Interactions between three weed biological control agents of Paterson's curse, *Echium plantagineum*

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Summary This study investigates the interspecific interactions between three biological control agents of *Echium plantagineum* L. (Paterson's curse; Boraginaceae): *Mogulones larvatus* Schultze*, M. geographicus* Goeze (Coleoptera: Curculionidae) and *Longitarsus echii* (Koch) (Coleoptera: Chrysomelidae). Previous research has suggested that the two *Mogulones* species do not interfere with each other in the same plant. However, it is not known how *L. echii* might affect or be affected by *M. geographicus* and *M. larvatus*. Furthermore, the effect of the agents in combination on the host plant has not been evaluated. In a glasshouse experiment, the impact of larvae from three different species on each other and on the host plant was evaluated. Plants were inoculated with larvae of the insects in one of the following combinations, no insects (control), *M. geographicus* alone, *M. larvatus* alone, *L. echii* alone, *M. geographicus* and *M. larvatus*, *M. geographicus* and *L. echii* or *M. larvatus* and *L. echii*. After seven weeks the mass of surviving larvae was measured. Various measurements of plant growth and reproduction were also taken. The average larval mass of *L. echii* decreased when attacking the same plant as either of the *Mogulones* species. Survival of *M. larvatus* was marginally reduced in the presence of *L. echii*. Plants with *M. geographicus* alone or with *L. echii* had reduced flower production and greater tap root mass and secondary root mass than the control, suggesting differential resource allocation. The results suggest that it is better to release *M. geographicus* and *M. larvatus* in combination because they will not affect each other's establishment or performance. *M. geographicus* may have a greater impact on the plant. Releases of *L. echii* should be made in areas where the other two agents are not established.

Keywords Survival, competition, biological control.

INTRODUCTION

Denoth et al. (2002) found an increase in the success rate of weed biocontrol when multiple agents were released. But, interspecific interactions occur in phytophagous insects (Denno *et al.* 1995) and these interactions may impact on the effectiveness of weed biological control programs. In the present study we investigated interactions and impact of three agents released for the biological control of Paterson's curse, *Echium plantagineum* L. (Boraginaceae), in Australia.

Paterson's curse is a winter annual of Mediterranean origin and was first introduced into Australia in the 1850s as an ornamental plant (Piggin and Sheppard 1995). It is now considered a noxious weed in every Australian state (Piggin and Sheppard 1995). Three of the seven agents released against *E. plantagineum* show the most promise. All of these three feed as larvae on below ground plant tissue. These agents are a crown feeding weevil (*Mogulones larvatus* Schultze [Coleoptera: Curculionidae]) a root feeding weevil (*M. geographicus* Goeze) and a flea beetle (*Longitarsus echii* (Koch) [Coleoptera: Chrysomelidae]). *Mogulones larvatus* was released in Australia in 1993, *M. geographicus* in 1994, *L. echii* in 1996, and since then have been extensively redistributed throughout Australia with variable success in establishment and impact on Paterson's curse populations (Smyth *et al.* 2004). As redistribution efforts continue it is not known whether it is appropriate to continue to release all three species in the same locations because competitive interactions between the three agents have not been evaluated.

In the case of the biological control agents on Paterson's curse, the larvae of each of these insects attack similar plant parts; *M. larvatus* larvae feed on the crown of the plant but also move down into the tap root, *M. geographicus* larvae feed on the main tap root, and *L. echii* feeds on the secondary roots and tap root surface. These resources are all related to each other in that the photosynthetic products flow from the leaves through the crown to the tap root and later to the secondary roots. Prior to their release in Australia, Vayssieres and Wapshere (1983) suggested that the two *Mogulones* weevils can coexist in their natural environment because they are temporally and spatially separated and Wapshere (1985) recommended that they both be released in Australia. Forrester (1993) investigated the resource partitioning between the two weevil species, *M. larvatus* and *M. geographicus*, in the field and in the laboratory in their native habitat. The study concluded that these two species do not compete for resources in their native environment because they were significantly separated in time and by their feeding niche. However, *Longitarsus echii* is a secondary root feeder and it is actively laying eggs in late autumn and its oviposition and feeding activity overlaps with both *Mogulones* species but the impact of these species on each other has not been investigated. We investigated these relationships and also the combined impact of these agents on the above and below ground components of the host plant, *E. plantagineum*.

MATERIALS AND METHODS

Echium plantagineum was grown in black plastic pots (20 cm diameter) filled with white sand. Seedlings were dug up from a plot at the Department of Agriculture Western Australia, South Perth and transplanted to the pots. Eight grams of slow release fertiliser was mixed into the top third of the sand before transplanting seedlings. Plants were grown until they were 20 cm in diameter. Forty-two plants with similar rosette size were chosen for this experiment. No plants had produced stems or flowers. Pots were rotated on the benches in the glasshouse throughout the experiment.

Eggs were collected from each insect species. All eggs were transferred onto moist filter paper in Petri dishes and put into an incubator at 24°C. Water was added to the filter paper every day to keep it moist and stop the eggs from drying out. When larvae hatched they were added, using a moist camel hair brush, to the petiole of the plants where there were fewer leaf hairs to injure the larvae. The petioles were checked the following day to make sure that the larvae had burrowed into the plant. If any dead larvae were found, another larva was added to replace it. Five *M. larvatus* larvae, ten *M. geographicus* and ten *L. echii* were added to a single plant in the following combinations: no insects (control, n = 7); *M. larvatus* alone (n = 6); *M. geographicus* alone (n = 5); *L. echii* alone (n = 6); *M. geographicus* and *M. larvatus* (n = 4); *M. geographicus* and *L. echii* (n = 6); *M. larvatus* and *L. echii* (n = 6). There was an uneven number of replicates due to a limited supply of eggs and high larval mortality that occurred during transfer to the plants.

The larvae were then left to feed on the plant for seven weeks. The plants were then removed from the pot and the soil sieved (0.5 × 0.5 mm sieve) to collect larvae. The plants were then destructively sampled and the fresh mass of the tap root and secondary roots were measured and the number of flowers (as indicator of potential reproduction) on each plant counted. The plants were then dissected to remove the larvae.

The larvae were identified. *L. echii* was easily distinguished from the *Mogulones* larvae and the two species of *Mogulones* larvae were identified using the width of their head capsule and other distinguishing characteristics described by Vayssieres (1986). After identification, larval mass and the percentage of larvae surviving were recorded. It was also noted if the insects had progressed to pupal or adult stage.

Statistical analysis The influence of treatment on the survival rate of both *Mogulones* species in the different treatments was analysed using a Kruskal-Wallis one-way analysis of variance (ANOVA) as the percent survival was not normally distributed even after transformation. Percent of *L. echii* larvae surviving in each treatment was arcsine transformed to meet the assumption of normality and then compared using a one-way ANOVA. Results presented are the untransformed values. An analysis of variance was used to test for significant effects of treatment on the average larval mass of all surviving larvae for each species. Fisher's unprotected LSD test was used for unplanned pairwise comparisons.

The influence of the treatments on the plants was analysed using multivariate analysis. The mass of the tap root, secondary roots, and number of flowers were included as variables (normalised) to construct a resemblance matrix using Euclidean distance. The matrix was then analysed to compare the effect of insect combination on plants (PERMANOVA+, Primer-E Ltd, 2007). Pairwise tests were also conducted.

RESULTS

Overall, the survival of *M. geographicus* was much lower than the other two species and *L. echii* had a higher survival rate than either of the *Mogulones* species (*L. echii* = 73 ± 4% > *M. larvatus* = 58 ± 9% > *M. geographicus* = 29 ± 5%). Both pupae and adult *M. geographicus* were recovered from plants. No pupae or adults of either *M. larvatus* or *L. echii* were collected from any of the plants. Survival of *L. echii* ($F_{2,15}$ = 0.14, P = 0.9), *M. geographicus* ($H_{5,6,4}$ = 0.9, P >0.05) and *M. larvatus* ($H_{4,6,6}$ = 1.56, P >0.05) was unaffected by the presence of other species. However, there appeared to be a trend towards reduced survival of *M. larvatus* larvae in the presence of *L. echii* (46 ± 9%) relative to survival of *M. larvatus* alone (64 ± 18%) or with *M. geographicus* (65 ± 22%).

The average mass of *L. echii* larvae decreased by 20% when there were other larvae added to the plant (Figure 1; $F_{2,15}$ = 5.77, P = 0.014). The average mass of *M. geographicus* (0.015 ± 0.002 g; $F_{2,9}$ = 0.1,

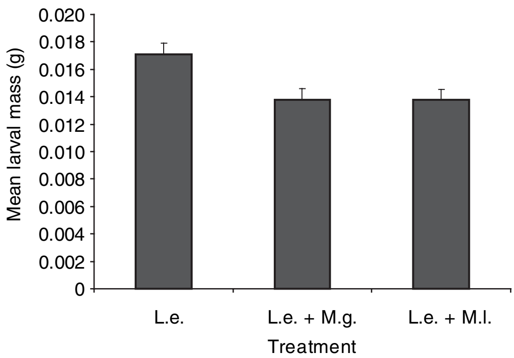

Figure 1. The mean (+SEM) weight of *L. echii* larvae in treatments with *L. echii* only and treatments with *L. echii* and another species; M.g. = *M. geographicus*, M.l. = *M. larvatus*, L.e. = *L. echii*.

$P = 0.91$) and *M. larvatus* (0.016 ± 0.002 g; $F_{2,11} = 1.03$, $P = 0.39$) were not affected by treatments.

Echium plantagineum plants were affected by the different combinations of insects (Pseudo– $F_{6,33} = 1.95$, $P = 0.03$). Those plants with *M. geographicus* and *M. geographicus* and *L. echii* (in combination) were significantly different that the control plants ($P < 0.05$). There was a 25% increase in tap root mass, a slight increase in secondary root mass and 83% fewer flowers in those plants with *M. geographicus* compared to the control. Plants with *M. geographicus* and *L. echii* had a 52% greater tap root mass, slightly greater secondary root mass and 33% fewer flowers compared to the control. Those plants with *L. echii* alone were different to those with *M. geographicus* alone ($P < 0.05$). Those with *M. geographicus* had 37% greater tap root mass, 57% greater secondary root mass, and fewer flowers compared to those plants with *L. echii* alone. Plants with *L. echii* had four times as many flowers as those with *M. geographicus*. All other plants were not significantly different to the control or from each other. None of the plants died or appeared unhealthy during the experiment.

DISCUSSION

The presence of either of the *Mogulones* sp. decreased the average mass of *L. echii* larvae. Although there was no effect on the survival of *L. echii*, the decrease in the average mass of *L. echii* larvae could mean that the larvae develop into adults with a lower fecundity than if they were not in competition with another species. A relationship between adult body mass and fecundity has been described for other beetles where low body mass females had lower fecundity (Credland *et al.* 1986, Sato *et al.* 2004). A lower fecundity in *L. echii* could reduce their ability to impact on *E. plantagineum* because the beetle population would have a slower rate of increase and spread. Furthermore, we have found that *L. echii* females do not discriminate between plants with *M. larvatus* larvae present and those without *M. larvatus* (Hawley *et al.* 2004). Therefore, it is possible that *L. echii* larvae might feed on the same plants as *M. larvatus* and to their detriment. Forrester (1993) concluded that the two *Mogulones* species did not compete for resources. Our results support his in that their co-occurrence did not seem to impact on larval mass or survival of either species at these densities. However, in our study *M. geographicus* tended to have an overall lower rate of survival than *M. larvatus* despite being able to successfully complete development within the experimental period. The reverse was measured by Forrester (1993): *M. larvatus* had a lower emergence rate than *M. geographicus*. He postulated that this was related to the phenology of the plant, that *M. larvatus* preferred younger plants as it emerges earlier in the field. As our plants were of similar size to those described by Forrester (1993) and we detected a different trend, further experimentation is clearly necessary to elucidate the factors influencing larval survival of these insects.

The below ground plant tissue seemed to be impacted more by the presence of *M. geographicus* and the combination of *L. echii* and *M. geographicus* than by any other combination of insects. There was greater root mass and fewer flowers in the plants with these insects than the control, suggesting that there is different resource allocation when these species are present on the plant. This may have consequences for plant reproduction. Smyth *et al*. (1997) found that the size of the roots positively correlated with the number of seeds that the plant produced. Unfortunately, we did not measure seed production and can not examine the effect of these agents in combination on actual reproductive output. The impact of the insects on *E. plantagineum* may in fact be greater under field conditions than we detected in our glasshouse study because field plants are likely to experience greater stress caused by drought and low nutrients in the soil.

Current infestations of *E. plantagineum* are quite dense and there is no shortage of host plants available for oviposition or development. Under these conditions, it is expected that the agents are unlikely to have much interspecific contact and therefore much less competition. As the infestations decrease in size then these interspecific interactions may become more important. However, rearing and releasing agents is an expensive and time consuming exercise. So, even if releases are to occur in areas with plenty of host plants available, careful consideration ought to be given to release strategies in which the agents are given the best opportunity to establish.

The results presented here are obtained from a single glasshouse study and therefore further work needs to be conducted in the field to confirm the patterns described. With this in mind we provide the following cautious recommendations. When future releases of insects for the biological control of *E. plantagineum* are to take place*,* we suggest that *L. echii* should be released in areas where the other two agents are not present or only in low numbers. This may be the case in Western Australia or South Australia where the late autumn rainfall conditions appear to negatively influence *M. larvatus* populations (Smyth *et al.* 2004). Further consideration might be given to the combination of insects released in grazed areas because *L. echii* populations do not appear to be negatively impacted by grazing whereas *M. larvatus* populations are (Smyth *et al.* 2004). If another species is to be released with *L. echii*, then we recommend co-releases with *M. geographicus* because this combination may have a greater effect on plant growth.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Agriculture Western Australia and the School of Animal Biology for the support of this project. This project was funded by an Honour's Scholarship by the CRC for Australian Weed Management.

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