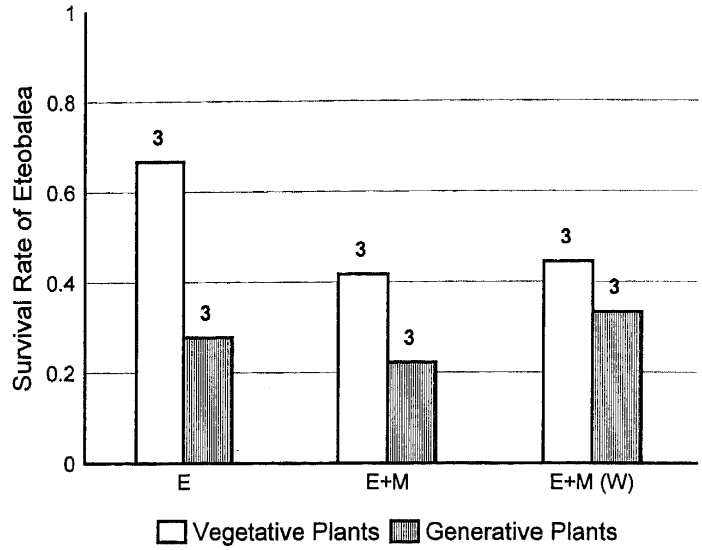

FIGURE 2. Effect of treatment and plant developmental stage on survival of *E. intermediella* (number of developed larvae/number of transferred larvae). The numbers on top of the bars represent the number of larvae transferred per plant. Abbreviations: E = *E. intermediella*; E + M = *E. intermediella* and *M. janthinus*; E + M(W) = *E. intermediella* and *M. janthinus*, and low water treatment.

larvae of *Eteobalea* have significant effects on regrowth of plants in the next season. This is supported by field observations in Yugoslavia, the collection site of the *E. intermediella* laboratory colony. Late instar larvae from the overwintering generation consume large amounts of basal stem tissue, and attacked *L. genistifolia* often produced only one flowering shoot instead of four to six (field observations personal and communication I. Toševski). Without the inclusion of the overwintering generation it is not possible to make meaningful predictions for the effects of *E. intermediella* on *L. dalmatica*.

Interaction among the Insects and the Developmental stage of *L. dalmatica*

Under the experimental conditions, *M. janthinus* and *E. intermediella* were specific to different developmental stages of *L. dalmatica* (Figures 1 and 2). Vegetative plants were highly unsuitable for larval development of *M. janthinus*. Weevil mortality in vegetative plants was likely caused by the inward collapse of dead prostrate stem through desiccation (in the treatment with additional water stress not a single weevil developed to maturity). The upright stems of flowering plants also tend to become dry, but unlike prostrate stems, did not collapse and in other studies it was shown that stem diameter may be of importance for successful development of *M. janthinus* (Jeanneret & Schroeder, 1992).

In contrast, more transferred larvae of *E. intermediella* survived on the vegetative plants. The most plausible explanation is found in the oviposition behaviour of *E. intermediella*. Unlike *M.*

TABLE 2. Survival of *E. intermediella*: results from a maximum likelihood analysis of the 2*2*2 table of developmental stage (vegetative/flowering), *M. janthinus* (presence/absence) and survival of *E. intermediella* (developed larvae/dead larvae). Relevant two-way interactions and the three-way interaction are presented

Source	χ^2	*P*
Developmental stage * survival of *E. intermediella*	7.79	0.0048
Presence of *M. janthinus* * survival of *E. intermediella*	0.32	0.57
Developmental stage * presence of *M. janthinus** survival of *E. intermediella*	< 0.01	0.96

janthinus, female moths lay their eggs on the stem surface and the freshly emerged larvae have to penetrate the plant epidermis. The flowering stems of *L. dalmatica* have a thicker epidermis than the vegetative stems. Probably, this affected the rate of successful penetration of first-instar larvae (head capsule width is only about 0.2 mm). It is conceivable that this effect occurs less often in nature due to females choosing to oviposit on the most suitable stems. However, observations in laboratory rearing indicate that females of *E. intermediella* do not discriminate between different stem qualities of *Linaria* spp. (Saner, 1991).

No host plant-mediated interactions between the two agents could be detected in this experiment. This could be explained by (1) an attack rate below a threshold where such effects would become apparent and/or (2) the observed partial specificity of the two species to different developmental stages of the host plant. Although a host plant-mediated interaction under field conditions cannot be excluded, these data do not justify concern for potential competitive interaction between the two agent species. Rather, this study indicates that the two agents will probably exhibit a complementary effect on the target population and this should increase the chance of successful control of *L. dalmatica*.

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REFERENCES

ALEX, J.F. (1962) The taxonomy, history, and distribution of *Linaria dalmatica*. *Canadian Journal of Botany* **40,** 296–307.

ANDERSEN, D.C. (1987) Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *Quarterly Review of Biology* **62,** 261–286.

BELSKY, A.J. (1986) Does herbivory benefit plants? A review of the evidence. *American Naturalist* **127,** 870–892.

BORROR, D.J., TRIPLEHORN, C.A. & JOHNSON, N.F. (1989) *An Introduction to the Study of Insects*, 6th Edn. Saunders, Philadelphia.

CRAWLEY, M.J. (1986) The population biology of invaders. *Philosophical Transactions of the Royal Society, London B* **314,** 711–731.

GANGE, A.C. & BROWN, V.K. (1989) Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia (Berl.)* **81,** 38–42.

HARRIS, P. (1984) *Linaria vulgaris* Miller, yellow toadflax, and *L. dalmatica* (L.) Mill., broad-leaved toadflax (Scrophulariaceae), in *Biological Control Programmes against Insects and Weeds in Canada 1969–1980* (KELLEHER, J.S. & HULME, M.A., Eds) Commonwealth Agricultural Bureaux, Slough, UK, pp. 179–201.

JEANNERET, P. & SCHROEDER, D. (1992) Biology and host specificity of *Mecinus janthinus* Germar (Col.: Curculionidae), a candidate for the biological control of Dalmatian and Yellow Toadflax, *Linaria vulgaris* (L.) Mill. and *Linaria dalmatica* (L.) Mill. in North America. *Biocontrol Science and Technology* **2,** 25–34.

JULIEN, M.H. (1987) *Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds*, 2nd Edn. Commonwealth Agricultural Bureaux, UK.

LANGE, A.W. & WOLFE, H.H. (1954) Dalmatian Toadflax. A Spreading Menace in Washington. *Institute of Agricultural Sciences, Extension Miscellaneous Publication* **21,** 3 pp.

LOHSE, G.A. (1983) In *Die Käfer Mitteleuropas, Bd. 11: Curculionidae* (FREUDE, H., HARDE, K.W. & LOHSE, G.A., Eds) Goecke & Evers, Krefeld, pp. 260–262.

MASCHINSKI, J. & WHITHAM, T.G. (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist* **134,** 1–19.

MCCLAY, A.S. (1992) Effects of *Brachypterolus pulicarius* (L.) (Coleoptera: Nitidulidae) on flowering and seed production of common toadflax. *Canadian Entomologist* **124,** 631–636.

MÜLLER, H. (1991a) Pflanzenreaktionen auf Wurzelherbivoren-Variationen, Ursachen, und Mechanismen: Experimente an zweijährigen Kompositen, in *Populationsbiologie der Pflanzen* (SCHMID, B. & STÖCKLI, J., Eds) Birkhäuser Verlag, Basel, pp. 281–297.

MÜLLER, H. (1991b) The impact of root herbivory as a function of plant density and competition: survival, growth and fecundity of *Centaurea maculosa* (Compositae) in field plots. *Journal of Applied Ecology* **28,** 759–776.

RIEDL, T. (1969) Matériaux pour la connaissance des Momphidae paléarctiques (Lepidoptera). Partie IX. Revue des Momphidae européennes, y compris quelques espèces d'Afrique du Nord et du Proche-Orient. *Polskie Pismo Entomologiczne* **39,** 635–919.

ROBOCKER, W.C. (1974) Life history, ecology, and control of Dalmatian toadflax. *Washington Agricultural Experiment Station, Technical Bulletin* **79,** 20 pp.

SANER, M., GROPPE, K. & HARRIS, P. (1990) *Eteobalea intermediella* Riedl and *E. serratella* Treitschke (Lep., Cosmopterigidae), two suitable agents for the biological control of yellow and Dalmatian toadflax in North America. *Final Report, IIBC, European Station, Delémont, Switzerland*, 44 pp.

SANER, M. (1991) Interactions of *Linaria vulgaris* Mill. and *L. dalmatica* (L.) Mill. (Scrophulariaceae) with insect herbivores. *Dissertation, Universität Basel*, 138 pp.

SANER, M.A. & MÜLLER-SCHÄRER, H. (1994) Impact of *Eteobalea* spp. on clonal growth and sexual reproduction of common toadflax, *Linaria vulgaris* Mill. *Weed Research* **34,** 199–204.

SAS INSTITUTE INC. (1988) *SAS/STAT User's Guide*, Release 6.03 Edition SAS Institute Inc., Cary, NC.

SCHERF, H. (1964) Die Entwicklungsstadien der mitteleuropäischen Curculioniden (Morphologie, Bionomie, Oekologie). *Abhandlungen der senckenbergschen naturforschenden Gesellschaft* **506,** 1–335 (121–122).

SOKAL, R.R. & ROHLF, F.J. (1981) *Biometry*, 2nd Edn. Freeman, San Francisco.

STEINGER, T. & MÜLLER-SCHÄRER, H. (1992) Physiological mechanisms underlying the effect of root herbivores, competition and nutrient shortage on growth and resource allocation of *Centaurea maculosa* (Asteraceae). *Oecologia (Berl.)* **91,** 141–149.

SUTTON, D.A. (1988) *A Revision of the Tribe Antirrhineae*. British Museum (Natural History), Oxford University Press, London.

WILSON, J.B. (1988) A review of evidence on the control of shoot:root ratio, in relation to models. *Annals of Botany* **61,** 433–449.