Longitarsus echii and its impact on *Echium plantagineum* (Paterson's curse): the insect for the Mediterranean rainfall range of the weed?

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Summary Since the release program for biological control of *Echium plantagineum* started, the establishment of insects in the drier Mediterranean rainfall areas of Australia has been poor. To rectify this poor establishment in low rainfall regions, the winter active flea beetle, *Longitarsus echii*, was imported from Mediterranean Europe, cleared through quarantine and insects supplied to collaborating State Departments for mass rearing by 1997. *L. echii* has now been released at 100 sites across temperate Australia and established at greater than 80% of sites. To confirm the ability of *L. echii* to limit the growth and reproductive potential of *E. plantagineum*, a fully replicated randomised block field experiment was conducted in the winter of 1998. The experiment was designed to measure insect feeding, pasture competition and their interaction. Plant reproductive effort and mortality was not effected by *L. echii* larval feeding alone but was reduced 90% by pasture competition. In combination with pasture competition larval feeding increased weed mortality by 26% and reduced reproductive effort a further 75% and 93% for moderate and high rates of larval feeding.
Keywords Biological control, below ground herbivory, plant competition, Boraginaceae, flea beetle.

INTRODUCTION

Echium plantagineum is a weed of temperate pastures in Australia, dominating a wide range of Mediterranean to continental climatic conditions (Piggin and Sheppard 1995). Its climatic range appears wide in Australia when compared to the native range, but subtle differences in rainfall patterns between Australia and the Mediterranean, in addition to reduced interspecific competition in the exotic environment, might explain this. The wide range of climatic conditions that *E. plantagineum* can dominate could make it difficult for any one species of insect to limit the vigour of the weed. The redistribution project for the biological control insects of *E. plantagineum* has been releasing the crown weevil, *Mogulones larvatus*, since 1993, the root weevil, *Mogulones geographicus*, since 1995 and the flea beetle, *Longitarsus echii*, since 1996. Following multiple releases of these insects in Australia, the success of establishing and damaging their host has varied widely across the country. Establishment rates, population growth rates and impacts have been observed to be lower, especially for *M. larvatus*, in areas of typically late autumn rainfall (Sheppard *et al*. 1999). Part of the reason for this is believed to be the limited capacity of *M. larvatus* to aestivate successfully when the summer break can be as long as six months and where adult weevil mortality can approach 100%, i.e. beyond April, which is typical of the autumn break in the states of Western and South Australia.

The mass rearing of *L. echii* in Canberra, Perth, Frankston and Mudgee has shown adults are most abundant in winter and early spring, a perfect time to become active in regions of late seasonal breaks. One of the concerns with *L. echii* as an effective biological control insect was that, even if it did emerge in large numbers after summer, would subsequent larval feeding in late winter and spring reduce *E. plantagineum* size and seed production? To confirm the ability of *L. echii* to successfully aestivate through an extended summer and to measure impact on its weedy host, controlled experiments were conducted to measure these factors.

MATERIALS AND METHODS

Biology of *L. echii* *L. echii* is a halticine beetle [Coleoptera: Chysomelidae] native to the Mediterranean regions of southern Europe and North Africa. *L. echii* is univoltine and adults emerge after autumn rain from earthen cells up to 20 cm below ground. Adults feed on the foliage of *E. plantagineum* and after 1–2 weeks lay eggs directly on the taproot of the plant. Larvae hatch after 2–3 weeks and feed in the cortex of the taproot and secondary roots. If there are sufficient larvae on a plant, the entire root system will be destroyed and feeding can continue into the crown, killing the plant. When feeding is complete, larvae leave the plant, form an earthen cell in the soil and pupate. Adults remain in the earthen cell until autumn rain stimulates them to emerge and start a new generation in the following autumn/winter (for more information see Wapshere 1982).

Laboratory experiment To accurately record adult emergence after summer, *L. echii* was reared in a fibreglass tub 1100 mm × 1100 mm by 300 mm deep at the CSIRO Black Mountain laboratory Canberra. The tub had 16 holes drilled in the bottom and placed on a pallet to improve drainage. The tub was filled with soil and 36 rosettes of *E. plantagineum* grown for two months in 150 mm pots were transplanted in the last week of June 2001. An aluminium cage covered with a thrip mesh, the same dimensions as the tub and 1000 mm high was secured to the tub. In the last week of July, 100 adults of *L. echii* were added to the tub that was also covered by a polycarbonate roof so rainfall and soil moisture could be regulated. In autumn, a potted plant of *E. plantagineum* was added to the tub and was actively watered from May to promote emergence and adults of *L. echii* were collected weekly.

Field experiment The trial was conducted at the CSIRO experimental station, Ginninderra ACT in 1998 in a fenced area. Seeds of *E. plantagineum* were germinated in a mixture of perlite/vermiculite in May. Seedlings were pricked out into 50 × 150 mm tubes and grown in a glasshouse at 10/20°C for three weeks when they were moved outside to harden off before transplanting into the field. Plants where selected for uniformity of size, at the six to eight leaf stage and 200 mm in diameter and dug into the field on 7 July using a 50 mm diameter tree-planting corer. The potted plant was removed from the tube and planted into the hole in the ground, in this way surrounding pasture plants were not disturbed. The pasture was a mix of perennial grasses (*Phalaris aquatica* and *Dactylis glomerata*), annual grasses (*Bromus hordeaceus*, *Lolium ridgium*, *Vulpia* spp.) and clovers (mainly *Trifolium subterraneum*).

Plants were arranged in a randomised split plot design with the four treatments allocated within the subplot and five subplots per block (see Figure 1). There were six blocks with 120 plants in total. The four treatments are as follows:

1. Control: no plant competition or *L. echii*
2. *L. echii*: feeding by *L. echii* and no plant competition
3. Plant competition: no *L. echii* and plant competition
4. Interaction: feeding by *L. echii* and plant competition

Plant competition was removed by spraying a 1 m diameter area with glyphosate three weeks before the tube plants were transplanted into the field. On the 10 July, to ensure attack by *L. echii*, six adults were caged

1	4	2	3	3	4	2	3	3	2
2	3	4	1	2	1	4	1	1	4

Figure 1. Block layout. Numbers 1–4 is an example of treatment allocation.

onto attacked plants for 18 days. Cages were made of a metal ring 100 mm wide and 250 mm in diameter with two wire hoops attached to the metal ring projecting 300 mm high. A muslin sleeve was glued to the metal ring and the cage was dug in around all the plants used in the experiment. Adults of *L. echii* were added to the 60 attacked treated plants and the muslin sleeve was then tied off above the wire hoops, holding the muslin sleeve off the transplanted rosette and securing *L. echii* adults inside the cage. Plants without *L. echii* were caged and sleeved but no adults were added. Once the cages were removed, rosettes that did not have *L. echii* added were sprayed fortnightly with a systemic insecticide (dimethoate 1 mL L^{-1} of water) to prevent larval feeding from eggs laid by adults that were now free to move around the experimental area. In spring once the first mature seeds were seen, seed production was recorded fortnightly as described in Smyth *et al.* (1997). At the end of seed production plants were dug up, cut into roots and shoots, rated for larval feeding (nil, moderate or high, as described in Forrester (1993), dried for 48 hours at 80°C and weighed. A sample of seed was collected from each of the plants and weighed. Plants affected by rabbit grazing were removed from the sample so all treatments finished with less than 30 plants.

Data from the impact experiment were analysed using Microsoft Excel. The $\log_{10}(x+1)$ transformation was used to normalise data and, for ease of comparison, values presented are back transformed means. Comparisons of factors in the impact experiment between all treatments were analysed using ANOVA. Once a statistical difference was confirmed by ANOVA, a difference between two treatments was confirmed by the students t-test.

RESULTS

Laboratory experiment In the over-summering trial, the emergence of *L. echii* from aestivation occurred over an extended period from May to August with peak emergence in July (Figure 2). In total, 2886 *L. echii* adults were collected from the rearing tub.

Field experiment Figure 3 shows the effect of the four treatments on plant size of *E. plantagineum*, which was highly significant ($F_{(3,81)} = 103.2$, $P < 0.0001$). After planting, the control plants grew vigorously with an average dry weight of 95 grams at the end of seeding. The addition of *L. echii* at the levels used in this experiment alone had little impact on *E. plantagineum*. Plant competition significantly reduced plant size by 94% ($t_{42} = 6.06$, $P < 0.0001$). The addition of *L. echii* to the plant competition treatment reduced plant size by a further 62% ($t_{39} = 2.97$, $P<0.01$). In addition when the

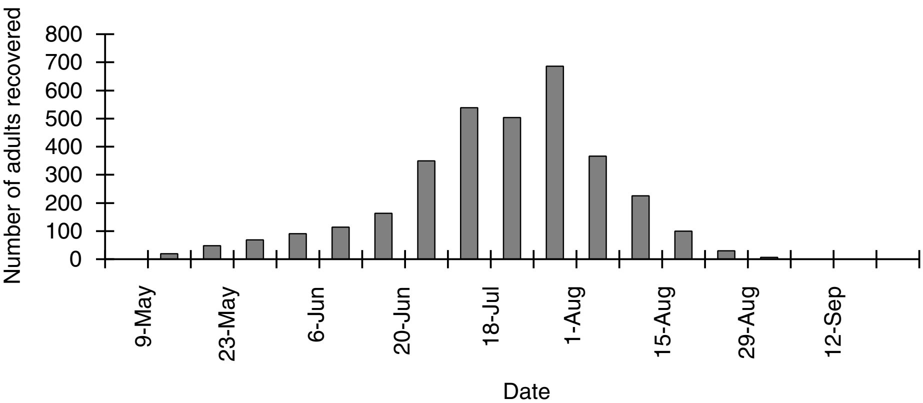

Figure 2. Emergence of *L. echii* from aestivation.

level of *L. echii* attack was taken into account, plants rated for a high level of larval feeding, shoot weight was reduced by 81% relative to the plant competition treated *E. plantagineum* (t_{37} = 3.97, P <0.001).

Figure 4 shows the effect of the four treatments on seed production of *E. plantagineum*, which was highly significant ($F_{(3,81)}$ = 30.5, P <0.0001). Seed number is closely associated with plant size and the treatment effects follow the same trend. *L. echii* feeding alone had no effect on *E. plantagineum* but pasture competition reduced seeding by 93% (t_{42} = 63.9, P <0.0001). The addition of *L. echii* in combination with pasture competition reduced seed production by a further 75% (t_{51} = 2.66, P <0.01). In addition when the level of *L. echii* attack was taken into account, plants rated for a high larval feeding, seed production was reduced by 93% relative to the plant competition treated *E. plantagineum* (t_{39} = 3.9, P <0.001).

Mean individual seed weights were not significantly different between treatments (P = 0.36). *E. plantagineum* mortality was nil for the control and *L. echii* but 8% and 26% for the plant competition and plant competition + *L. echii* treatments respectively.

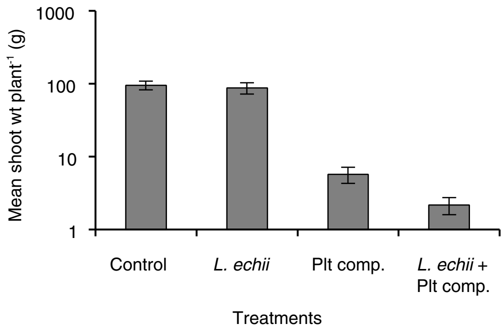

Figure 3. Mean shoot weight (grams) per plant of *E. plantagineum*.

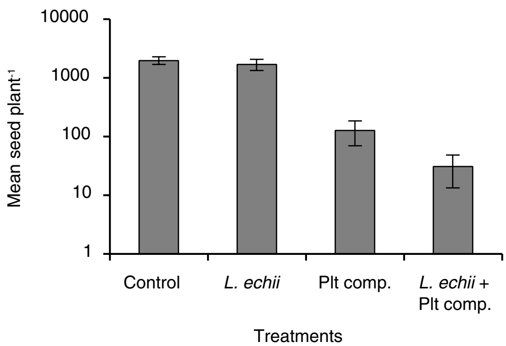

Figure 4. Mean seed production per plant of *E. plantagineum*.

DISCUSSION

The rearing and aestivation experiment confirms what had been seen in mass rearing throughout the country, namely that peak emergence of *L. echii* occurs through the middle of winter, with 63% of adults emerging in July (Figure 2). Starting with a parental generation of 100 adults (50 females) a total of 2886 adults successfully emerged the following year. A total of 58 adults per female, potentially giving the *L. echii* field populations a high intrinsic rate of increase even in climatic regions where the seasonal break can occur as late as July. The ability to successfully survive late season breaks has been confirmed by field observation in Western Australia where 84% of *L. echii* releases have established, even after the driest summer/autumn on record in 2001. Compared to the establishment rate of 16% for *M. larvatus* and 47% for *M. geographicus* in Western Australia, *L. echii* is clearly the best insect for the climatic conditions experienced in that State or any region with late autumn breaks. The ability of *L. echii* to reproduce efficiently on *E. plantagineum* will have to be achieved under grazing conditions. *L. echii* will tolerate grazing better than the root and crown feeding weevils because only the adult stage of its life history is above ground and exposed to livestock (Smyth *et al*. 2000). Protection from grazing can only benefit the population growth of *L. echii*.

Whether *L. echii* population can successfully breed into large numbers or not, larval feeding will still need to reduce the growth and seeding of *E. plantagineum* significantly to reduce the dominance of the weed in Australian temperate pasture. In the field experiment, by itself, *L. echii* did not reduce the size or seeding of *E. plantagineum* (Figures 3 and 4). When *E. plantagineum* plant size was limited by pasture competition, the same level of *L. echii* attack reduced plant size by 62% and seeding by 75%. Mean individual seed weight was not significantly different between treatments, so reproductive effort was also reduced by 75%.

This experiment shows that once larval numbers per plant weight are high enough, plant growth and seeding can be significantly reduced. Encouragingly, when the level of larval attack was accounted for, plants rated for a high level of larval feeding had shoot weight and seed production reduced by 81% and 93%, respectively. If this level of reduced seed production is maintained through several seasons, soil seed banks and subsequent germination and recruitment of *E. plantagineum* will be reduced (Smyth *et al.* 1997). Importantly, the larval feeding damage to *E. plantagineum* in this experiment occurred at the same time as it would in the field, since peak adult emergence is in July (Figure 2) and adults were added to the plants in this experiment on 10 July. So, even though larval feeding of *L. echii* occurs late in the season (August–November) if sufficient numbers are present not only is plant size and seeding reduced, but also plant mortality is increased.

Making broad predictions about the likely success of a biological insect is difficult to do accurately. Many biological, climatic and management factors can affect the likely impact an insect herbivore may have on its host. However, in the case of *L. echii*, based on the data presented here, the prospects for this insect having an impact on *E. plantagineum* are very encouraging and its release across the widest possible range is the primary aim of national redistribution project for the biological control insects of *E. plantagineum.*

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