

Population development and impact of the bridal creeper leafhopper *Zygina* sp. in Western Australia

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Summary Bridal creeper, *Asparagus asparagoides* (L.) W.Wight, is an environmental weed in southern Australia, infesting bushland and roadside remnant vegetation. Three biological control agents have been approved to manage the weed; a leafhopper *Zygina* sp. in 1999, a rust fungus *Puccinia myrsiphylli* (Thuem.) Wint. in 2000 and a leaf beetle *Crioceris* sp. in 2002.

During 2001, *Zygina* sp. populations were monitored at three locations in Western Australia, two at Yanchep National Park and one at Bold Park. *Zygina* sp. populations ranged during autumn to spring between 1992 and 162421 m⁻² at Bold Park, 584 and 4833 m⁻² and 784 and 1148 m⁻² at the two Yanchep sites respectively. Populations were moderately parasitised by an egg-parasite, identified in 2000 as *Stethynium* sp. (Hymenoptera: Mymaridae), parasitising between 28 to 59% of eggs at all sites. An impact experiment on potted seedlings showed *Zygina* sp. at higher densities reduced new tuber and cladode production.

Keywords *Zygina* sp., *Asparagus asparagoides*, *Stethynium* sp., bridal creeper, parasitoid, leafhopper, impact, population monitoring.

INTRODUCTION

Bridal creeper, *Asparagus asparagoides*, is an exotic weed that poses a major threat to biodiversity and conservation in Australia's temperate natural ecosystems (Raymond 1995). Originally introduced as a garden plant in the 1850s, it became naturalised in the early 1900s and is now listed as a Weed of National Significance. In the early 1990s surveys for biological control agents in the weed's native range, South Africa, identified several potential agents (Scott and Kleinjan 1991). One of these, the leafhopper *Zygina* sp., had undergone preliminary host-testing in South Africa (Witt and Edwards 2000) and was subsequently imported into quarantine in Perth in 1996 for detailed host testing. Permission was granted by AQIS and EA for the leafhopper's release into the Australian environment in 1999. It has been extensively released across southern Australia (Batchelor and Woodburn 2002).

The leafhopper damages *A. asparagoides* by reducing its ability to photosynthesise. The leafhopper nymphs and adults feed on mesophyll cells, damage being seen as white spotting on the leaf surface (Witt

and Edwards 2000). When the leaf is depleted of mesophyll, it senesces and falls from the plant. Continual attack by *Zygina* sp. (and the rust, Morin *et al.* 2002) has been observed to cause premature defoliation, which is also expected to lead to a gradual depletion of stored energy from the tubers, thus affecting the plant's reproductive and regenerative abilities.

By the end of 2001, the leafhopper had been released at 480 southern Australian locations. It is relatively easy to establish and community landcare groups were involved with setting up many of the release sites: it will slowly disperse from the release sites.

This paper presents the results of population development of the leafhopper, and its impact on tuber and cladode production, in 2001. The leafhopper was studied at three sites near Perth, Western Australia, one at Bold Park and two at Yanchep National Park. A preliminary laboratory impact experiment was undertaken to determine the impact of the leafhopper on new tuber and foliage production on 1.5 year old *A. asparagoides* plants.

MATERIALS AND METHODS

Leafhopper field sampling The leafhopper was sampled at Bold Park (31°56'S, 115°46'E) and two sites in Yanchep National Park (31°52'S, 115°41'E), 'Boomerang Gorge' and 'Rangers', between February and October 2001. Leaf samples were collected every 2–3 weeks using a 'column'. The column comprised of a 15 × 15 cm aluminium rod square with four 80 cm rods attached at the corners (like legs). The columns were placed randomly at the field site, with the legs being pressed lightly into the soil. All *A. asparagoides* cladodes (but not the stems) within the column were collected and put into plastic bags. Five column samples were collected at each field site. The height of *A. asparagoides* in the column was also noted.

A sub-sample of cladodes (min. 50) from each column was examined microscopically in the laboratory and the number of leafhopper eggs, nymphs and cast skins was counted. Within 12 months of *Zygina* sp.'s release, it was noted that the eggs were being attacked by a mymarid (Woodburn and Batchelor, unpublished), which was subsequently identified as an undescribed *Stethynium* sp., native to Australia, (John Huber, personal communication). Hence leafhopper eggs were

partitioned into the following five categories:

1. *Clear*. Newly laid eggs with no visible signs of parasitoid or leafhopper development.
2. *Grey/opaque*. Eggs parasitised but no visible parasitoid characteristics discernible.
3. *Yellow with eyespots*. Unparasitised leafhopper egg, with developing nymph.
4. *Parasitised*. Parasitised eggs with parasitoid larvae or pupae visible. Usually black or reddish brown in colour
5. *Ex-parasitised*. Egg case with a parasitoid emergence hole. Usually black (pupal case remaining in egg).

The number of 'clear eggs' collected in the sample was partitioned to the unparasitised or parasitised category based on the ratio of unparasitised to parasitised (i.e., Categories 3: (2+4) above) ratio in the sample. If the sample ratio was zero, the 'clear eggs' were partitioned based on the average ratio of the other samples for that site. If the ratio of unparasitised:parasitised was zero for the whole sample, then the 'clear eggs' were categorised on the average ratio of unparasitised:parasitised on the closest sampling date.

The number of eggs per 100 cladodes and percentage eggs parasitised were calculated over a three month period during sampling i.e., February to April 'Autumn', May to July 'Winter', August to October 'Spring'.

Leafhopper preliminary impact experiment The experiment ran from January to June 2001. The number of tubers was counted on seventy 1.5 year old *A. asparagoides* seedlings, which then had their stems cut off. The tubers were potted in University of California (UC) mix, and the pots placed in an air-cooled glasshouse. The experiment started when there were at least five fully expanded cladodes on each plant. Plants were assigned to replicates based on similar counts of foliage. The treatments were as followings: 1♀1♂ (n=10), 2♀1♂ (n=10), 4♀1♂ (n=10), and control (n=40). Before the insects were added, all the plants were enclosed in a plastic bottle cage (PET drink bottle) with two side windows covered with fine nylon mesh for ventilation. Newly emerged leafhoppers were added to the treatment plants through the bottle lid and allowed to feed and lay eggs etc until all the cladodes had defoliated (about five to six weeks). After defoliation, all plants were un-potted and the number cladodes and new tubers recorded. Plants were then re-potted and treatments re-applied when there were at least five fully expanded cladodes on each

treatment plant. The experiment was terminated early before the third inoculation of leafhoppers as several plants failed to re-grow after transplanting. In the initial experiment design, 10 control plants were to be destructively sampled after each defoliation (commencing with the second) until the all leafhopper treated plants died. As the experiment was terminated early, this only occurred once.

RESULTS

Leafhopper field sampling Leafhoppers numbers m⁻² were significantly higher in Bold Park during autumn and spring than those found in Yanchep National Park (P<0.05, Table 1). There was no significant difference in leafhoppers per m² during autumn and winter at all sites, and the population only increased significantly in spring at Bold Park and Yanchep 'Boomerang Gorge'. (P<0.05). Leafhoppers m⁻² did not significantly change over the year at Yanchep 'Rangers' (P<0.05). Number of leafhoppers per 100 cladodes was significantly higher in autumn than in winter at all sites (P<0.05). Parasitism ranged from 42–57% at Bold Park, 33–59% at Yanchep 'Boomerang Gorge' and 27–45% at Yanchep 'Rangers'. From September onwards, leafhoppers in Bold Park were observed to cause early defoliation in the sampling area and large numbers of adults were seen. Leafhoppers totally defoliated *A. asparagoides* by late October. Cladodes in adjacent parts of Bold Park not infested by the leafhopper remained green until late November.

At Yanchep National Park leafhoppers did not cause notable defoliation until late October and adults were rarely seen during the year. Cladodes in sections of Yanchep not infested by the leafhopper remained green until December.

Table 1. Average leafhopper eggs m⁻², per 100 cladodes and per cent parasitism at three sites.

Site	Period	Leafhopper per 100 cladodes (± SE)	Leafhopper m ⁻² (± SE)	% Parasitism (± SE)
Bold Park	Autumn	56.4 ± 2.7	1992.6 ± 383.4	42.0 ± 1.1
	Winter	9.5 ± 3.4	1238.4 ± 535.4	57.1 ± 1.6
	Spring	328.8 ± 127.6	162421.6 ± 57721.1	47.6 ± 1.6
Yanchep-Boomerang Gorge	Autumn	13.7 ± 3.9	584.2 ± 166.1	33.4 ± 1.1
	Winter	1.9 ± 0.7	428.2 ± 232.5	59.0 ± 1.8
	Spring	15.1 ± 4.9	4833.2 ± 1919.9	48.4 ± 1.9
Yanchep-Rangers	Autumn	10.3 ± 2.1	784.5 ± 227.9	45.5 ± 1.1
	Winter	3.2 ± 1.0	833.0 ± 273.0	27.7 ± 1.7
	Spring	5.4 ± 1.5	1148.2 ± 298.0	33.6 ± 1.9

Leafhopper preliminary impact pot experiment

The numbers of cladodes produced were significantly lower in the leafhopper treatments than in the controls ($P < 0.05$, Figure 1). There was no significant difference in cladode production between densities 1♀1♂ and 4♀1♂ ($P < 0.05$). Cladode production at density 2♀1♂ was significantly lower than density 1♀1♂ after the second inoculation ($P < 0.05$). The number of cladodes produced by all plants was greater after the second inoculation, including the controls.

There was no significant difference in the number of new tubers produced between the control and density 1♀1♂ after each inoculation ($P < 0.05$, Figure 2). The production of new tubers at densities 2♀1♂ and 4♀1♂ was significantly lower than the control after each inoculation ($P < 0.05$). No new tubers were produced after the second inoculation at densities 2♀1♂ and 4♀1♂.

DISCUSSION

Sampling leafhoppers using the 'column' enabled the population to be quantitatively measured. As *A. asparagoides* climbs over existing vegetation, the column enabled the cladodes at height to be sampled. Also, as the leafhoppers are shade loving insects, they are more prevalent in the lower cladodes of the infestation which are not likely to be well represented using random leaf sampling. The column was not as suitable for sampling adults, and may not be a reliable way of sampling nymphs, as many could be dislodged during the collection process. Hence population levels presented here are based on eggs counts.

Leafhopper populations increased in size during the year, being largest in spring at all sites (Table 1). The smallest increase occurred at the Yanchep 'Rangers' site. This site was treated with herbicide late in 2000, which resulted in less foliage growth during 2001, and may have been responsible for the lower increase observed. Populations at Bold Park increased massively, showing an 81 fold increase from autumn to spring. The increase observed at Yanchep 'Boomerang Gorge' was a much more modest one at eight fold. The rapid foliage growth of *A. asparagoides* with the onset of winter seemingly diluted the occurrence of leafhoppers when they are expressed as numbers per 100 cladodes, but not the actual densities of insects as there was no significant change in the number of leafhoppers when they are expressed as numbers m^{-2} (Table 1).

Studies have commenced on the biology of *Stethynium* sp. (Joder *et al.* 2002). In this present study, leafhopper populations would appear to be greatly affected by this parasitoid accounting for between 27.7–59.0% of eggs at all sites. Egg parasitism at

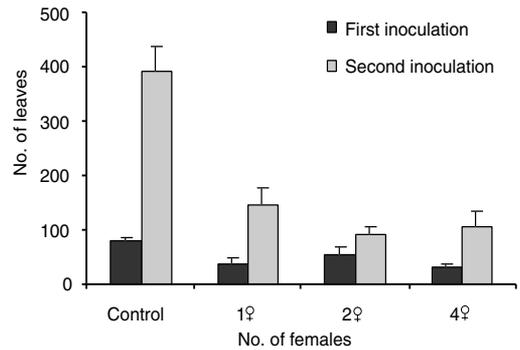


Figure 1. The effect of leafhopper density on foliage production.

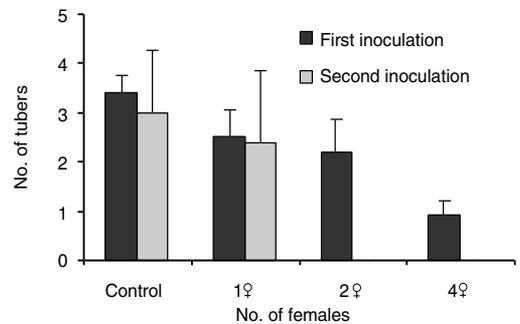


Figure 2. The effect of leafhopper density on new tuber production.

Bold Park was between 42–57%, but this did not prevent the leafhopper from causing early defoliation. Egg parasitism was lowest at Yanchep 'Rangers' during winter, being only half that observed at the nearby 'Boomerang Gorge' site. This is reflected in the population growth at 'Rangers', which showed a 1.3 fold increase compared to that observed at 'Boomerang Gorge'. Lower populations at Yanchep National Park compared to Bold Park could not be attributed to parasitism alone as the percentage parasitism at Bold Park was not significantly lower than at Yanchep. Other factors that could reduce population growth at Yanchep National Park are predation, adult/nymph parasitism, humidity and disease, all factors not investigated by this study. Another parasite was detected at all sites, an unidentified ectoparasitic larva of the family Dryinidae (Hymenoptera). The incidence of this ectoparasite was not studied. The leafhoppers were observed to cause early defoliation at Bold Park at a time when *A. asparagoides* was flowering and fruiting. It is not known if the leafhoppers had any effect on fruit production at this site, but feeding pressure during this period is likely

to have reduced new tuber production. At Yanchep National Park, leafhoppers were not observed to cause defoliation until late October and their effect on new tuber production is likely to be not as great.

The leafhopper was shown to significantly reduce new tuber and foliage production in the glasshouse pot experiment. Continual feeding by the leafhopper reduced the area of the foliage available for photosynthesis and therefore it seems reasonable to postulate that this reduction was responsible for the decrease in tuber production. Cladode production increased after the second inoculation in all plants, but this may not be a clear measure of impact as measurements were not made of the size/quality of cladodes produced. This experiment was a preliminary trial to refine the design of a more detailed impact experiment, which was commenced in April 2001.

These results from the field indicate that *Zygina* sp. does readily colonise bridal creeper in Australia, and that populations can build to very high numbers during a growing season. Laboratory experiments in South Africa demonstrated that feeding by *Zygina* sp. significantly reduced flowering and fruiting (Kleinjan *et al.* in press). Both the laboratory and field findings presented here indicate that the leafhopper should impact negatively on vegetative growth and tuber production of its target weed, *Asparagus asparagoides*. When these impacts are combined with those of the rust fungus (Morin *et al.* 2002) the prospect of successful biocontrol of the target weed looks promising indeed. Permanent field sites in WA (and other states) were set up before the release of any biocontrol agent. Pre- and post-release data have/are being collected at these sites on the impact of both the rust and leafhopper on bridal creeper growth and reproduction.

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REFERENCES

- Batchelor, K.L. and Woodburn, T.L. (2002). Community involvement in distributing the bridal creeper leafhopper *Zygina* sp., a biocontrol agent for bridal creeper, *Asparagus asparagoides*. Proceedings of the 13th Australian Weeds Conference, Perth.
- Kleinjan, C.A., Edwards P.B. and Hoffmann J.H. (in press) Impact of foliage feeding by *Zygina* sp. on tuber biomass and reproduction in *Asparagus asparagoides* (L.) – relevance to biological control in Australia. *Bulletin of Entomological Research*.
- Joder, A., Spafford Jacob, H., Batchelor, K.L. and Woodburn, T.L. (2002). Biology of *Stethynium* nov. (Hymenoptera: Mymaridae) an egg parasitoid of *Zygina* sp. (Hemiptera: Cicadellidae). Proceedings of the 13th Australian Weeds Conference, Perth.
- Morin, L., Willis, A.J. and Armstrong, J. (2002). Spread, epidemic development and impact of the bridal creeper rust in Australia: summary of results. Proceedings of the 13th Australian Weeds Conference, Perth.
- Raymond, K.L. (1995). The autecology of bridal creeper – how does it work? Weeds of Conservation concern: Seminar and workshop papers 5th-6th April 1995, Adelaide, South Australia, eds D. Cooke and J. Choate, pp. 17-21.
- Scott, J.K. and Kleinjan, C.A. (1991). Bridal creeper (*Myrsiphyllum asparagoides*) in Australia and developments towards biological control. *Plant Protection Quarterly* 6, 116-119.
- Witt, A.B.R. and Edwards, P.B. (2000). Biology, distribution and host range of *Zygina* sp. (Hemiptera: Cicadellidae) a potential biological control agent for *Asparagus asparagoides*. *Biological Control* 18(2), 101-109.