The Impact of a Root-Crown Weevil and Pasture Competition on the Winter Annual Echium plantagineum

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The impact of a root-crown weevil and pasture competition on the winter annual *Echium plantagineum*

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Summary

1. Root-feeding insect herbivores may suppress their host populations by altering the competitive balances within the plant community of the host-plant. In this study, repeated field experiments using caged and natural populations of a root-crown weevil, *Mogulones larvatus*, on the annual weed *Echium plantagineum* (Boraginaceae) were conducted with and without pasture competition in its exotic range.

2. The experiment used a split-plot design, excluding weevils with insecticide and plant competition through weeding.

3. The greatest effect of the weevils was observed when natural densities of weevils occurred on naturally growing *E. plantagineum* rosettes in the field. Weevils reduced plant survival by 43%, and the size and seed weight of survivors by 58% and 74%, respectively.

4. Pasture competition had a similar impact on *E. plantagineum* as the weevils for plant size and seed weight, except that there was no impact on rosette survival. There were also no significant interactions between the effects of weevils and competition. Most impact occurred in the second half of the growing season.

5. The number of weevil eggs and larvae and all plant reproductive parameters measured per unit biomass were independent of plant size.

6. Such impact experiments help develop simplified impact assessment methodologies for assessing the success of biological control projects in the field.

Key-words: below-ground herbivory, biological control, Boraginaceae, Paterson's curse, plant competition, *Mogulones larvatus*.

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Introduction

Root-feeding insect herbivores have been shown to have considerable impact on the survival (Müller-Schärer 1991; Tscharntke & Greiler 1995; Maron 1998), reproductive capacity (Cartwright & Kok 1985; Prins, Nell & Klinkhamer 1992; Woodburn 1997) and population dynamics of their hosts, either directly (McEvoy, Cox & Coombs 1991; Setchfield 1999) or indirectly (Brown & Gange 1989, 1990). Insects in guilds that feed in root systems (Müller *et al.* 1989) are increasingly being used as biological control agents against broad-leaved pasture weeds (Dunn & Rizza 1976; Rizza *et al.* 1988; Blossey 1993; Woodburn 1997). This has been encouraged by evidence that they can be considerably effective (Kok & Mays 1991; McEvoy, Cox & Coombs 1991; Senft & Cooke 1994).

Classical biological control of introduced weeds within pasture ecosystems aims to reduce the competitive ability of the weeds using natural enemies, reducing either their vigour or their reproductive potential, to a level where they can no longer dominate. Therefore, it is the interaction between the top-down effects of natural enemies and the lateral effects of plant competition that underlies plant population control (McEvoy *et al.* 1993). Despite this, the nature of these interactions, particularly for different natural enemy types, is still poorly understood (Rees & Brown 1992; Sheppard 1996). Some studies have suggested possible synergy in this interaction (Groves & Williams 1975; Windle & Franz 1979; Parker & Salzman 1985), where the combined effect is greater than the sum of the separate effects, but this does not occur frequently (Sheppard 1996). A better understanding, however, might lead to an ability to manipulate the weed's environment to increase the effectiveness of biological control (Groves 1995) and prevent weed substitution

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following successful weed control (Burdon, Groves & Cullen 1981).

Where manipulative field studies have been used to compare the relative and interactive nature of herbivory and competition on plants, competition was the dominant factor eight times more often than the natural enemy (Sheppard 1996). In most of these cases, however, the target plant was not the dominant component of the community and the relative importance of competition may have reflected this. Three such studies used root-feeding herbivores. One study detected no effect of the root herbivore (Müller-Schärer 1991), one found the root herbivore effect to be of similar magnitude to competition (McEvoy *et al.* 1993), and the third found a greater impact from the root herbivore (Schroeder, Thomas & Murray 1993).

This study compared the effects of pasture competition and the root-crown feeding weevil, *Mogulones larvatus* Goeze, on Australia's economically most important temperate broad-leaved pasture weed, the winter annual *Echium plantagineum* L., following the release of the weevil as a biological control agent for this weed.

Materials and methods

Echium plantagineum is an introduced toxic pasture weed in wet-summer temperate and Mediterranean climate regions of Australia, South America and South Africa (Piggin & Sheppard 1995). In southern Australia, where *E. plantagineum* has invaded 33 million ha, it is abundant because of affinities in soil type (particularly super-phosphate enriched low pH soils), climate (particularly summer rains) and land management. A lack of abundant strongly competitive pasture species and natural enemies, however, is considered to be the reason for its dominance in some areas (Piggin & Sheppard 1995). Such *E. plantagineum*-rich pasture is most often a low diversity mixture of introduced European species (Smyth *et al.* 1992) with only *Trifolium subterraneum* L. and other weeds in competition for establishment (Piggin & Sheppard 1995). The same is not true in its native Mediterranean range, where, in addition to natural enemies, the plant competes with many winter annual species (Noy-Meir, Gutman & Kaplan 1989) during a short autumn recruitment period.

Mogulones larvatus is a univoltine weevil that starts oviposition in late summer to autumn. Larval feeding can be observed in meristems until the onset of stem elongation in spring. Oviposition occurs in the leaf petioles and the larvae mine into the central rosette meristem (Sheppard, Smyth & Swirepik 1999). This weevil is specific to the genus *Echium*, the larvae causing most damage (Vayssières & Wapshere 1983). In native communities, the root-cortex feeding *Mogulones geographicus* Schultze (Forrester 1993), another biological control agent, is also present, but *M. larvatus* is the only insect found restricted to the root-crown region (Wapshere 1985).

FIELD EXPERIMENTS

An experiment was conducted in each of two consecutive years in a mown 1-ha field covered by a turf of exotic grasses on 'Black Mountain' in Canberra, Australia (altitude 570 m, 35°19'S 149°12'E), dominated by *Vulpia myuros* (L.) C.C. Gmel., *Vulpia bromoides* (L.) Gray, *Bromus catharticus* Vahl. and *Phalaris aquaticus* L., with *Plantago lanceolata* L. and *Hypochaeris* spp. being the other common forbs. In autumn of each year, 200 *E. plantagineum* were planted out into the grassland 1·5 m apart in 20 rows of 10 plants. The plot was divided into five blocks containing four rows of 10 plants and segmented into five subplots (of eight plants) per block. The following standard treatments were applied in a split-plot design. A 'no plant competition' hand-weeding treatment (hereafter called a competition treatment) was applied to one half of each subplot (four plants), where plants were kept free of vegetation to a 20-cm radius from the edge of the rosette using a trowel to cut any roots of competitors entering this area. The vegetation in the remaining half of each subplot was left untouched throughout the experiment. The relative positions of this treatment alternated between adjacent subplots. An insecticide treatment of *Rogor®* (= Dimethoate) plus the wetting agent *Chemspread®*, both at a rate of 1 ml l⁻¹, was applied using a hand-pump sprayer to one randomly selected plant within each half subplot (hereafter termed sprayed plants), and the same concentration of wetting agent solution was applied to all other plants (hereafter termed unsprayed plants) on a fortnightly basis throughout the experiments. During the experiments the field was left unmown during the growth period of *E. plantagineum*, i.e. from February to September, but the field was exposed to grazing by rabbits *Oryctolagus cuniculus* L.

In 1994, before any *M. larvatus* had been released on site, 6-week-old glasshouse-grown rosettes were planted out into the design in February. After a further 6 weeks, during which the plants were watered when necessary, a male–female pair of *M. larvatus* was caged onto each of two unsprayed plants per half subplot for a week and then removed. Laboratory studies suggested this should lead to about 20 eggs laid per plant (M.J. Smyth, unpublished data). The fourth plant in each half subplot was used as a 'no insecticide' control to assess the effect of the insecticide on the test plants.

In 1995, naturally occurring seedlings were transplanted from surrounding areas into the grid design in March to obtain a mean plant size more typical of naturally occurring *E. plantagineum* in the field. In this year, *M. larvatus* had naturally established at the site, so three unsprayed plants per competition treatment were exposed to *M. larvatus* oviposition.

Plant survival and the presence of weevil larvae (attack rate) were monitored monthly. In early July of each year, four randomly selected rosettes per block were up-rooted irrespective of treatment and dissected to record the number of *M. larvatus* eggs and larvae (attack level).

This time was selected to correspond to peak numbers of eggs and larvae per damaged rosette (Sheppard, Smyth & Swirepik 1999). These plants were then dried (85 °C for at least 36 h) and weighed to an accuracy of 0·01 g in the laboratory. During flowering (November–January), flowers and seeds are progressively produced and shed on elongating cymes. A fortnightly method, described in Smyth, Sheppard & Swirepik (1997), was used to obtain accurate estimates of total cyme length and the total number of flowers and seeds for each plant. Where possible seeds were collected from each plant as they matured and turned black. Towards the end of the flowering period all naturally senescent plants were dug up and divided into roots and shoots. The shoots (with any attached seeds removed) and roots were dried and weighed as above. For each plant, all removed seeds were counted and weighed together (to an accuracy of 0·001 g) to calculate mean individual seed weight, which was itself used to calculate total seed weight for each plant from the total seed number estimated during the fortnightly sampling. To test for treatment effects on seed viability and dormancy, all removed seeds were checked for viability by germination after 3 months (to allow after-ripening of the seed to be completed; Piggin & Sheppard 1995) in Petri dishes on filter paper soaked with distilled water. After 2 weeks, ungerminated seeds were tested with tetrazolium chloride to check for viability (Moore 1962); percentage viability was used to estimate the number of viable seeds per plant.

PLANT SIZE AND ATTACK LEVEL

In March 1996, 40 natural seedlings were transferred as in 1995 into one block of the 1994–95 experimental grid and left exposed to natural oviposition by the resident *M. larvatus* population. Ten plants were sampled destructively at random every 2 weeks from early June until mid-July across the peak in the number of eggs and larvae per plant (Sheppard, Smyth & Swirepik 1999). All plants were dissected as in previous years, recording the number of eggs and larvae and measuring plant dry weight.

ANALYSIS

GLIM statistical software (Payne 1986) was used to analyse the experiments, taking account of the split-plot design (Crawley 1993). The effects of *M. larvatus* on individual plants and the whole plot were analysed separately.

The effects of weevils on individual plants were analysed using only experimental plants in the weevil treatments where weevil damage was confirmed during the course of the experiment. The effects of the treatments on plant mortality (lethal effects) and on vegetative and reproductive parameters of survivors (sublethal effects) were analysed separately. The number of plants dying was analysed in GLIM using binomial errors, while the data were arcsine-transformed for presenta-

tion. Analysis of sublethal effects used all positive plant weight and reproductive measurements, which were log-transformed to homogenize variances. We also looked for any effects of treatments on reproductive effort (Klinkhamer, de Jong & Meelis 1990) using log-log regressions of vegetative weight (plant weight – seed weight) against total cyme length, seed weight, flower and seed number and the regressions among these reproductive parameters (flowers per unit cyme length, seeds per flower, etc.). Regression coefficients of log-log plots were also used to test treatment effects on root/shoot ratios (Hunt 1978).

A separate analysis of the effects of weevils on *E. plantagineum* at the level of the whole plot (i.e. exposure to weevils rather than just plants that were attacked) on plant mortality, and then the combined lethal and sublethal effects on mean plant biomass and seed number, were also made.

Regression analysis was used to relate plant dry weight at harvest and sample number to weevil attack level for data collected in 1996.

Results

Glasshouse-propagated plants dug into the experiment in 1994 grew very quickly and were soon well over the average size of most naturally occurring rosettes around the experimental field. Their large size and the dry season in 1994 led to these flowering plants attracting the attention of a flock of crimson rosella parrots *Platycerus elegans* (Gmelin) that nipped off a significant number of flowering cymes across all blocks of the experiment. Using naturally occurring seedlings in 1995 gave a more representative mean plant size, although six plants in that year were destroyed by rabbits.

None of the insecticide-treated plants destructively sampled during this experiment were found to have been attacked by *M. larvatus*. In 1994, plants treated with insecticide produced 43% more seeds than unsprayed plants found without weevil damage elsewhere in the experiment ($F_{1,66} = 6·53$, $P < 0·05$). There was no significant difference in plant weight between these groups. While this may have indicated a fertilizer effect of the insecticide treatment, no such effect was found in 1995 when field-grown plants were used and no parrot damage occurred. Analysis of the experiment in 1994 therefore included only data from undamaged unsprayed plants as the control treatment.

Mogulones larvatus attack rates of destructively sampled unsprayed plants in the experiment in July were 55% in 1994 and 42% in 1995, with mean of 5·1 ± 1·1 and 4·8 ± 0·8 (± SE) larvae per attacked plant in 1994 and 1995, respectively. By the end of the experiment, attack rates had increased to 81% in 1994 and 82% in 1995.

PLANT-LEVEL LETHAL EFFECTS
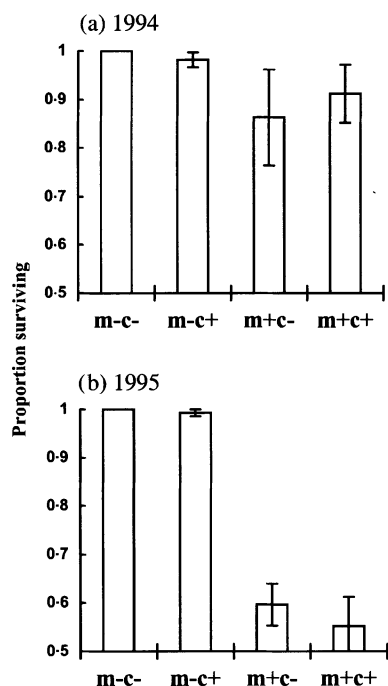
Mortality analysis across years detected a significant

(a) 1994

(b) 1995

Fig. 1. Proportion of the *E. plantagineum* experimental plants surviving in the four treatment combinations (m–c–, control; m–c+, with pasture competition; m+c–, with root-crown weevil; m+c+, with pasture competition and root-crown weevil) in the experiment in (a) 1994 and (b) 1995 (± SE back-transformed from arcsine-transformed data).

(a) 1994

(b) 1995

Fig. 2. Mean total *E. plantagineum* dry weight per plant (log scale) from the four treatment combinations (Fig. 1) in both July of each year (grey bar) and at senescence (white bar) in the experiment in (a) 1994 and (b) 1995 (± SE).

effect of damage by *M. larvatus* ($F_{1,36}$ = 67·9, P < 0·001) but no effect of competition treatment, block or year. There was a significant *M. larvatus*–year interaction ($F_{2,36}$ = 4·37, P < 0·05) as far more plants died following damage in 1995 (Fig. 1).

PLANT-LEVEL SUBLETHAL EFFECTS

Significant effects of the treatments on total plant dry weight (i.e. including shoot, root and seed weights) were not evident in July of each year (i.e. approximately 4 months after germination) when 20 experimental plants were destructively sampled (Fig. 2), although the trends were starting to become evident. The mean weight of control plants did not change much between the first July sample and the end of the experiment, suggesting final weight had already been attained by July (Fig. 2). However, this was probably due to some foliage being shed and lost between sampling dates (see the Discussion). In 1995, all plants in treatments were significantly smaller by the end of the experiment (Fig. 2b). The long-term effects of competition and *M. larvatus* appeared to have resulted in a negative growth rate

In 1994 there was no direct effect of *M. larvatus* on any measured plant parameter, while competition significantly reduced both seed number per plant by 16% and seed weight per plant by 12% (Tables 1 and 2 and Fig. 3). Significant interactions between these treatments indicated that combined *M. larvatus* and
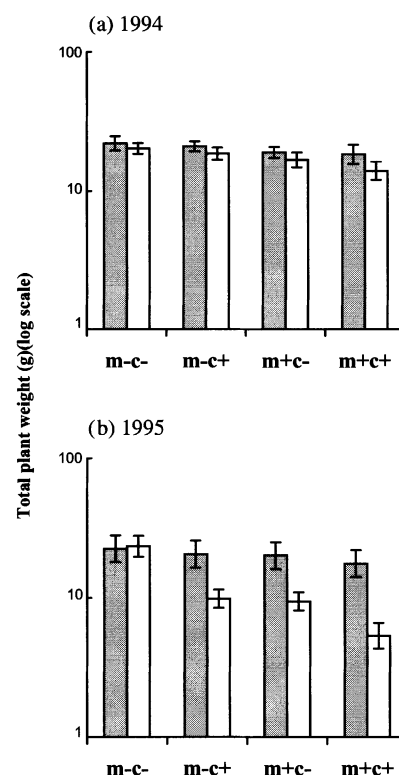
competition caused greater reductions in seed number (59%, $F_{1,84}$ = 5·70, P < 0·05) and seed weight (62%, $F_{1,84}$ = 5·54, P < 0·05; Fig. 3). In 1995 the sublethal effects of competition and *M. larvatus* were greater than in 1994 and significant for all plant parameters in Table 1 (Fig. 3). Competition and *M. larvatus* had sublethal effects of similar magnitude, reducing plant parameters by 44–67% and 41–74%, respectively, and reducing shoot weight more than root weight (Table 2). *Mogulones larvatus* reduced reproductive parameters by 61–74% (Table 2). Mean individual seed weight (3·0 ± 0·5 mg, ± SE), germinability (46 ± 2·1%) and overall viability (89 ± 3·5%) did not vary with any treatment in either year.

There were significant block and subplot effects on all plant parameters in Table 1 in 1994 and subplot effects on all plant reproduction parameters in 1995. These were largely due a fivefold and sixfold variation in plant weight and a 30-fold and 20-fold variation in seed production across subplots in 1994 and 1995, respectively. There were no interaction effects between the treatments and blocks or subplots, which, given this degree of plant size variation across the experiment, indicated that the effects of competition and *M. larvatus* were independent of plant size. The combined effects of *M. larvatus* and competition in 1995 caused greater reductions (45–62% in 1994 and 80–84% in 1995) in plant performance (Tables 1 and 2 and Fig. 3).

Table 1. Back-transformed means of the plant vegetative and reproductive parameters of *E. plantagineum* in 1994 and 1995 for the different treatments: m–c–, control; m–c+, with pasture competition; m+c–, with root-crown weevil; m+c+, with pasture competition and root-crown weevil. Ln-transformed means ± SEs are given in parentheses and *F*-ratios are given for the main treatment effects under the means

Year/ treatment	Vegetative parameters			Reproductive parameters			
	Plant weight (g)	Root weight (g)	Shoot weight (g)	Total cyme length (cm)	Flower number	Seed number	Seed weight (g)
1994							
m–c– (control)	19·1 (2·95 ± 0·09)	3·3 (1·21 ± 0·10)	15·5 (2·74 ± 0·09)	317 (5·76 ± 0·16)	453 (6·12 ± 0·16)	275 (5·62 ± 0·18)	0·71 (−0·34 ± 0·19)
m–c+	17·6 (2·87 ± 0·09) $F_{1,17} = 1·41$	3·2 (1·17 ± 0·09) $F_{1,17} = 0·06$	13·9 (2·63 ± 0·10) $F_{1,17} = 2·13$	273 (5·61 ± 0·15) $F_{1,34} = 3·90$	406 (6·01 ± 0·13) $F_{1,34} = 2·81$	242 (5·49 ± 0·18) $F_{1,34} = 4·21*$	0·62 (−0·48 ± 0·19) $F_{1,34} = 4·82*$
m+c–	15·7 (2·75 ± 0·12) $F_{1,84} = 3·64$	2·9 (1·05 ± 0·10) $F_{1,84} = 3·13$	12·6 (2·53 ± 0·13) $F_{1,84} = 3·14$	273 (5·61 ± 0·19) $F_{1,84} = 0·42$	375 (5·93 ± 0·17) $F_{1,84} = 0·67$	230 (5·44 ± 0·24) $F_{1,84} = 2·17$	0·63 (−0·47 ± 0·23) $F_{1,84} = 1·80$
m+c+	13·4 (2·60 ± 0·14)	2·8 (1·02 ± 0·29)	10·4 (2·34 ± 0·17)	151 (5·02 ± 0·21)	246 (5·51 ± 0·22)	112 (4·72 ± 0·31)	0·27 (−1·30 ± 0·33)
1995							
m–c– (control)	17·5 (2·86 ± 0·17)	2·1 (0·74 ± 0·11)	15·1 (2·72 ± 0·18)	918 (6·82 ± 0·18)	1686 (7·40 ± 0·18)	1457 (7·23 ± 0·17)	5·18 (1·59 ± 0·19)
m–c+	7·4 (2·00 ± 0·15) $F_{1,23} = 26·89***$	1·2 (0·16 ± 0·12) $F_{1,23} = 22·32***$	6·1 (1·81 ± 0·15) $F_{1,23} = 25·49***$	302 (5·71 ± 0·17) $F_{1,23} = 47·24***$	583 (6·37 ± 0·16) $F_{1,23} = 45·59***$	537 (6·29 ± 0·18) $F_{1,23} = 27·35***$	1·89 (0·64 ± 0·20) $F_{1,23} = 22·47***$
m+c–	7·3 (2·00 ± 0·15) $F_{1,92} = 25·24***$	1·2 (0·21 ± 0·12) $F_{1,92} = 24·93***$	6·0 (1·78 ± 0·16) $F_{1,92} = 24·74***$	356 (5·87 ± 0·19) $F_{1,101} = 21·95***$	598 (6·39 ± 0·19) $F_{1,101} = 26·47***$	402 (6·00 ± 0·24) $F_{1,101} = 25·85***$	1·37 (0·31 ± 0·25) $F_{1,101} = 23·54***$
m+c+	4·0 (1·40 ± 0·21)	0·6 (0·52 ± 18)	3·4 (1·21 ± 0·22)	165 (5·11 ± 0·24)	270 (5·60 ± 0·25)	270 (5·60 ± 0·26)	1·02 (0·02 ± 0·26)

*$P < 0·05$; ***$P < 0·001$.

Table 2. Percentage reductions of the plant size and plant reproductive performance variables of *E. plantagineum* due to treatments (Table 1) in 1994 and 1995 using means back-transformed from the logged data

Year/factor	Plant weight (g)	Root weight (g)	Shoot weight (g)	Total cyme length (cm)	Flower number	Seed number	Seed weight (g)
1994							
m−c+	8	4	10	14	10	12	13
m+c−	18	14	19	14	17	16	12
m+c+	30	17	33	52	46	59	62
1995							
m−c+	58	44	60	67	65	63	64
m+c−	58	41	61	61	65	72	74
m+c+	77	72	78	82	84	81	80
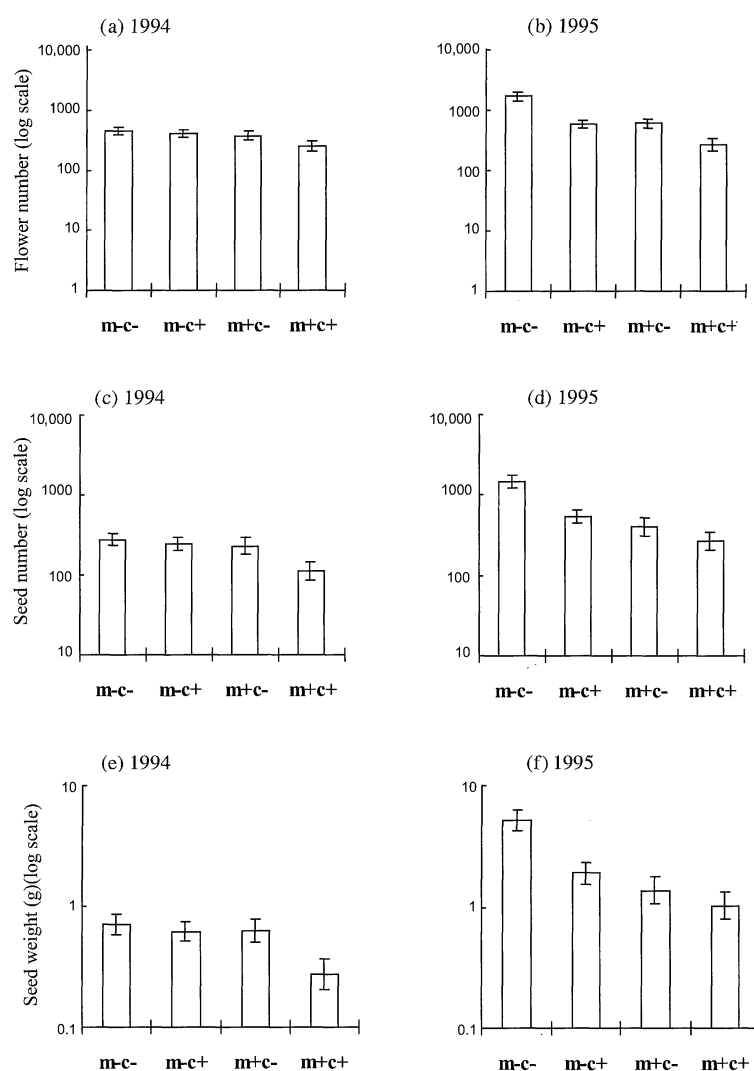
Fig. 3. Mean *E. plantagineum* flower number (a, b), seed number (c, d), and seed weight (e, f), per plant (log scale) from the four treatment combinations (Fig. 1) in the experiment in 1994 (a, c, e) and 1995 (b, d, f) (± SE).

All analyses of reproductive effort using log-log regressions between vegetative weight and the four measures of reproductive performance in Table 1 had slopes not significantly different from unity, indicating that plant weight had no effect on these relationships in both years. A similar result was obtained for the log-log relationships between (i) total cyme length and flower number, (ii) flower number and seed number, and
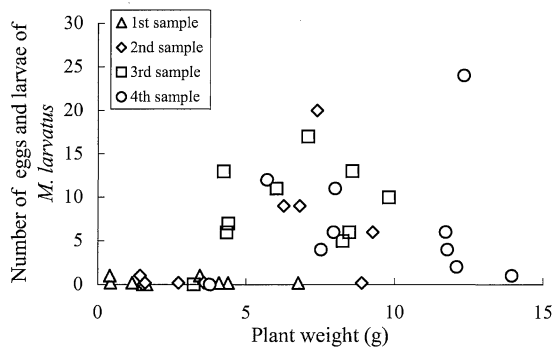
Fig. 4. Graph of plant dry weight at harvest against the number of eggs plus larvae of *M. larvatus* (attack level) for 40 plants of which 10 were harvested each fortnight from mid-June to the end of July.

(iii) root weight and shoot weight. Analysis of co-deviance also indicated no treatment effects on any of these relationships in either year.

PLOT-LEVEL EFFECTS

At the level of the whole experimental plot, a similar attack rate between years (i.e. *c.* 80%) caused 10% plant mortality and reduced vegetative weight and seed number per plot by 45% ($F_{1,95} = 7{\cdot}86$, $P < 0{\cdot}01$) and 64% ($F_{1,99} = 8{\cdot}33$, $P < 0{\cdot}01$), respectively, in 1994, and 40% plant mortality and reduced vegetative weight and seed number per plot by 70% ($F_{1,148} = 42{\cdot}77$, $P < 0{\cdot}001$) and 96% ($F_{1,156} = 35{\cdot}81$, $P < 0{\cdot}001$), respectively, in 1995.

PLANT SIZE AND ATTACK LEVEL

The data relating plant weight at harvest and the level of attack by *M. larvatus* from the experiment in 1996 across the four sampling dates are given in Fig. 4. Both plant weight ($F_{3,39} = 11{\cdot}1$, $P < 0{\cdot}01$; means $\pm$ SE for sampling dates 1–4: $2{\cdot}5 \pm 0{\cdot}7$, $5{\cdot}0 \pm 1{\cdot}0$, $6{\cdot}4 \pm 0{\cdot}7$, $9{\cdot}5 \pm 1{\cdot}1$) and the number of eggs and larvae ($F_{3,39} = 4{\cdot}6$, $P < 0{\cdot}05$; means $\pm$ SE for sampling dates 1–4: $0{\cdot}2 \pm 0{\cdot}1$, $4{\cdot}5 \pm 2{\cdot}1$, $8{\cdot}8 \pm 1{\cdot}6$, $7{\cdot}0 \pm 2{\cdot}3$) were positively related to the sample number, but there was no significant effect of plant size on the attack level ($F_{1,35} = 3{\cdot}07$, $P < 0{\cdot}05$) whether or not a log transformation was used.

Discussion

PLANT-LEVEL HERBIVORY AND PLANT COMPETITION

Natural densities of *M. larvatus* on naturally growing *E. plantagineum* plants in the 1995 experiment reduced plant survival by up to 43%, final half-year growth by 62%, plant size by 58% and seed weight by 74%. In 1994, *M. larvatus* only affected plant survival, except when accompanied by pasture competition (Fig. 3). These between-year differences are most likely to be due to the greater average plant size achieved by the

glasshouse-propagated plants used in 1994 and additional error in these data resulting from parrot damage. Even though weevil attack rates and levels were similar between years, this contrast may also have been in part due to the caged vs. natural weevil density methods used in the different years as the natural activity period of the weevils in 1995 started 6 weeks before weevils were caged in 1994. The plant growing conditions did not appear to be any better in 1994, as this was the drier of the two years at the site.

Pasture competition reduced only *E. plantagineum* growth, size and total seed weight, although the magnitude of these effects was similar to those of *M. larvatus* in both years. The qualitative difference in the effect of the weevil, i.e. its capacity to kill its host, gave it a greater impact on *E. plantagineum* overall.

The comparison of experimental plant weights at 4 months after the start of the experiment, with final weights at 10 months (Fig. 2), suggested that control plants had reached maximum size by the first sample date and that all treatments caused negative growth in the last 6 months. This was probably a sampling artefact. Final plant weights were likely to have been underestimates, because many rosette and cauline leaves had withered and fallen from the plant prior to final harvest and the leaves can make up approximately 40% of the shoot dry weight (M. J. Smyth, unpublished data). Nonetheless the similar trends in the data for the different treatment combinations between sample dates suggest that (i) *M. larvatus* affects plant growth in a similar manner to competition, and that (ii) both factors are having a much greater impact in the second half of the experiment, i.e. in spring.

Significant interactions between weevil and plant competition treatments were only observed in 1994 and reflected the relatively weak effect of *M. larvatus* in that year. No interaction between treatments in 1995 indicated that the combined effect was not synergistic but multiplicative (i.e. additive on a log scale; Fig. 3; Rees & Brown 1992), in common with most other studies of this type (Paul & Ayres 1990; McEvoy *et al.* 1993; Schroeder, Thomas & Murray 1993; Weiner 1993; Willis & Ash 1996).

Rees & Brown (1992) argued that such multiplicative effects would be expected when natural enemies and competition are affecting different components of biomass accumulation, i.e. a herbivore removing growth units and competition reducing growth rate, hypothesizing that a natural enemy that also affects the growth function should lead to an interaction between these factors. In this case, *M. larvatus*, the root feeder, also affected growth rate, so multiplicative effects can be expected even if both factors act in a similar manner (cf. McEvoy *et al.* 1993).

The lack of interaction effects is in itself revealing. No interaction between competition and weevils for weevil-induced mortality suggests that the capacity to kill its host is independent of the competitive state of the pasture. Field observations of such plant mortality from both the native range and more recently in

Australia support this, as does the fact that mortality is not confined to small plants (Vayssières & Wapshere 1983; Sheppard, Smyth & Swirepik 1999). Our analysis indicated that sublethal effects of the treatments were independent of plant weight.

The data from 1996 indicated no relationship between the level of attack and plant weight (Fig. 4). While this might suggest that heavier plants were not attracting more weevils, a simpler conclusion would be that there is high variation in weevil attack levels. Assessing any clear relationship between plant size and attack level will be confounded by a long weevil oviposition period, during which plant size will be increasing as a function of the number of larvae already present.

The experimental design attempted to minimize variation in plant size by controlling pretreatment growing conditions (1994) and using plants of similar age (both years). Despite this, a five- to sixfold variation in final plant weight was observed in all treatments, presumably caused by local differences in soil fertility. If true, then edaphic conditions relating to plant growth do not appear to alter the treatment effects. This contrasts with other studies which have found that root-feeding insect herbivores can have a greater impact in less fertile soils (Müller-Schärer 1991; Steinger & Müller-Schärer 1992).

The treatments had no effect on four measures of reproductive effort (i.e. cyme length, flower number, seed number and seed weight per unit vegetative weight) or on flowers per unit cyme length, seeds per flower or the root–shoot ratio, all of which were independent of plant weight. It is surprising that the high levels of root-crown damage observed in this study did not disrupt at least the root–shoot allometry of the plant (cf. Cartwright & Kok 1985; Steinger & Müller-Schärer 1992). Despite feeding in the root-crown area, *M. larvatus* does not appear to interfere with the transfer of plant reserves from root to shoot.

The effects of weevils and pasture competition measured in this study need to be placed in an environmental context (Burger & Louda 1994). In most *E. plantagineum* populations, *M. larvatus* has to contend with livestock grazing, trampling, etc., important but complex factors we had to leave out of our experiment. Our results therefore probably overestimate the effect of this weevil on *E. plantagineum* under livestock-grazed conditions. This will also be true for plant competition. Grazed pasture generally has less of an effect on *E. plantagineum* growth than we observed in our system (Grigulis 1999; Grigulis *et al.* 2001), where grass growth was kept in check by some rabbit grazing and mowing outside the main growth period of the plant. Nonetheless, significant damage to natural weedy *E. plantagineum* populations has been observed both with and without grazing (Sheppard, Smyth & Swirepik 1999). *Mogulones larvatus* has been released at over 800 sites so far across southern Australia, and the next phase of the impact evaluation process is to compare effectiveness across different grazing systems and stocking rates, and in different environments.

PLOT-LEVEL EFFECTS AND EVALUATING BIOLOGICAL CONTROL

It is worthwhile considering how the detailed results from these experiments can be used to assess the impacts of biocontrol agents on weed populations exposed to them following release, thereby circumventing the need for complex experiments at all release sites (McEvoy & Coombs 1999). The plot-level assessment in 1995, using naturally occurring seedlings and oviposition by an unmanipulated over-summering weevil population, led to an overall 95% loss in the number of seeds. By comparison, in an established *E. plantagineum* infestation, Smyth, Sheppard & Swirepik (1997) removed 90% of *E. plantagineum* seed production for 4 successive years and found germination levels were reduced after 2 years and seed bank density was reduced by 90% after 4 years.

A first step in impact assessment will be to accurately estimate the amount of seed production lost to the agent over several years. As both treatment effects from this study were independent of plant size, the results suggest ways such assessments might be simplified. The weevils, however, may respond in a more complex way to variation in plant population density or size. Some recent studies aimed at assessing the oviposition response of *M. larvatus* to this suggest that larval loads may indeed be largely independent of both patch size and plant density within a patch once the early colonization phase of the weevil is complete (Sheppard, Smyth & Swirepik 1999; Shea *et al.* 2000; G. Pons, unpublished data). Field observations of attack rate in relation to host density of *M. larvatus* and related species tend to support this (Forrester 1993; Schwarzlaender 1997). If larval loads are also independent of the density and patch size of its host-plant, then *M. larvatus* impact could be simply estimated from data on mean plant size and weevil attack level, although estimating variation in attack level is also likely to be important. Further, it is probable that *M. larvatus* attack level and attack rate are also correlated across a number of weed populations. Studies are currently under way in Australia to explore these issues using data from release sites where the weevils are killing a proportion of the *E. plantagineum* population (Sheppard, Smyth & Swirepik 1999). This corroborating evidence from release sites indicates that hopes for the successful biological control of *E. plantagineum* in Australia should remain high.

Acknowledgements

References

Blossey, B. (1993) Herbivory below ground and biological weed control: life history of a root-boring weevil on purple loosestrife. *Oecologia*, **94**, 380–387.

Brown, V.K. & Gange, A.C. (1989) Differential effects of above- and below-ground herbivory during early plant succession. *Oikos*, **54**, 67–76.

Brown, V.K. & Gange, A.C. (1990) Insect herbivory below ground. *Advances in Ecological Research*, **20**, 1–58.

Burdon, J.J., Groves, R.H. & Cullen, J.M. (1981) The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Australia. *Journal of Applied Ecology*, **18**, 957–966.

Burger, J.C. & Louda, S.M. (1994) Indirect versus direct effects of grasses on the growth of a cactus (*Opuntia fragilis*): insect herbivory versus competition. *Oecologia*, **99**, 79–87.

Cartwright, B. & Kok, L.T. (1985) Growth responses of musk and plumeless thistles (*Carduus nutans* and *C. acanthoides*) to damage by *Trichosirocalus horridus* (Coleoptera: Curculionidae). *Weed Science*, **33**, 57–62.

Crawley, M.J. (1993) *GLIM for Ecologists*. Blackwell Scientific Publications, Oxford, UK.

Dunn, P.H. & Rizza, A. (1976) Bionomics of *Psylliodes chalcomera*, a candidate for biological control of musk thistle. *Annals of the Entomological Society of America*, **69**, 395–398.

Forrester, G.J. (1993) Resource partitioning between two species of *Ceutorhynchus* (Coleoptera: Curculionidae) on *Echium plantagineum* in a Mediterranean habitat. *Bulletin of Entomological Research*, **83**, 345–351.

Grigulis, K. (1999) *The comparative population dynamics of Echium plantagineum L. between its native and invaded ranges*. PhD Thesis. Australian National University, Canberra, Australia.

Grigulis, K., Sheppard, A.W., Ash, J.E. & Groves, R.H. (2001) The comparative demography of the pasture weed *Echium plantagineum* between its native and invaded ranges. *Journal of Applied Ecology*, **38**, 281–290.

Groves, R.H. (1995) Biological control of weeds – past, present and future. *Proceedings of the VIII International Symposium on Biological Control of Weeds, 2–7 February 1992* (eds E.S. Delfosse & R.R. Scott), pp. 1–5. Lincoln University, Canterbury, New Zealand and CSIRO Publications, Melbourne, Australia.

Groves, R.H. & Williams, J.D. (1975) Growth of skeleton weed (*Chondrilla juncea* L.) as affected by growth of subterranean clover (*Trifolium subterraneum* L.) and infection by *Puccinia chondrillina* Bubak & Syd. *Australian Journal of Agricultural Research*, **26**, 975–983.

Hunt, R. (1978) *Plant Growth Analysis*. Edward Arnold, London, UK.

Klinkhamer, P.G.L. & de Jong, T.J. & Meelis, E. (1990) How to test for size dependant reproductive effort. *American Naturalist*, **135**, 291–300.

Kok, L.T. & Mays, W.T. (1991) Successful biological control of plumeless thistle, *Carduus acanthoides* L. [Campanulatae: asteraceae (= Compositae)], by *Trichosirocallus horridus* (Panzer) (Coleoptera: Curculionidae) in Virginia. *Biological Control*, **1**, 197–202.

McEvoy, P.B. & Coombs, E.M. (1999) Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecological Applications*, **9**, 387–401.

McEvoy, P., Cox, C. & Coombs, E. (1991) Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications*, **1**, 430–422.

McEvoy, P.B., Rudd, N.T., Cox, C.S. & Huso, M. (1993) Disturbance, competition and herbivory effects on ragwort *Senecio jacobaea* populations. *Ecological Monographs*, **63**, 55–75.

Maron, J.L. (1998) Insect herbivory above- and below-ground – individual and joint effects on plant fitness. *Ecology*, **79**, 1281–1293.

Moore, R.P. (1962) Tetrazolium as a universally acceptable quality test for viable seed. *Proceedings of the International Seed Testing Association*, **27**, 795–805.

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

Müller, H., Stinson, C.S.A., Marquardt, K. & Schröder, D. (1989) The entomofaunas of roots of *Centaurea maculosa* Lam., *C. diffusa* Lam. and *C. vallesiaca* Jordan in Europe. *Journal of Applied Entomology*, **107**, 83–95.

Noy-Meir, I., Gutman, M. & Kaplan, Y. (1989) Responses of Mediterranean grassland plants to grazing and protection. *Journal of Ecology*, **77**, 290–310.

Parker, M.A. & Salzman, A.G. (1985) Herbivore exclosure and competitor removal: effects on juvenile survivorship and growth in the shrub *Gutierrezia microcephala*. *Journal of Ecology*, **73**, 903–913.

Paul, N.D. & Ayres, P.G. (1990) Effects of rust infection of *Senecio vulgaris* on competition with *Capsella bursa-pastoris*. *New Phytologist*, **114**, 667–674.

Payne, C.D. (1986) *The GLIM System Release 3·77 Manual*. Numerical Algorithms Group Limited, Oxford, UK.

Piggin, C.M. & Sheppard, A.W. (1995) *Echium plantagineum* L. *The Biology of Australian Weeds*, Vol. 1 (eds R.H. Groves, R.C.H. Shepherd & R.G. Richardson), pp. 87–110. R.G. and F.J. Richardson, Melbourne, Australia.

Prins, A.H., Nell, H.W. & Klinkhamer, P.G.L. (1992) Size-dependent root herbivory on *Cynoglossum officinale*. *Oikos*, **65**, 409–413.

Rees, M. & Brown, V.K. (1992) Interactions between invertebrate herbivores and plant competition. *Journal of Ecology*, **80**, 353–360.

Rizza, A., Campobasso, G., Dunn, P.H. & Stazi, M. (1988) *Cheilosia corydon* (Diptera: Syrphidae), a candidate for the biological control of musk thistle in North America. *Annals of the Entomological Society of America*, **81**, 225–232.

Schroeder, J., Thomas, S.H. & Murray, L. (1993) Yellow and purple nutsedge and chile peppers host southern root-knot nematode. *Weed Science*, **41**, 150–156.

Schwarzlaender, M. (1997) Bionomics of *Mogulones cruciger* (Coleoptera: Curculionidae), a below-ground herbivore for the biological control of hound's-tongue. *Environmental Entomology*, **26**, 357–365.

Senft, D. & Cooke, L. (1994) Leafy spurge is reunited with old enemy. *Agricultural Research Washington*, **42**, 20–22.

Setchfield, R.P. (1999) *Insect herbivores and the population dynamics of ragwort* (Senecio jacobaea L.) PhD Thesis. University of London, London, UK.

Shea, K., Smyth, M., Sheppard, A.W., Morton, R. & Chalimbaud, J. (2000) Effect of patch size and plant density of a weed (*Echium plantagineum*) on the oviposition of a biological control agent (*Mogulones larvatus*). *Oecologia*, **124**, 615–621.

Sheppard, A.W. (1996) The interaction between natural enemies and interspecific plant competition in the control of invasive pasture weeds. *Proceedings of the IX International Symposium on Biological Control of Weeds, 19–26 January 1996* (eds V.C. Moran & J.H. Hoffmann), pp. 47–54. University of Cape Town, Stellenbosch, South Africa.

Sheppard, A.W., Smith, M. & Swirepik, A. (1999) Impact of the root-crown weevil (*Mogulones larvatus*) and other biological control agents on Paterson's curse in Australia: an update. *Proceedings of the 12th Australian Weeds Conference, 12–16 September 1999* (eds A.C. Bishop, M. Boersma & C.D. Barnes), pp. 343–346. Tasmanian Weed Society, Devonport, Australia.

Smyth, M.J., Moorhouse, J., Sheppard, A.W. & Swirepik, A. (1992) Factors affecting the dominance of *Echium plantagineum* in annual pastures. *Australian Weeds Research Newsletter*, **41**, 11–14.

Smyth, M.J., Sheppard, A.W. & Swirepik, A. (1997) The effect of grazing on seed production in *Echium plantagineum*. *Weed Research*, **37**, 63–70.

Steinger, T. & Müller-Schärer, H. (1992) Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nutrient availability. *Oecologia*, **91**, 141–149.

Tscharntke, T. & Greiler, H.-J. (1995) Insect communities, grasses and grasslands. *Annual Review of Entomology*, **40**, 535–558.

Vayssières, J.F. & Wapshere, A.J. (1983) Life histories and host specificities of *Ceutorhynchus geographicus* (Goeze) and *C. larvatus* Schultze (Coleoptera: Curculionidae), potential biological control agents for *Echium*. *Bulletin of Entomological Research*, **73**, 431–440.

Wapshere, A.J. (1985) The phytophagous insect guild on *Echium* in the western Mediterranean and the biological control of the weed *Echium plantagineum*. *Acta Oecologia et Applicata*, **6**, 331–338.

Weiner, J. (1993) Competition, herbivory and plant size variability: *Hypochaeris radicata* grazing by snails (*Helix aspersa*). *Functional Ecology*, **7**, 47–53.

Willis, A.J. & Ash, J.E. (1996) Combinations of stress and herbivory by a biological control mite on the growth of target and non-target native species of *Hypericum* in Australia. *Proceedings of the IX International Symposium on Biological Control of Weeds, 19–26 January 1996* (eds V.C. Moran & J.H. Hoffmann), pp. 93–100. University of Cape Town, Stellenbosch, South Africa.

Windle, P.N. & Franz, E.H. (1979) Effects of insect parasitism on plant competition: greenbugs and barley. *Ecology*, **60**, 521–529.

Woodburn, T.L. (1997) Establishment in Australia of *Trichosirocallus horridus*, a biological control agent for *Carduus nutans*, and preliminary assessment of its impact on plant growth and reproductive potential. *Biocontrol Science and Technology*, **7**, 645–656.