Biological control: a promising tool for managing bridal creeper, *Asparagus asparagoides* (L.) Druce, in Australia

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Summary

Three agents of South African origin have been released in Australia for the biological control of bridal creeper (*Asparagus asparagoides*): the leafhopper *Zygina* sp. in 1999, the rust fungus *Puccinia myrsiphylli* in 2000 and the leaf beetle *Crioceris* sp. in 2002. Community groups and land managers across Australia have embraced the biological control program against bridal creeper with enthusiasm and are actively involved in the large-scale redistribution of the first two agents, now released at more than 2500 locations across southern Australia. In contrast, establishment and growth of leaf beetle populations have so far been disappointing, possibly due to predation and limited availability of young shoots at time of release. Heavy attacks by the leafhopper are evident at some sites but high population levels are not necessarily sustained across years. The rust fungus is currently the most effective agent and has already demonstrated its ability to reduce populations of this invasive species, particularly in coastal areas. A major reduction in bridal creeper populations, combined with appropriate strategies to prevent invasion by other weeds, will significantly enhance the protection and restoration of key natural assets.

Introduction

The widespread form of *Asparagus asparagoides* (L.) Druce, commonly referred to as bridal creeper in Australia (Kleinjan and Edwards 1999), is one of southern Australia's worst environmental weeds (Thorp 1999). It is a climber that smothers large areas of natural vegetation and threatens biodiversity, including rare and unique native plants, such as orchids and *Pimelea spicata* R.Br. (Groves and Willis 1999, Willis *et al.* 2003). It is also found climbing trees in irrigated orchards in New South Wales (NSW) and Victoria, where chemical control is difficult to implement (Kwong *et al.* 2002, Kwong and Holland-Clift 2004). Plants develop 'mats' of rhizomes and tubers under the soil surface, representing close to 90% of the weed's biomass, that enable survival during dry summer months (Raymond 1999). Bridal creeper's foliage begins to grow after the first autumn or winter rains and fruits in late November. Birds that feed on the bright red berries spread the seeds and are responsible for the establishment of satellite populations away from main infestations (Stansbury 1996, 2001, Siderov and Ainsworth 2004). Hobbs (1991) stated that contrary to most other environmental weeds, bridal creeper's invasion of new sites appears unrelated to disturbance events. A similar observation was recently made in a study of the invasion process of bridal creeper in Victoria (Siderov *et al.* 2005).

Bridal creeper became naturalized and invaded Australia's bushland soon after its introduction from South Africa in the mid 1800s. It is, however, relatively uncommon in South Africa where its populations appear to be regulated by natural enemies. A research program was initiated in the late 1980s to identify potential biological control agents in the native range of *A. asparagoides* (Scott and Kleinjan 1991, Edwards 1995, Kleinjan 2000, Witt and Edwards 2000, 2002, Kleinjan *et al.* 2004b, Kleinjan and Edwards 2006). Following detailed host-specificity testing to demonstrate limited risks towards non-target plant species (Scott *et al.* 1999, Morin 1999, Batchelor and Woodburn 2001), three agents were approved by the relevant authorities for release in Australia: the leafhopper *Zygina* sp. in 1999, the rust fungus *Puccinia myrsiphylli* (Thuem.) Wint. in 2000 and the leaf beetle *Crioceris* sp. in 2002.

All agents have a narrow host range. The rust fungus has been found on five *Asparagus* species in South Africa, but the strain released in Australia only infects *A. asparagoides* and none of the other native, naturalized or cultivated *Asparagus* spp. present (Morin 1999, Kleinjan *et al.* 2004b). In contrast, the leafhopper can attack, albeit negligibly, a few other species within and outside the *Asparagus* genus, but can only complete normal development on *A. asparagoides* (Scott *et al.* 1999, Witt and Edwards 2000). The leaf beetle can complete normal development on a range of *Asparagus* species, but cultivated asparagus (*Asparagus officinalis* L.) and *Asparagus racemosus* Willd., the only native species in Australia, are not suitable hosts (Batchelor and Woodburn 2001, Witt and Edwards 2002). All agents were selected from the winter rainfall zone of the Western Cape Province of South Africa (100–600 km east of Cape Town), which climatically matches southern Australia. As anticipated, the rust fungus and leafhopper have coped with the Australian climate after their release and their phenology is well-synchronized with that of bridal creeper (Batchelor and Woodburn 2002b, Morin *et al.* 2002). It is too early, however, to draw conclusions with regards to the establishment of the leaf beetle across Australia.

In this paper, we will summarize the biological characteristics of each agent, provide an update on releases and establishment, and discuss their impact on bridal creeper. The extent and success of community engagement with the release and redistribution of the agents will also be discussed.

Leafhopper

Characteristics

The bridal creeper leafhopper is an undescribed typhlocybine species, *Zygina* sp. that belongs to the family Cicadellidae. Its adults are small (~2.5 mm long), winged and yellowish-white. Eggs are laid into mature leaves and hatch after two to three weeks depending on ambient temperature (Witt and Edwards 2000). The leafhopper has five nymphal instars, each significantly larger than the previous (Figure 1). The wingless nymphs do not disperse readily, unless disturbed or after depleting their food supply.

Many generations of the insect occur within a year, as long as living host material is available. In most bridal creeper infested regions of Australia, the leafhopper is active from approximately March to November, with populations peaking in the spring (Batchelor and Woodburn 2002b). In contrast, adults are present all year in the central part of its distribution in South Africa where bridal creeper grows continuously (Witt and Edwards 2000). In winter-rainfall areas where bridal creeper senesces during summer, adults are believed to aestivate during the dry months with occasional feeding on surrounding non-host plants (Batchelor *et al.* 1999). However, searches in South Africa and Australia for leafhopper over-summering sites have been unsuccessful.

Releases

The leafhopper has been released at more than 850 sites across Australia in collaboration with the community (Figure 2A). Whilst the leafhopper readily establishes at most sites, this is often difficult to confirm in the first year because individuals

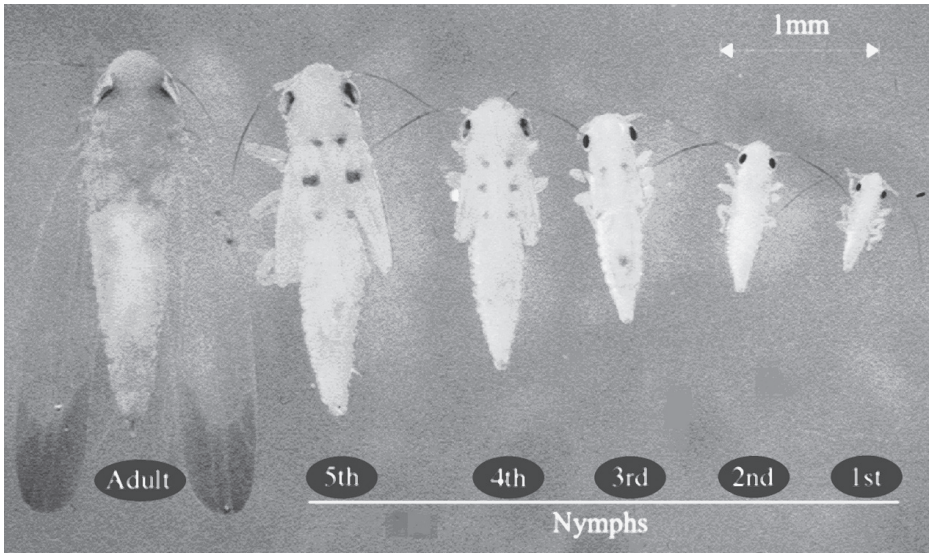

Figure 1. Developmental stages of the bridal creeper leafhopper (*Zygina* sp.) (indicative sizes).

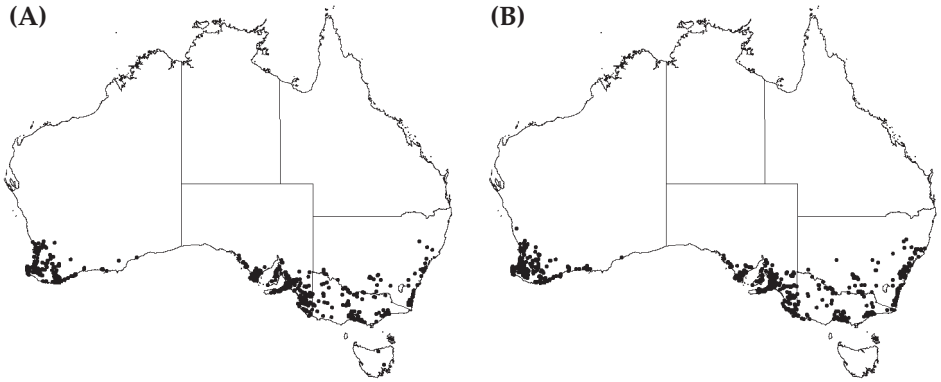

Figure 2. Location of sites where (A) the leafhopper (*Zygina* sp.) and (B) the rust fungus (*Puccinia myrsiphylli*) have been released since the beginning of the biological control program for bridal creeper (based on information currently available in the CSIRO database (http://www.ento.csiro.au/weeds/bridalcreeper/project.html).

disperse quickly and populations take time to build up.

The performance of the leafhopper has been highly variable. At many release sites populations have remained extremely low (e.g. Figures 3B, 3C) and at others the leafhopper has performed extremely well, spreading considerable distances and contributing to significant bridal creeper defoliation (e.g. Figure 3A). Substantial population fluctuations have been recorded at some well-established sites and could be due to increased parasitism over time (Joder *et al.* 2002, Batchelor and Woodburn 2002b). For example, in the 2001 growing season between 28 to 50% of eggs at study sites in Western Australia were parasitized (Batchelor and Woodburn 2002b).

Damage and impact

The leafhopper feeds on mesophyll cells of leaves (cladodes) and young stems, using a stylet to 'suck out' the content of cells. Feeding damage initially appears as small chlorotic flecks on the upper surface of leaves, followed by chlorotic zigzag patterns that coalesce and completely discolour leaves. Damage is generally more prevalent on fully developed leaves occurring in the shade (Witt and Edwards 2000) and heavy attacks often result in early defoliation.

The impact of the leafhopper on bridal creeper has been experimentally assessed in the glasshouse and in the field using standardized plants (Batchelor and Woodburn 2002b, Kleinjan *et al.* 2004a). In the field experiment conducted in South Africa, flower and fruit production was significantly lower in plants attacked by leafhopper than insect-free plants (Kleinjan *et al.* 2004a). Plants exposed to leafhopper feeding had a significantly lower tuber biomass than that of control plants by the end of the experiment. However, it was not possible to determine whether the leafhopper actually reduced tuber reserves and/or reduced the rate of new tuber production (Kleinjan *et al.* 2004a). A preliminary glasshouse experiment by Batchelor and Woodburn (2002b) demonstrated that leafhopper feeding on bridal creeper foliage reduced the rate of tuber production, although it did not show whether the agent depleted existing tuber reserves.

The impact of the leafhopper and/or rust fungus on populations of bridal creeper is being monitored yearly at sites in Western Australia, South Australia, Victoria and NSW where permanent quadrats and trellises have been set up, in collaboration with several researchers from these states. Base-line data on several growth parameters of bridal creeper was gathered at most sites in the 2–3 years prior to the release of the agents (L. Morin and collaborators unpublished). Although the rust fungus is the most active agent, the leafhopper has contributed to the reduction of bridal creeper populations at some sites (Figure 3). This work is in progress and will be published in full elsewhere.

Rust fungus

Characteristics

The rust fungus associated with bridal creeper is identified as *Puccinia myrsiphylli* (Basidiomycota; Uredinales; Teliomycetes). It is a biotrophic pathogen, or obligate parasite, that infects the leaves and stems of bridal creeper and obtains nutrients from living plant cells. It is autoecious, completing its life cycle on a single host (bridal creeper), and macrocyclic, producing all five typical spore states of rust fungi (Kleinjan *et al.* 2004b) (Figure 4). The bridal creeper rust fungus does not spread internally throughout plants and therefore it must reinfect plants every growing season.

In early to late autumn the first signs of the rust fungus appear in the field as small, wart-like, orange structures on the upper surface of leaves (spermagonia = pycnia) and cup-shaped, orange fruiting bodies (aecia) on the under surface of leaves (Kleinjan *et al.* 2004b) (Figure 4). Aecia produce spores called aeciospores, which infect plants to produce uredinia, the repetitive and most common stage of the rust fungus. Uredinia (orange pustules) and telia (browning-black pustules) occur on the under surface of leaves and on stems. Uredinia produce spores called urediniospores that are dispersed by wind. Telia develop several weeks after the appearance of uredinia and produce thick-walled resting spores called teliospores, ensuring survival of the rust during the dry summer months when bridal creeper naturally senesces. These two spore stages of the rust fungus can be observed for most of the growing season on bridal creeper.

The occurrence of *P. myrsiphylli* in South Africa is dependent on the presence of

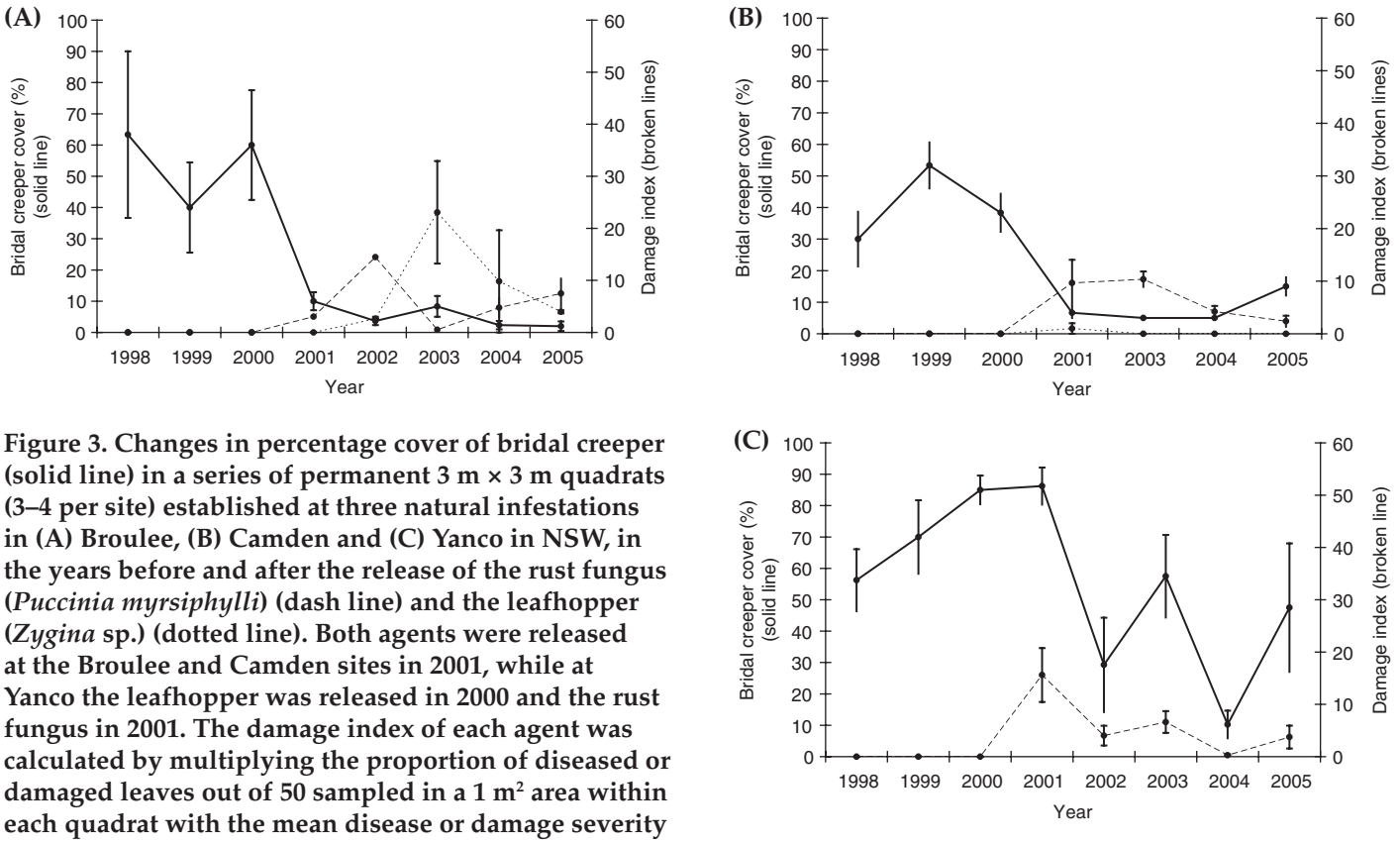

Figure 3. Changes in percentage cover of bridal creeper (solid line) in a series of permanent 3 m × 3 m quadrats (3–4 per site) established at three natural infestations in (A) Broulee, (B) Camden and (C) Yanco in NSW, in the years before and after the release of the rust fungus (*Puccinia myrsiphylli*) (dash line) and the leafhopper (*Zygina* sp.) (dotted line). Both agents were released at the Broulee and Camden sites in 2001, while at Yanco the leafhopper was released in 2000 and the rust fungus in 2001. The damage index of each agent was calculated by multiplying the proportion of diseased or damaged leaves out of 50 sampled in a 1 m² area within each quadrat with the mean disease or damage severity of affected leaves. Yearly assessments at each site were made after fruits had formed but before they had ripened. Each data point represents the mean value of all quadrats at a site and bars indicate ± one standard error of each mean. Data for 2002 is missing for the Camden site.

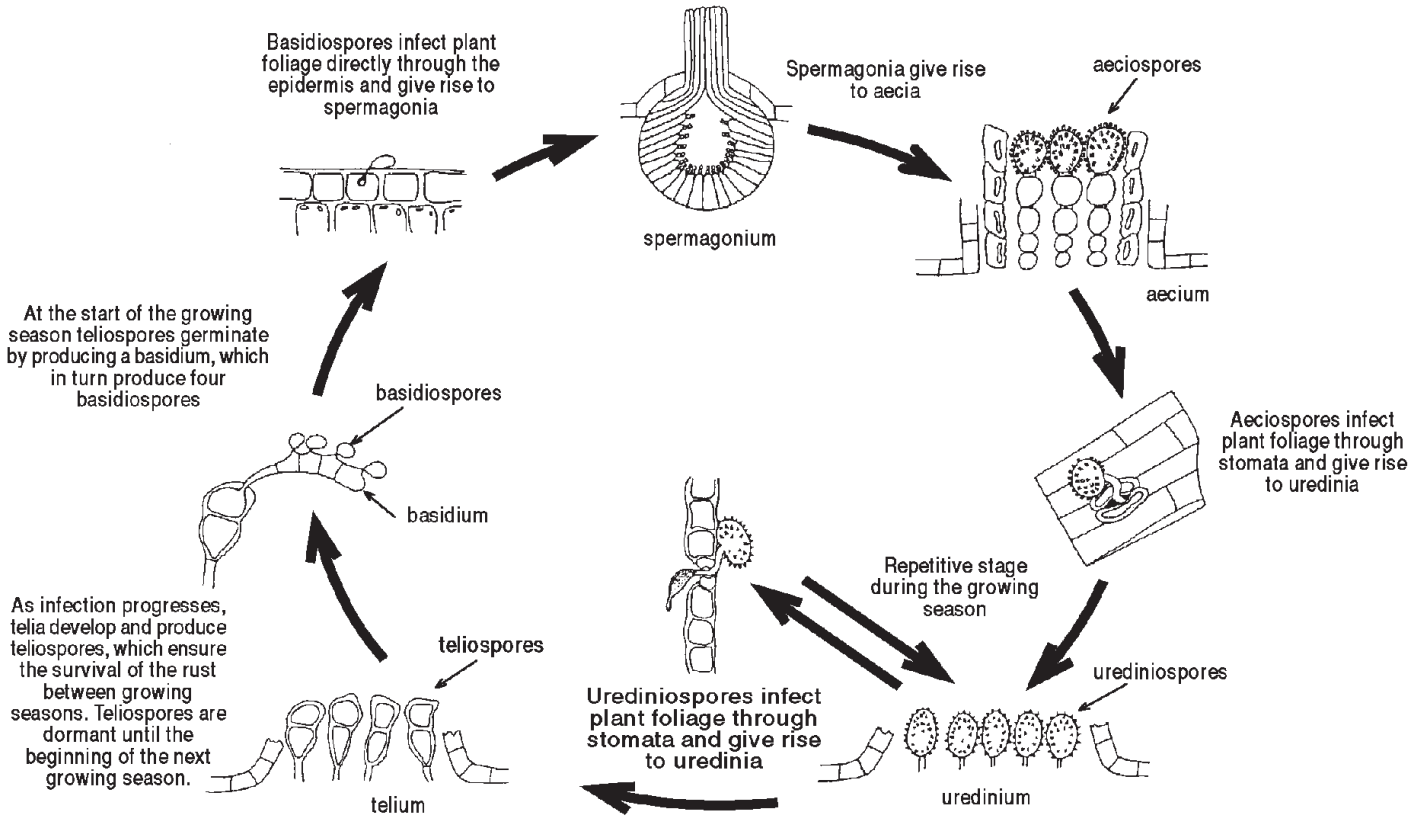

Figure 4. Schematic illustration of the life cycle of *Puccinia myrsiphylli* on bridal creeper.

living foliage of its host and rainfall pattern (Kleinjan *et al.* 2004b). Similar observations have been made in Australia, where the rust fungus generally appears within 4–8 weeks after the emergence of bridal creeper shoots and the first autumn rains (around May-June in Western Australia and South Australia, and March in NSW) (Morin *et al.* 2002). From then on, the rust fungus repeatedly infects plants during the growing season, if environmental conditions are suitable. In laboratory experiments, urediniospores required at least eight hours of leaf wetness and temperatures between 16 to 20°C to infect plants (L. Morin unpublished data). Repeated infections result in a steady increase of disease incidence and severity, which peak in the spring when plants are flowering and fruiting (Figure 5).

Releases

In collaboration with community groups and land managers, the rust fungus has to date been released at more than 1700 sites across the distribution of bridal creeper in temperate Australia (Figure 2B). It has established readily at most sites, particularly those in moist coastal areas. In inland areas recently stricken by drought, *P. myrsiphylli* exceeded expectations by surviving and recolonizing sites between seasons, albeit at low densities (L. Morin unpublished).

Puccinia myrsiphylli has spread relatively slowly at sites in southern NSW that were closely monitored following release in July 2000 (Morin *et al.* 2002). At one site near Narooma, the rust fungus only spread about 30 m by the end of the growing season, four months after release. Low wind turbulence and high moisture levels during the bridal creeper winter growing season probably restricted dispersal of urediniospores at these sites. In the following growing season, the rust fungus reappeared at all sites and continued to spread from the recolonized infection foci (Morin *et al.* 2002).

Several community members across southern Australia who released the rust fungus in 2001 and 2002 followed a protocol that we developed to measure maximum natural spread in the year following release. Four transects radiating from the initial release point were inspected for rust infections every 5 m for the first 50 m, every 10 m between 50–100 m and every 20 m until no more bridal creeper or rust was encountered. Maximum spread of *P. myrsiphylli* within one year of release varied greatly between sites, ranging from 35 to 360 m at sites where releases were made 2001 and 2.5 to 65 m for the 2002 releases (Figure 6).

A Cecidomyiidae larva, similar to that found feeding on urediniospores of *P. myrsiphylli* in South Africa (Kleinjan *et al.* 2004b), has also been observed on infected leaves in NSW, particularly during very wet conditions (L. Morin unpublished). However, grazing by this natural enemy did not appear to restrict the development of rust epidemics.

Damage and impact

Puccinia myrsiphylli, like other rust fungi, acts as a resource sink through the continuous absorption or diversion of plant nutrients and detrimentally affects plant development and reproduction (reducing stem, fruit, rhizome and tuber production). The fungus also destroys leaf tissue by producing chlorotic spots and fruiting bodies that reduce the photosynthetic surface of the plant. It infects young and old leaves and stems, but disease symptoms are generally not as severe in stressed or unhealthy plants. Severely diseased plants shed infected leaves prematurely and produce few or no fruits.

Severe infections by the rust fungus adversely affected the production of new tubers and foliage during a glasshouse experiment (Morin *et al.* 2002). After 20 weeks of fortnightly inoculation with *P. myrsiphylli*, the tuber number, rhizome length and shoot mass of infected bridal creeper plants were reduced by more than 60% compared to control plants. While the latter regrew extensively after foliage removal, severely diseased plants did not regrow in the following 20 week period, demonstrating the exceptional indirect impact of the fungus on belowground biomass.

The impact of the bridal creeper rust fungus has been particularly impressive in coastal areas, where climatic conditions are ideal for epidemic development. For example, a drastic decline in bridal creeper coverage was recorded following the release of the rust fungus and leafhopper at study sites in Broulee on the southern NSW coast and Camden in western Sydney (Figure 3A, 3B). The two agents occupy the same broad feeding niche and their combined attack lead to an additive reduction in the relative growth rates of bridal creeper (Turner 2003, Turner *et al.* 2004). However, the rust fungus is considered the most effective agent in the field because its populations do not fluctuate as widely as the leafhoppers.

Unfortunately, the impact of the rust fungus has not been as remarkable at drier inland sites. For example, the bridal creeper population at Yanco has not been severely affected by the rust fungus, as demonstrated by fluctuating percentage

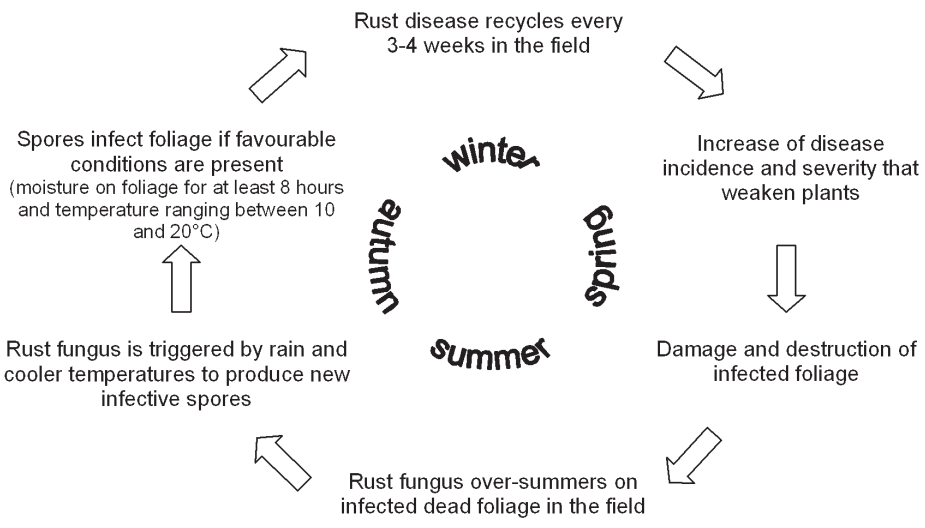

Figure 5. Schematic illustration of the various stages in the development of the bridal creeper rust disease during a year.

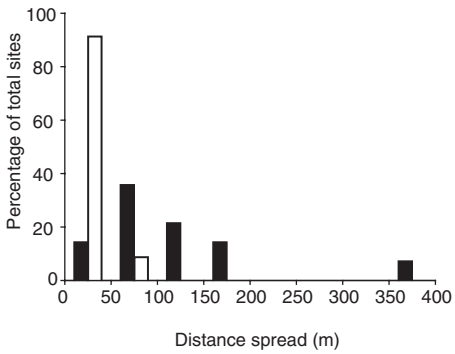

Figure 6. Distance spread by the rust fungus (*Puccinia myrsiphylli*) one year after its release in 2001 (solid column) and in 2002 (open column) at 14 and 23 sites, respectively. Sites were monitored by community members in Western Australia, South Australia and NSW by walking from the point of release along four transects in opposite compass directions to a distance where either no bridal creeper or rust could be detected.

cover since the release (Figure 3C). The three-year drought in this region has prevented the development of rust epidemics early enough in the season to reduce bridal creeper growth.

We are continuing to monitor the impact of the rust fungus and document the success of this biological control agent. A fully controlled, three-year exclusion experiment has been initiated to test more rigorously the direct impact of the rust fungus on bridal creeper at six sites in NSW and Western Australia. At each site, bridal creeper aboveground biomass and fruit production will be assessed towards the end of each growing season in permanent quadrats infected with the rust fungus and others that have been maintained free of the fungus by regular fungicide applications (Folicur™). It has previously been established that this fungicide does not affect bridal creeper growth (Turner 2003). Changes in plant community composition will also be monitored to determine whether sustained control of bridal creeper over consecutive years leads to natural restoration of native vegetation or invasion by other weed species (e.g. weed substitution).

Only the first year's data has so far been collected, but reductions in bridal creeper cover, aboveground biomass and fruiting are already being observed in rust-infected quadrats compared to rust-free plots. A comprehensive methodology with the full set of results will be published elsewhere at the completion of the experiment. Results from this study will form the basis of management plans for the restoration of native vegetation following successful control of bridal creeper.

Leaf beetle

Characteristics

The bridal creeper leaf beetle is an undescribed *Crioceris* sp. (Chrysomelidae: Criocerinae). The adults are light brown, winged and long lived (>6 months) and males are slightly smaller (3.9 mm body length) than females (4.1 mm long) (Witt and Edwards 2002) (Figure 7). Although capable of flight, adults are more frequently seen walking across shoots (Witt and Edwards 2002). Females can copulate immediately after eclosion and lay eggs, singly or in groups, on young growing shoots of bridal creeper. Based on a controlled environment experiment, eggs maintained at 15, 20 and 30°C hatched after 8.7, 5.1 and 3.6 days, respectively (Witt and Edwards 2002). The leaf beetle has four larval instars, light to dark brown in colour, which take 8–21 days to develop, depending on temperature, from eclosion to pupation. Mature larvae drop to the ground and pupate by producing a hardened protective cocoon.

The leaf beetle has one to two generations per year and over-summers as an adult in the pupal cocoon. Laboratory results indicated that wetting of pupal cocoons serves as a stimulus for adult

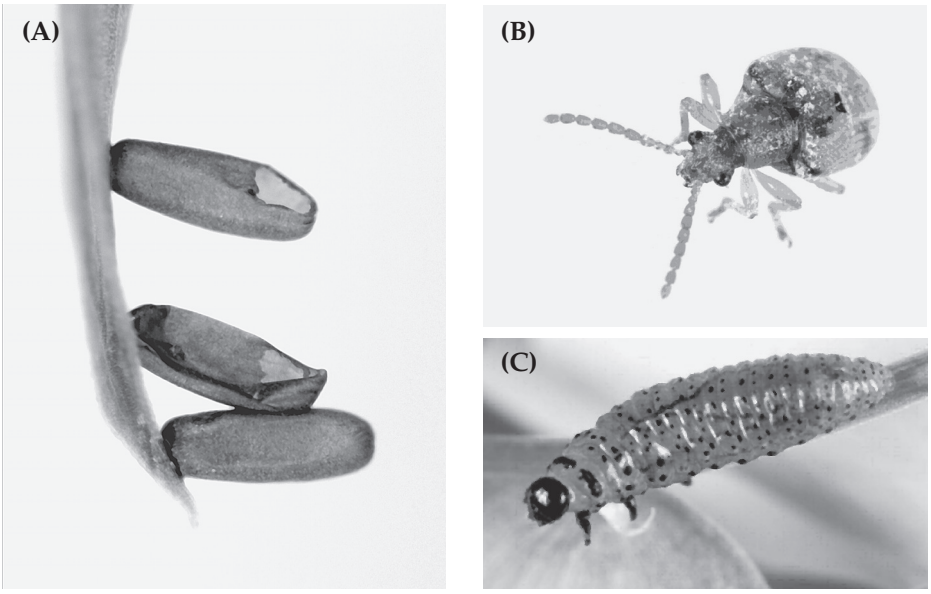

Figure 7. Bridal creeper leaf beetle *Crioceris* sp. (A) Eggs (~1 mm long; ~0.4 mm wide) laid perpendicularly on a shoot of bridal creeper. (B) Adult (~4 mm long). (C) Fourth-instar larvae (~8 mm long). Photos CSIRO, David McClenaghan.

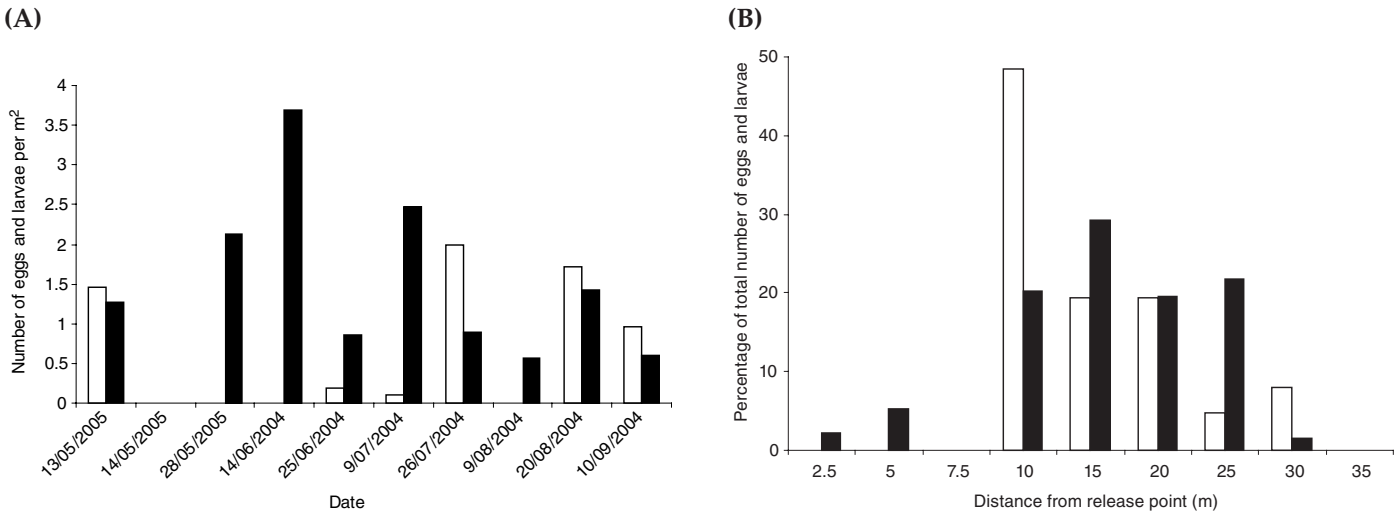

Figure 8. Bridal creeper leaf beetle emergence pattern and spread during the 2004 growing season at Woodman Point Regional Park in Western Australia. More than 1000 leaf beetles were released at this site in April 2003. (A) Average number of leaf beetle eggs (open column) and larvae (solid column) per metre square at different visits. (B) Percentage of total eggs (open column) and larvae (solid column) counted at various distances from the release point.

emergence, although not all adults emerge simultaneously (Witt and Edwards 2002). In South Africa winter rainfall regions, the leaf beetle is mainly active from April to June when bridal creeper resumes growth from its belowground reserves.

To date, only limited observations of the phenology and spread of the leaf beetle have been made in Australia. For example, we monitored the leaf beetle between May–October 2004 at the Woodman Point Regional Park in Western Australia, where more than 1000 adults were released in April 2003 (Figure 8). At each visit, the number of leaf beetles (eggs/larvae/adults) was recorded in six 50 cm^2 quadrats at 0, 2.5, 5, 7.5, 10, 15, 20 metres from the initial release point. Eggs were not detected at every visit but larvae were present on most occasions (Figure 8). Most leaf beetles were recorded between 10–30 m from the release point and none were found beyond this distance (Figure 8).

Releases

Leaf beetles have so far been released (mostly by CSIRO) at a total of 46 sites across southern Australia, including eight releases made by colleagues from the Department of Primary Industry in Victoria in 2005. The number of adults released at each site ranged from 40 to 1020, with an average release size of 400. Of the 16 releases made in 2002 and 2003, only three, all in Western Australia, resulted in leaf beetle establishment, i.e. leaf beetle populations sustained over more than one year after release. However, one of those established sites (Boomerang Gorge, Yanchep National Park) was destroyed by a bush fire in December 2004.

The leaf beetle does not appear to have established following any of the nine releases made in 2004 (one of those sites was also destroyed by bushfire eight months after the release). The drought of recent years may have detrimentally affected establishment of the leaf beetle at several sites by limiting production of young bridal creeper shoots, which are crucial for egg laying and as a food source for larvae. It is too early to confirm if the leaf beetles have established following releases made in 2005, although our initial field observations are promising.

There is scope to improve the release strategy for the leaf beetle to increase establishment rates. Leaf beetle releases should be targeted to areas where the other agents have not been exceptionally damaging, such as inland sites where rainfall and humidity may not be optimal for rust fungus development. Leaf beetle females should also not be released for 7–10 days after being manually removed from their pupal cocoons. This simple tactic would prevent predation of female adults during the pre-oviposition period and ensure maximum egg laying occurs shortly after release.

We also recommend that releases are made in the first part of the bridal creeper growing season when high quality young shoots are plentiful and temperatures are not too cold. We have shown that temperature can significantly affect the number of eggs laid by the leaf beetle in the laboratory. In this experiment, adults were manually removed from pupal cocoons and placed in small cages (one couple of leaf beetles per cage), each containing an actively growing bridal creeper plant, and a small bouquet of young shoots. Cages were then exposed to two different temperature regimes (10 replicate cages per temperature) and eggs were counted at regular intervals for a three-month period. Plants and young shoots were replaced regularly to ensure that suitable material for egg laying were present at all times. The number of eggs laid by insects subjected to an average temperature of 16.4°C (max 26.3°C; min 8.6°C) (mean no. eggs ± SE: 156.2 ± 26.7) was almost seven times greater than that of insects exposed to an average temperature of 9.3°C (max 14.5°C; min 8.6°C) (mean no. eggs ± SE: 22.9 ± 8.8).

Parasitism of leaf beetle pupae by species of Tachinidae and Eulophidae has been recorded in South Africa (Witt and Edwards 2002). In Australia, we observed low levels of larval parasitism (by an unknown wasp species) in one of our rearing colonies of the leaf beetle, but parasitism has not yet been seen in the field. In contrast, predation of larvae and pupae is suspected, based on results from a recent field experiment in Western Australia (Reilly *et al.* 2004). In this experiment, the highest number of larvae and pupae were recorded when adults were placed within exclusion cages, possibly due to protection from natural enemies and prevention of adult dispersal. Ant baiting at open experimental plots did not result in higher numbers of leaf beetle larvae and pupae compared to untreated plots, suggesting that predatory ants do not greatly affect leaf beetle populations.

We conducted a preliminary experiment during the 2005 growing season to explore further if parasitism and predation affects leaf beetle establishment (Figure 9). We will describe our initial experiment in

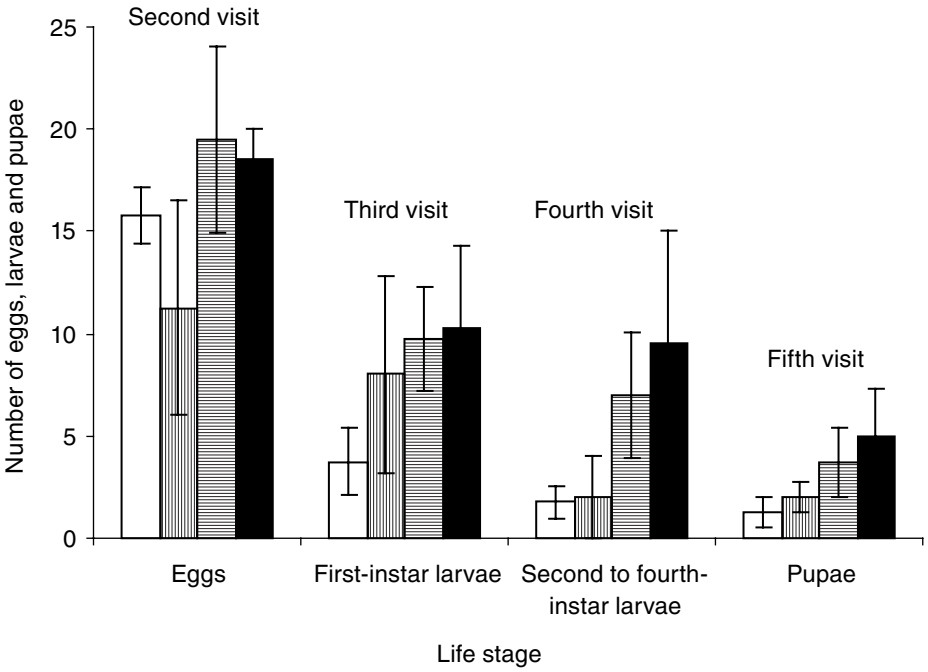

Figure 9. Results from a preliminary experiment conducted at a naturally infested bridal creeper site in Leeton, NSW in 2005. Numbers of eggs, larvae and pupae recorded following the release of ten males and ten females in each experimental plot (0.785 m^2). The treatments consisted of: an open control (no cage) (open column), a partial predator and parasitoid exclusion (using green nylon mesh; 1 mm^2 holes) (vertical hatching column), a complete predator and parasitoid exclusion until after the peak of the first larval instar stage (using white nylon mesh; 0.11 mm^2 holes) (horizontal hatching column), and a complete predator and parasitoid exclusion (using fine white nylon mesh for the entire experiment) (solid column). The site was visited every fortnight for 10 weeks. Each column represents the mean value of four replicates per treatment and bars indicate ± one standard error of each mean.

this paper and the possible implications for further leaf beetle releases. However, further experiments are planned to clarify the mechanisms behind these initial observations. In these experiments, we will optimize uniformity and monitor abiotic conditions between the treatments to reduce confounding effects and assist with interpretation of results.

In the preliminary experiment, the treatments were applied in a randomized block design with four replications. These treatments consisted of: an open control (no cage), a partial predator and parasitoid exclusion (using green nylon mesh with 1 mm^2 holes), a complete predator and parasitoid exclusion until after the peak of the first larval instar stage (using while nylon mesh with 0.11 mm^2 holes), and a complete predator and parasitoid exclusion (using fine while nylon mesh for the entire experiment). Experimental plots were circular (1 m diam.) and cages were made of two 1 m diameter steel rings connected by four 2 m long steel rods, covered with the different mesh fabrics. The base steel ring was placed 10–15 cm deep in the soil. Ten males and ten females were released into each plot immediately after manual removal from pupal cocoons. The site was visited every fortnight for 10 weeks to count eggs and larvae. At the final visit, the top 5 cm of soil/leaf litter in each plot was removed and sieved to separate and count pupae.

Eggs were only found during the second visit and the numbers were similar between treatments (Figure 9). There were significantly less first-instar larvae found in the open treatment compared to the fine nylon mesh treatments, during the third visit. This suggested that predation or parasitism of the small first-instar larvae may have occurred in the open control treatment. Some predation and parasitism of second to fourth-instar larvae may have also occurred, although the complete exclusion treatment was not significantly different to the treatment where complete exclusion was maintained until after the peak of the first instars stage. Carabid beetles, spiders and several genera of ants including *Iridomyrmex*, *Pheidole* and *Rhytidaponera* were caught in pitfall traps during the experiment and may have opportunistically taken leaf beetle larvae.

If our initial observations are confirmed in subsequent, more robustly designed experiments, the use of exclusion cages at the time of release will be recommended to enhance the initial increase of leaf beetle populations. Alternatively, releases of large numbers of beetles may compensate for any predation or parasitism that may occur.

Damage and impact

Adult leaf beetles feed on the growing tips and young leaves of bridal creeper but do not inflict as much damage as larvae, which strip young shoots and leaves and reduce bridal creeper's ability to climb. Older foliage is avoided by all leaf beetle stages. The impact of the leaf beetle is most likely to be on very young bridal creeper shoots emerging at the beginning the growing season before the other agents become active. No experiments have been carried out to quantify the impact of the leaf beetle on the belowground biomass of bridal creeper.

Community engagement - redistributing the agents

The natural spread process of both the rust fungus and leafhopper can be enhanced by redistributing diseased or insect-infested foliage (collected from established sites) to new sites. Simple protocols have been developed to assist the community in undertaking such activities and have been made available on a web site (http://www.ento.csiro.au/weeds/bridalcreeper/project.html). The CSIRO has published its database of agent release sites, in the form of an interactive online map that can be used to locate source sites for agents in specific regions. Community members have been urged to complete and forward a release site form to CSIRO every time a new release is made so that the database is kept up-to-date. Once the agents, and in particular the rust fungus, have established their populations should be sustainable over subsequent years unless major events (e.g. fire) or prolonged unfavourable climatic conditions occur.

Community groups, land managers and primary schools across Australia have been enthusiastically participating in the redistribution program of biological control agents for bridal creeper (Batchelor and Woodburn 2002a, Woodburn *et al.* 2002, Kwong 2002, Batchelor *et al.* 2004) (Figure 2). Several community groups have extended their commitment by organizing field days to tell others of the program. Other groups decided to speed up the spread of the rust fungus by suspending spores in water using infected material collected from established sites and applying it with conventional herbicide spray units onto large bridal creeper infestations (Overton and Overton 2006).

Community members have also been encouraged to monitor the long-term impact of the agents at their release sites to detect if other weeds are invading areas previously occupied by bridal creeper. Regular monitoring activities will allow them to take proper and timely management actions to address these new weed problems. A simple monitoring protocol was developed in 2002 in collaboration with members of the National Bridal Creeper Steering Committee and made available on the CSIRO web site (see above) to assist community groups who want to be actively involved in gathering impact data of the biological control program. The protocol involves recording the percentage cover of different vegetation types and presences of the bridal creeper biocontrol agents along transect lines at the release sites. With a few repeated measures made in September over a period of 5–10 years it will be possible to see if bridal creeper is declining, and if native plants or other exotic weeds are subsequently increasing. Unfortunately only one community group in South Australia collected base-line data in 2002 before the release of biocontrol agents at 18 sites (I. Abbott personal communication) and as far as we are aware these sites have not yet been reassessed. Although the protocol is relatively simple to follow, it appears that most land managers and community groups do not have the time to monitor release sites in such detail.

Conclusion

The biological control program for bridal creeper has already shown its potential to contribute to the long-term management of this nationally significant environmental weed. With additional efforts the entire distribution of the weed will be targeted by biological control. A major reduction in the impact of bridal creeper, combined with appropriate strategies to prevent invasion by other weeds, will significantly enhance the protection and restoration of key natural assets.

The rust fungus is currently the most effective agent, having already demonstrated its ability to reduce populations of this invasive species, particularly in coastal areas. The leafhopper has been outstanding in some years and locations, but it is unpredictable because its populations have a tendency to fluctuate. The leaf beetle could complement the other agents, but it is too early to make a proper assessment of its potential because it has not widely established.

An estimated investment of approximately $6 000 000 (in 2002's dollars) has been required to cover the cost of the bridal creeper biological control program from the early exploration phases in South Africa (1989/90) to the release of the third agent in 2002. This does not include the extensive and outstanding contribution of volunteer community members to the on-ground implementation of the program.

It is now crucial to document the success of the program in terms of environmental outcomes so that returns on investments can be demonstrated to the community. Biological control is a long-term weed control strategy and reductions in bridal creeper populations across the continent are likely to take some time (5–15 years). The agents will not eradicate bridal creeper but based on initial observations could substantially reduce infestations.

Acknowledgements

This biological control program would not have been possible without the financial support of CSIRO, the CRC for Australian Weed Management, CONCOM (later known as ANZECC), the South Australian Animal and Plant Control Commission and the Australian Government Natural Heritage Trust. Special thanks are due to staff of CSIRO Entomology based in Canberra and Perth for their continued support over the years. We are also grateful to all our collaborators across Australia who have played an important role in releasing and monitoring the biological control agents for bridal creeper. We would also like to thank John Scott (CSIRO Entomology) and an anonymous reviewer for their valuable comments on earlier drafts of the manuscript.

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The development of 'spore water' on Kangaroo Island for rapid spread of bridal creeper rust fungus

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Summary

An overview is given of the development and use of 'spore water' on Kangaroo Island, South Australia. Spore water is a solution of spores of the bridal creeper rust fungus, *Puccinia myrsiphylli*, which is sprayed onto bridal creeper, *Asparagus asparagoides*, using standard pesticide application equipment. It has been found to be a very successful, non labour-intensive method of introducing biological control into the bridal creeper populations.

Introduction

'Spore water' is a solution of rainwater and viable bridal creeper rust fungus (*Puccinia myrsiphylli* (Thuem.) Wint.). The rust is an effective seasonal biological control, and both this control vector and bridal creeper (*Asparagus asparagoides* (L.) Druce) are native to southern South Africa. The rust fungus is one of a small number of biological control vectors specific to bridal creeper (see Morin *et al*. 2006).

The rust fungus was first distributed to South Australian Animal and Plant Control Officers and delegates in August 2000. In spite of being effective, the initial method to spread the rust fungus amongst bridal creeper infestations with pots of infected plants and cuttings of infected shoots proved to be extremely time consuming and financially expensive for paid staff and volunteers, and it was also taking too long to see positive results. Once a small area (<1 m^2) was inoculated, it was taking another two or three bridal creeper growing seasons for rust fungus to spread several metres from the inoculation sites into new areas.

A quicker way to spread rust fungus was investigated. Trials were conducted in autumn 2003 to test spore water, comparing different weights of rust fungus infected bridal creeper shoots in a specific volume of rain water. Applications in winter 2003 on the Kingscote coastal foreshore found that spore water was a quick and cost effective way to spread rust fungus across the bridal creeper-infested landscape.

From then on, it has been our intention to spread the word (about spore water) as well as the rust fungus as far and as wide as possible across areas of Australia affected by bridal creeper.

What is spore water?

Spore water is a different way to spread a biological control. It is made by washing bridal creeper leaves infected with rust fungus with rainwater, and adding this concentrated mix to a clean spray unit containing rainwater.

It has proven necessary to always use rainwater as the chemicals in mains water and minerals in bore water would adversely affect the rust fungus spores. It is necessary to have a clean tank, lines and spray gun as otherwise residual pesticides may also adversely affect the rust fungus spores. Spore water solution also needs to be kept gently agitated during the spray operation to keep the spores suspended.

Rust requires humidity as well as mild temperatures to establish. It was not always humid when the spore water was sprayed out, so the spray jet was angled so that spore water landed into sheltered and warmer areas.

How to make spore water

A method is given at the web site: http://www.weeds.org.au/docs/BC_How_to_make_spore_water.ppt

Advantages of using spore water

Spore water is not an herbicide, uses rain water and the rust fungus is specific to bridal creeper. Hence it can be sprayed over any areas of native vegetation, forests or orchards infested with bridal creeper with no off-target damage.

Spore water can be sprayed when the day is mild or cold, when it is windy or just breezy, or when misty rain is falling and even a few hours before rain is expected. However, weather conditions will affect the establishment rate of the rust fungus. Any sized spray unit can be used, from a hand held two litre pump action unit a fixed wing crop duster aircraft.

Where was spore water sprayed and how successful has it been?

On Kangaroo Island, spore water has been sprayed on over 250 km of roadside vegetation, farm shelterbelts, coastal verges and other areas where bridal creeper is growing (Figure 1). Spore water has been