Progress on boneseed (*Chrysanthemoides monilifera* subsp. *monilifera* (L.) Norlindh) biological control: the boneseed leaf buckle mite *Aceria* (Keifer) sp., the lacy-winged seed fly *Mesoclanis magnipalpis* Bezzi and the boneseed rust *Endophyllum osteospermi* (Doidge) A.R.Wood

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Introduction

So far six exotic organisms have been released in Australia as potential biological control agents for the environmental weed boneseed (*Chrysanthemoides monilifera* subsp. *monilifera* (L.) Norlindh (Asteraceae)). None of these has established on boneseed although one, the bitou tip moth (*Comostolopsis germana* Prout (Lepidoptera: Geometridae)) has colonized all of the major infestations of the closely-related invasive species bitou bush (*C. monilifera* subsp. *rotundata* (DC.) Norlindh). Currently three organisms are under investigation for possible use as biological control agents for boneseed. They are the boneseed leaf buckle mite (*Aceria* (Keifer) sp. (Acari: Eriophyidae)) (BLBM), the lacy-winged seed fly (*Mesoclanis magnipalpis* Bezzi (Diptera: Tephritidae)) (LWSF) and the rust fungus (*Endophyllum osteospermi* (Doidge) A.R.Wood) that induces witches' brooms. They are all endemic natural enemies of boneseed in South Africa and each has the potential to suppress boneseed vigour and/or seed production.

Boneseed Leaf Buckle mite

Characteristics

The BLBM (Figure 1) is vermiform (worm-like), about 0.15 mm long and its preferred host is boneseed (Morley 2004). The BLBM induces formation of leaf galls known as erinea. Erinea are composed of abnormally dense patches of hair-like structures that resemble felt. Initially erinea are white but turn brown within a few weeks. Erinea may develop anywhere on a leaf, have irregular shapes and size and may range from one to several hundred square millimetres. Erineum formation is induced by BLBM feeding at shoot meristems and commences before a leaf becomes visible. Erineum growth interrupts the normal expansion of affected leaves, resulting in leaf disfigurements ranging from small dimples to gross distortion of mature leaves. The BLBM feeds and breeds in erinea until overcrowding or erineum deterioration stimulates dispersal. Dispersive mites can walk to adjacent uncolonized shoot tips or can be wind-dispersed to other boneseed plants.

Introduction to Australia

Following host specificity testing in South Africa in 2002 that showed the BLBM would be safe to introduce to Australia (Morley 2004), the mite was imported into quarantine at the Department of Primary Industries – Frankston, Victoria in 2006 and was due for field release in Spring 2007. In collaboration with local land managers, community groups and researchers, releases are planned for core boneseed infestations in Victoria, South Australia and Tasmania and may be extended to New South Wales in the future. There are no plans to release in Western Australia as very few boneseed infestations are present and State law requires eradication of plants when found.

Chrysanthemoides seed flies

Three *Mesoclanis* spp. have been considered for biological control of boneseed and bitou bush in Australia (Edwards and Brown 1997; Neser and Morris 1985). These flies lay their eggs into *Chrysanthemoides* flowerheads and the larvae can destroy substantial proportions of developing ovules, thus suppressing seed production. When introduced to Australia in 1996, the bitou seed fly (*Mesoclanis polana* Munro) (BSF) rapidly colonized almost the entire range of bitou bush (Edwards *et al*. 1999). However, in South Africa the BSF does not utilize boneseed and prevails at latitudes much closer to the equator than the more southerly bitou bush infestations in Australia. The BSF was therefore considered unlikely to effectively suppress seed production of Australian boneseed or the more southerly bitou bush infestations, and this appears to be true (Robin Adair personal communication and Morley unpublished observations). Conversely, in South Africa the lacy-winged seed fly (LWSF) (Figure 2) utilizes a range of *C. monilifera* subspecies (Munro 1950, Edwards and Brown 1997) including boneseed, bitou bush (most prosperously at latitudes similar to southerly Australian bitou bush infestations (Edwards and Brown 1997)), subsp.

Figure 2. Lacy-winged seed fly *Mesoclanis magnipalpis*.

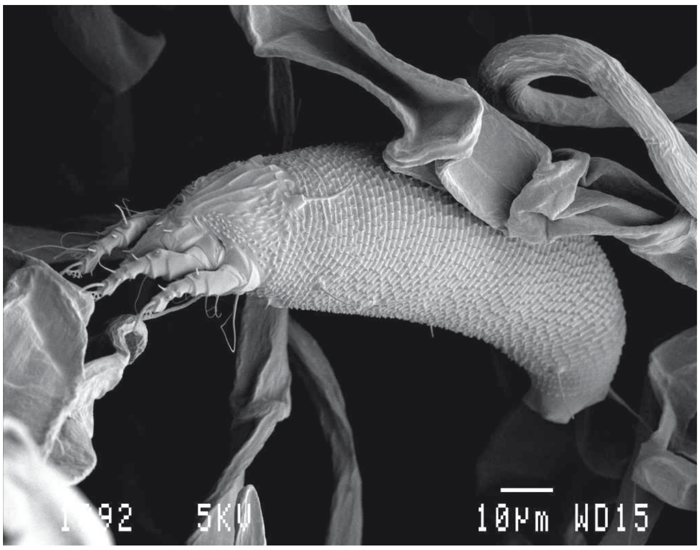

Figure 1. Boneseed leaf buckle mite *Aceria* sp. (Courtesy Charnie Craemer, Plant Protection Research Institute, Pretoria, and Alan Hall, University of Pretoria, South Africa).

pisifera (L.) Norlindh and *Chrysanthemoides incana* (Burm.f.) Norlindh. Thus the LWSF seemed a good candidate biological control agent.

Failure of LWSF to establish

Boneseed

Attempts to establish the LWSF in Australia have been in progress for nearly a decade. It was first released on boneseed in 1998 and also in 1999, 2000 and 2005. These efforts involved LWSF reared from imported *C. monilifera* subsp. *pisifera* fruit using a special AQIS-approved direct release protocol to ensure the released individuals were free of pests and diseases. Releases were made in the You Yangs, on the Mornington Peninsula and at Frankston, Victoria. The LWSF did not establish but on three occasions offspring were recovered in the same season as the release (Aline Bruzzese and Robin Adair personal communications, Morley unpublished data). This confirmed the apparent suitability of boneseed as a host for LWSF ex *C.m. pisifera*. The reason for establishment failure is not known but it appears that LWSF ex *C.m. pisifera* is not able to survive between the end of boneseed's fruiting season in early summer and the beginning of the next flowering season in late winter. In South Africa, LWSF ex *C.m. pisifera* might not be constrained in this way either because the interval between *C.m. pisifera* fruiting and flowering is sufficiently short or there are alternative *Chrysanthemoides* hosts whose flowerheads are suitable and available at critical times. Importation and release of the LWSF ex *C.m. monilifera* has been previously considered but not attempted largely because boneseed's relatively short flowering season combined with the logistic and mandatory delays of passage through Australian quarantine make this option difficult. Populations of LWSF on *C.m. monilifera* are also often difficult to find in South Africa (Robin Adair personal communication). Notwithstanding these difficulties, in light of the failure of LWSF ex *C.m.* subsp. *pisifera* to establish on boneseed in Australia it might now be worthwhile investigating the introduction of LWSF ex. *C.m. monilifera* in case it exists as a biotype specifically adapted to tolerate boneseed's flowerhead phenology.

Bitou bush

In 2005, the LWSF ex *C.m. pisifera* was also released on bitou bush on the New South Wales south coast. This was done with the view that LWSF could be redistributed from these release sites to boneseed infestations in Victoria, South Australia and Tasmania, in much larger numbers than it is practical to import, thereby perhaps enhancing the possibility of establishment on boneseed. However, the LWSF has not been recovered from bitou bush post-release (although sampling of release sites has yielded nearly 4000 BSF (Morley unpublished data)). The reason for this outcome is not known but the following speculations seem plausible:

1) LWSF is present but in such low numbers that it has not yet been detected. If this is the case then further sampling should eventually detect it.
2) There are distinct biotypes of LWSF in South Africa that have particular *Chrysanthemoides* preferences and LWSF ex *C.m. pisifera* is a biotype not suited to the bitou bush populations found in Australia. Intraspecific variation of *Mesoclanis* has not been studied but based on the experience described above and an observation by Munro (1950), biotypism seems a real possibility. Munro (1950) observed that flies of a collection of *M. magnipalpis* ex *C. incana* were 'larger than usual'. Importation of LWSF ex *C.m. rotundata* at a latitude comparable to Australia's southerly infestations (e.g. St Francis Bay, South Africa, where large populations of LWSF can be found (Edwards and Brown 1997)) would address the biotype theory. This could perhaps improve the chance of establishment on bitou bush and is the next most obvious strategy to try.
3) Competition with the BSF has prevented or impeded LWSF establishment. If this is the reason for failure of the LWSF to establish on bitou bush, then it might be overcome by releasing larger numbers and/or using other techniques to promote establishment (e.g. exclusion of BSF from bitou bush at release points).

Boneseed rust

The South African rust fungus *E. osteospermi* is a microcyclic species only recorded on a small group of related plants of the genera *Chrysanthemoides* and *Osteospermum* (Calenduleae: Asteraceae) in South Africa (Doidge 1926, Morris 1982, Wood 1998, Wood and Crous 2005b). It is thought to have considerable potential for the biological control of boneseed, and to a lesser extent bitou bush, in Australia (Morin 1997). Although the disease has been recorded on a few occasions on bitou bush in South Africa, this was only at the southernmost part of the plant's range (Wood and Crous 2005b). Consequently, while the rust may attack bitou bush in the southern part of its New South Wales range, should it be released in Australia, it is not expected to have a significant impact on most Australian bitou bush populations. Climate modelling has shown that the rust would likely have its greatest impact in Tasmania and southern Victoria (Wood *et al*. 2004).

Infection

E. osteospermi systemically infects its hosts via immature foliage and stems. After 1–2 years, infected plants develop witches' broom branches with multiple swollen stems, short internodes and smaller and slightly chlorotic leaves (Wood 2002, Wood and Crous 2005a) (Figure 3). The rust produces fruiting bodies on leaves of witches' brooms when conditions are conducive, generally in winter to late spring (Wood 2002).

Impacts

The rust has been shown to have a significant impact on boneseed populations in South Africa, reducing growth and reproduction and in some instances killing plants (Wood 2002, Wood and Crous 2005a). Deformed infected branches of diseased plants produced far fewer buds, flowers and fruits than branches of healthy plants and usually die within 1–4 years. The rust also has an indirect effect on the

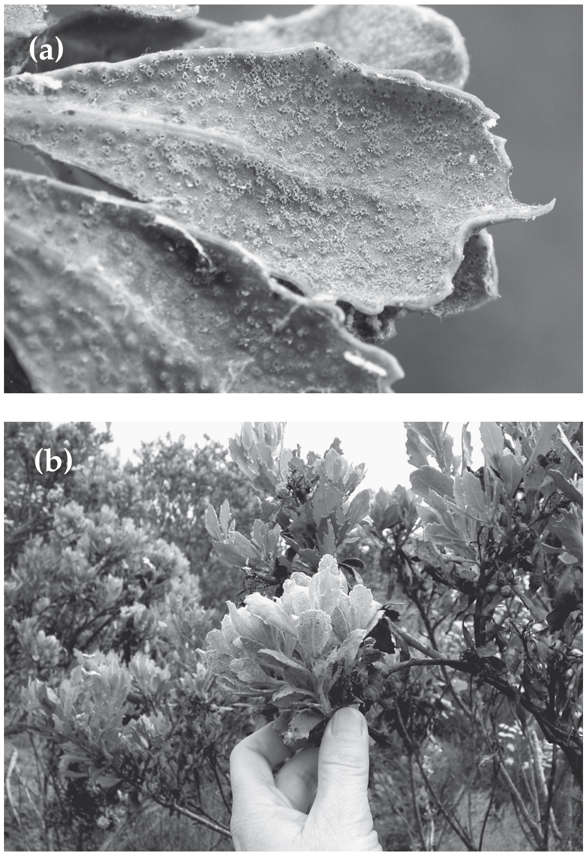

Figure 3. Boneseed rust, *Endophyllum osteospermi*; (a) fruiting bodies on leaves and (b) witches' brooms caused by the rust infection.

growth and reproduction of non-infected branches of diseased plants. Field measurements showed that the production of flowers and fruits on non-infected branches decreased as the severity of the disease on plants increased (Wood 2002, Wood and Crous 2005a). The systemic nature of *E. osteospermi* is a desirable characteristic for biological control purposes, as once the fungus is established within the host, the infection is retained until the death of infected branches (Morin 1997).

Risk assessment

Because of the nature of the rust, which develops visible symptoms only 1–2 years after infection of its host, an initial series of host-specificity tests were performed on detached leaves of test plant species. This was done to determine, using microscopy techniques, whether the rust was capable of penetrating epidermal cells of non-target plant species (as it successfully does on its host *C. monilifera*) (Wood 2006). Successful penetration was observed on boneseed and its close relative species tested within the Calenduleae tribe, but also on four other species outside the Calenduleae. Additional tests were carried out on leaves still attached to plants of some of the non-target plant species as well as the target weed species boneseed and bitou bush, to confirm accuracy of results obtained with detached leaves (Wood 2006). Since penetration of epidermal cells does not necessarily imply that the infection process will continue and be successful, more tests on whole plants of the species where penetration occurred in initial tests are currently underway to determine if the fungus is capable of colonizing tissue of these species. Results from these additional tests will provide the necessary information to fully assess the risk of significant impact on these non-target species should the rust be released in Australia.

If permission is granted to release *E. osteospermi* in Australia, an acceptable approach for its release from quarantine will have to be negotiated with AQIS and Biosecurity Australia. Because of the rust's long generation time, it will not be possible to maintain infected plants in quarantine until spores are produced and therefore an alternative strategy will be required (e.g. surface decontamination of infected plants before transfer from quarantine facility to ordinary glasshouse to provide optimal sporulation conditions; or direct release of spores collected in South Africa).

Summary

BLBM was due for release in Australia in Spring 2007. Further attempts to establish LWSF on: 1) bitou bush in Australia with collections ex *C.m. rotundata* from St Francis Bay, South Africa and 2) boneseed in Australia with collections ex *C.m. monilifera* in South Africa would be worthwhile. The boneseed rust has a significant impact on *C.m. monilifera* populations in South Africa. Substantial progress on risk assessment has been made and if approval for release is granted then an importation strategy that accommodates the rust's unusually long life cycle will need to be negotiated with AQIS and Biosecurity Australia.

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