Attempts to establish biological control agents for boneseed in Tasmania

John E. Ireson, Jamie T. Davies, Wade S. Chatterton and Richard J. Holloway
Tasmanian Institute of Agricultural Research, 13 St. John's Avenue, New Town, Tasmania 7008, Australia

Summary Boneseed, *Chrysanthemoides monilifera* ssp. *monilifera* is a significant environmental weed in Tasmania. Three foliage feeding biological control agents have been released in Tasmania between 1991 and 1997. These were the black boneseed beetle (*Chrysolina* sp. 1), the painted boneseed beetle (*Chrysolina* sp. 2) and the bitou tip moth (*Comostolopsis germana*). Despite repeated and often large releases, none of these agents established. Biotic resistance by indigenous invertebrates has been suspected as a key factor in preventing their establishment. Releases of a fourth agent, the *Chrysanthemoides* leaf roller, '*Tortrix*' sp., began in 2000. Surveys in 2001 failed to recover '*Tortrix*' sp. at any of the nine release sites. At one of these sites, the survival of protected and unprotected '*Tortrix*' sp. egg batches were compared to determine the possible impact of natural enemies on the agent's establishment. About 70% of the unprotected egg batches were damaged compared to only 4% of the protected batches. Of the eggs that hatched, significantly more '*Tortrix*' sp. (16%) were recovered on protected branches compared to unprotected branches (only 1%). A complex of mainly generalist predators were found to exist on boneseed in Tasmania and a predatory mite, *Abrolophus* sp. (Erythraeidae), was observed feeding on the unprotected '*Tortrix*' sp. eggs. Predators are believed to be a key factor in either restricting or preventing the establishment of '*Tortrix*' sp. in Tasmania.

Keywords Boneseed, *Chrysanthemoides monilifera* ssp. *monilifera*, biological control, predation, Tasmania.

INTRODUCTION

Two subspecies of the South African shrub, *Chrysanthemoides monilifera* (L.) Norl. (Asteraceae), have become serious environmental weeds in Eastern Australia. *C. monilifera* ssp. *rotundata* (DC.) Norl. (bitou bush) occurs mainly in coastal regions of NSW, however, outbreaks have also been reported from Queensland and Victoria. *C. monilifera* ssp. *monilifera* (L.) Norl. (boneseed) occurs predominantly in coastal regions of south-eastern Australia, with major infestations in Victoria and South Australia (Weiss *et al.* 1998). Boneseed is also a significant weed in Tasmania, and is widely distributed in north, east and south-east coastal regions (Figure 1). However, the largest infestations are found in northern Tasmania along the Tamar River and in the south-east around suburban Hobart. Boneseed still has the potential to invade extensive areas of Tasmanian coastline.

Following nomination by the NSW National Parks Service, both subspecies of *Chrysanthemoides monilifera* were accepted as targets for biological control in 1987 by the Standing Committee on Agriculture (Holtkamp *et al.* 1999). Six biological control agents have since been released on boneseed in Australia: *Chrysolina* sp. 1 (black boneseed beetle), *Chrysolina* sp. 2 (painted boneseed beetle), *Chrysolina picturata* (Clark) (blotched boneseed beetle), *Comostolopsis germana* Prout (bitou tip moth), the seed fly, *Mesoclanis magnipalpis* Bezzi (Adair and Edwards 1996, Edwards *et al*. 1999) and, most recently, the *Chrysanthemoides* leaf roller moth '*Tortrix*' sp. Four of these species, *Chrysolina* sp. 1 and 2, *C. germana* and '*Tortrix*' sp. have been released in Tasmania.

The aim of this paper is to present the results of attempts to establish boneseed biological control agents in Tasmania together with a comparative exclusion experiment to investigate the possible role of predators in the establishment of '*Tortrix*' sp. The possible impact of natural enemies on the establishment of foliage feeding biological control agents on boneseed in Tasmania is discussed.

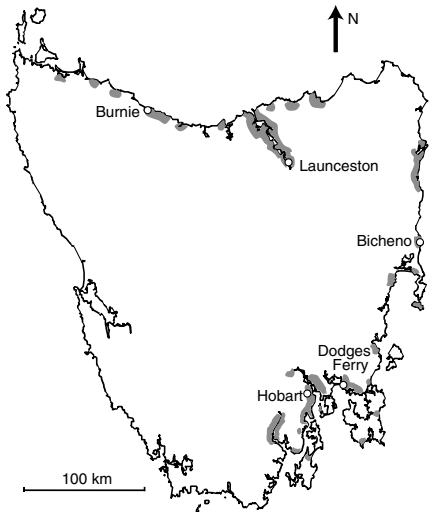

Figure 1. Distribution of boneseed in Tasmania.

MATERIALS AND METHODS

Propagation of boneseed Boneseed plants, 30–60 cm in height, were collected from the field by hand pulling. Soil was washed from the roots and the plants placed singly in 15 cm diameter pots containing a peat, pinebark and sand mix (pH 6.5) together with a slow release fertiliser. Plants were watered as required and fumigated fortnightly with Dichlorvos (Insectigas-DDVP Insecticide®) to reduce attack from pests. The plants were given at least three weeks to re-establish in the pots under glasshouse conditions and fumigated at least five days prior to their use in cultures. The potted plants were then transferred to rearing cages in a controlled temperature glasshouse (18–24°C).

Mass rearing and release of *Chrysolina* spp. Starter cultures of adult *Chrysolina* sp. 1 and *Chrysolina* sp. 2 were received from Keith Turnbull Research Institute (KTRI), Victoria in May 1991 and March 1995 respectively. Rearing cages were 1 m high, 750 cm wide and 500 cm in depth and consisted of insect proof nylon mesh set in aluminium framing with an aluminium base. Initially six and up to 12 boneseed plants were used per cage with the fresh plants being added as feeding activity increased. Vermiculite was spread over the base of the cages to a depth of *ca*. 1 cm to provide a pupation substrate additional to the potting soil. Fifty mature adults (sex ratio 1:1) were placed in each cage for a minimum of seven days or longer depending on the amount of oviposition. Adults commenced emergence after seven to nine weeks and were collected from the cages every two to three days. They were then transferred to another cage (max. 350 per cage), onto fresh sprigs of boneseed supported by a wire mesh platform. This foliage was changed three times per week and examined for eggs. The preoviposition period lasted about a fortnight and adults were used for field release at the onset of egg laying. Adults (with the foliage) were transported to selected sites and released amongst boneseed without restraint. Occasionally, larval infested foliage was removed from culture cages and released with the adults (Table 1).

Mass rearing and release of *C. germana* Larval infested boneseed tips were received from KTRI in April and May 1993. Larval infested tips of field hardened material collected in NSW were received in October 1996. Culturing was carried out using potted plants and the cages described above. Rearing was also carried out in a polytunnel containing up to 500 plants. The techniques used to infest culture plants included tying tips infested with larvae to the plants with 'twist ties' to allow larval transfer, releasing small numbers of newly emerged moths into cages (two to four male and female moths per cage) or using pots with larval infested boneseed. Field releases (Table 1) were carried out using the larval transfer technique.

Mass rearing and release of '*Tortrix*' sp. Larval infested leaf material was received from KTRI in October 2000. Cage cultures were initiated using eight late instar larvae per cage or four male and four female moths per cage. Larval infested potted plants were used to initiate a polytunnel culture.

Two releases were made by clipping egg masses to leaves and another seven were made using boneseed foliage infested with larvae and pupae (Table 1).

'*Tortrix*' sp. natural enemy exclusion experiment The first egg release was used to examine the possible impact of natural enemies on an establishing '*Tortrix*' sp. population in south-east Tasmania at Dodges Ferry,

Table 1. Summary of releases of boneseed biological control agents in Tasmania.

Agent	No. of release sites	No. of releases*	Release periods	Stages released	Estimated Nos. per release (range)	Result
Chrysolina sp. 1 (black boneseed beetle)	10	29	Oct. 1991–Dec. 1993; Nov. 1995–Nov. 1996	Adults, larvae	100–1000	Not established
Chrysolina sp. 2 (painted boneseed beetle)	2	2	May 1995; Sept. 1995	Adults, larvae	500–5000	Not established
Comostolopsis germana (bitou tip moth)	7	14	Sept. 1993–Sept. 1995; Oct. 1996–Sept. 1997	Larvae	100–3000	Not established
'*Tortrix*' sp. (*Chrysanthemoides* leaf roller moth)	9	9	Oct. 2000–Mar. 2001	Eggs, larvae, pupae	3600 eggs (2 sites each); 150–500 larvae/pupae	No recoveries to date

*Multiple releases were carried out at some sites, but not all sites received the same number of releases and some sites received only one release.

(43°7.9'S, 147°36.8'E) (Figure 1). In December 2000, twenty haphazardly selected boneseed plants were randomly allocated to either a protected or unprotected treatment. Thirty branches on each of 10 plants (three branches per plant) were then protected from natural enemies by enclosing them in 300 × 400 mm nylon mesh bags (mesh size 0.2 × 0.8 mm). Each enclosed branch was then sprayed with a synthetic pyrethroid insecticide (Mortein®, active constituents 3.7 g kg^{-1} bioalletrin and 0.7 g kg^{-1} bioresmethrin) for five seconds to kill or encourage existing arthropods to vacate branches. Bags were then sealed with 'twist ties'. Finally, a band of Tanglefoot® was applied at the base of each branch to prevent arthropods from crawling up branches. Ten plants (30 branches) were not protected from natural enemies.

In mid-summer (8.1.01), three weeks following the application of the insecticide, a mean ± SE of 60 ± 1.64 (range 40–86) eggs per batch of glasshouse reared '*Tortrix*' sp. were clipped onto each protected and unprotected branch. Egg batches were either orange or further developed with larval heads just visible, indicating that they were in the later stages of development.

Weekly monitoring was conducted to follow larval development and observe signs of parasitism and predation. Twenty-three days following egg introduction, egg batch chorions were collected from protected and unprotected branches and scored as either intact (at least half the chorions present) or damaged (less than half the chorions present). Fifty-seven days following egg introduction (6.3.01) both protected and unprotected branches were removed and taken to the laboratory where counting of larvae and pupae took place. Additionally, all boneseed plants in a radius of one metre surrounding the unprotected branches were searched for the distinctive leaf-rolling damage of '*Tortrix*' sp. Larvae and pupae found on unprotected branches and any surrounding plants were reared to adults for identification.

The egg batch damage scores were subjected to a chi-squared test. Data from the comparative exclusion study was converted to a percentage survival from the egg stage then subjected to a t-test.

Limb jarring of 10 boneseed plants within the study site was conducted on three occasions to sample the fauna on the bushes themselves. Boneseed shoots and branches were collected from six plants in mid-summer (17.1.01) and arthropods extracted using Tullgren funnels.

RESULTS

Despite widespread, multiple and often large releases, *Chrysolina* sp. 1 *Chrysolina* sp. 2. and *C. germana* have failed to establish in Tasmania (Table 1). Surveys at nine release sites in November 2001, nine to 12 months post-release have so far failed to recover '*Tortrix*' sp.

After 23 days of field exposure at the Dodges Ferry site, a significantly higher proportion of damage was recorded for egg batches on the unprotected branches (70% damaged) than for eggs on the protected branches (4% damaged) ($\chi^2 = 55.06$, $P<0.001$).

The number of '*Tortrix*' sp. found on branches protected from natural enemies exceeded those on unprotected branches by a factor of eighteen ($t = 8.34$, $P<0.001$, Table 2). This comparison also includes nine '*Tortrix*' sp. larvae that had moved off the control branches and were collected within a radius of one metre.

A range of potential predators was collected from the boneseed plants during the exclusion experiment (Table 3).

DISCUSSION

Establishment of *Chrysolina* sp. 1 and *Chrysolina* sp. 2 on boneseed or bitou bush has also been unsuccessful in NSW, Victoria and South Australia (Adair and Edwards 1996, Holtkamp *et al.* 1999). *C. germana* has also failed to establish on boneseed in Victoria. Poor climate matching may be a factor responsible for limiting the establishment of *C. germana* in parts of southern Australia by restricting its reproductive capacity (Adair and Scott 1989) which would restrict its ability to overcome the effects of any predation. In NSW, *C. germana* is now widely established on bitou bush and is having a significant impact on flower and seed production. At some sites, high densities

Table 2. Number and stages of live '*Tortrix*' sp. collected from Dodges Ferry 57 days after egg releases.

Treatment	No. larvae	No. pupae	No. adults	Total	% stages recovered*
Protected branches	262	32	1	295	16.4
Unprotected branches	16	0	0	16	0.9

* calculation based on the mean number of 60 eggs in each batch clipped onto each of 30 protected and unprotected branches (i.e. 1800 eggs per treatment).

exceeding 400 larvae m^{-2} have been recorded (Holtkamp *et al.* 1999). Although parasitoids have been recorded attacking *C. germana* in NSW (Holtkamp 1993), there is no evidence that parasites or predators have caused any significant interference in establishing populations (Holtkamp pers. comm.).

The predators (ants, mites and spiders) identified in this study, are common on boneseed throughout Tasmania (Ireson pers. obs.) and all may have contributed to the failure of *Chrysolina* spp. and *C. germana* to establish. In Victoria, Meggs (1995) showed that ants and spiders had played a role in preventing *Chrysolina* sp. 1 from establishing and discussed the possibility of a facultative mutualism between the ants and the honeydew secreting nigra scale, *Parasaissetia nigra* (Nietner). He showed that higher levels of *Chrysolina* sp. 1 egg loss occurred on plants possessing both ants (including a species of *Iridomyrmex*) and scales. In Tasmania, *P. nigra*, is also common on boneseed and occurred at all releases sites (Ireson and Davies personal observations).

The low survival of '*Tortrix*' sp. after egg hatch, even on protected branches at Dodges Ferry, indicates that a range of environmental factors other than predation may have been involved. The extent to which the arboreal mites, ants and spiders identified contributed to the low survival was beyond the scope of this study and further work will be required to determine their impact under field conditions. However, the destruction or damage to 70% of the unprotected egg batches and the observation that predatory mites attack these eggs, suggests that the impact of the mites and possibly ants and spiders was high. These predators could also have taken a heavy toll on immature larvae.

Manual placement of the eggs used in this study may have significantly reduced the survival of '*Tortrix*' sp. on the protected as well as unprotected branches. For instance, under natural conditions, female '*Tortrix*' sp. moths may oviposit on the plant in locations that are less likely to be found by predators. All releases of '*Tortrix*' sp. now being carried out in Tasmania involve transplanting of infested plants containing late instar larvae or pupae so that adults can emerge and oviposit under natural conditions. Tasmanian releases of '*Tortrix*' sp. are scheduled for completion in September 2002, by which time it will have been released at over 40 sites. It will therefore be at least 12-18 months before there is some indication whether the species will become permanently established in Tasmania. However, the *Chrysolina* spp., *C. germana* and '*Tortrix*' sp., all spend large parts of their life cycle exposed on the foliage of boneseed which makes them particularly vulnerable to predation. The abundance of generalist predators that are associated with boneseed habitats in Tasmania may have enabled boneseed to resist the establishment of *Chrysolina* spp. and *C. germana* and could prevent or restrict the establishment of '*Tortrix*' sp.

Although other biological control agents for boneseed are being evaluated, including the leaf buckle mite, *Aceria neseri* Meyer, and a rust fungus, *Endophyllum osteospermi* (Doidge) (Holtkamp *et al*. 1999), failure of '*Tortrix*' sp. to establish in Tasmania would further reduce the biological control options for boneseed in this State.

Table 3. Predators collected from boneseed at Dodges Ferry on which '*Tortrix*' sp. was present.

Predator	Comments
Mites (Acarina): European whirligig mite, *Anystis baccarum* L. (Anystidae) Velvet mite, *Abrolophus* sp. (Erythraeidae)	Anystid and erythraeid mites are generalist predators (Krantz 1971). A. baccarum was observed feeding on '*Tortrix*' sp. neonate larvae in the laboratory; Abrolophus sp. was observed feeding on '*Tortrix*' sp. egg masses on an unprotected branch at the study site
Spiders (Arachnida: Araneida): Amaurobiidae (2 species) Araneidae (1 species) Clubionidae (2 species: *Sidmella* sp., cf. *Clubiona* sp.) Mysmenidae (1 species) Salticidae (1 species: *Opisthoncus* sp.) Thomisidae (3 species: *Stephanopis lata*, *cf. Diaea* sp. A, *cf. Diaea* sp. B)	Generalist predators (Savory 1977) Form webs Hunting species
Ants (Hymenoptera: Formicidae): *Myrmecia* sp. *Iridomyrmex* sp. *Camponotus consobrinus* (Erichs.)	Generalist predators (Anderson 1991)

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