
Biological Control of Mist Flower (*Ageratina riparia*, Asteraceae): Transferring a Successful Program from Hawai'i to New Zealand

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Abstract

Mist flower (*Ageratina riparia* (Regel) R. King and H. Robinson: Asteraceae) is an invasive weed that is widespread in tropical and warm temperate regions. It was introduced to New Zealand in the early 1930s and today is widespread in the North Island. Mist flower invades a range of habitats including native forests, where it can displace rare species and limit regeneration. New Zealand is undertaking a biological control program for mist flower based on 3 agents that were used successfully against the weed in Hawaii: a white smut fungus (*Entylooma ageratinae* Barreto and Evans), a gall fly (*Procecidochares alani* Steyskal), and a plume moth (*Oidaematophorus beneficus* Yano and Heppner). The white smut fungus and the gall fly were the most effective agents in Hawai'i, and so are the initial focus of the New Zealand program. The host specificity of these biological control agents was tested prior to their use in Hawai'i, Australia and South Africa, but additional tests, of plants significant in New Zealand, were conducted as part of this program. Results were consistent with previous findings: both agents are highly specific to mist flower, and are unlikely to damage any other plant species. The white smut fungus was released in New Zealand in November 1998 and has successfully produced secondary infections at all release sites. Permission to import and release the gall fly has been requested. A monitoring program has been established so that the impact of the biological control agents in the field can be determined.

Keywords: mist flower (*Ageratina riparia*), New Zealand, Hawai'i, white smut, gall fly.

Mist flower [*Ageratina riparia* (Regel) R. King and H. Robinson: Asteraceae] is a shrubby, perennial herb that grows to about 1m in height and produces large numbers of small, white flowers in spring. It is an aggressive, fast-growing, moderately shade-tolerant plant with seeds that are dispersed by wind and water. Mist flower is native to central America but has become a serious invasive weed in many tropical and warm temperate regions of the world. In 1925, the plant was introduced to Hawai'i, where it is known as Hamakua pamakani. By 1972 it had infested about 52,000 ha of range land (Morin *et al.*

1997). A highly successful biological control program against mist flower was implemented in Hawai'i using a white smut fungus (*Entyloma ageratinae* Barreto and Evans), a gall fly (*Procecidochares alani* Steyskal), and a plume moth (*Oidaematophorus beneficus* Yano and Heppner). These biological control agents were introduced in the mid 1970s and 10 years later, infested range land had returned to productive use (Trujillo 1985).

Mist flower was introduced to New Zealand in the early 1930s (Webb *et al.* 1988) and has since become a serious problem in a wide range of habitats such as forest margins, stream edges, pastures, and road sides, in the northern half of the North Island. The weed forms dense mats of semiwoody stems that smother indigenous vegetation, and limit regeneration (ARC 1999). Effective management using physical or chemical means would be difficult given the nature of the areas affected; some are prone to erosion and others are adjacent to waterways which can easily be contaminated through herbicide runoff. The option of using biological control was investigated.

Morin *et al.* (1997) studied the feasibility of transferring the successful biological control programme in Hawai'i to New Zealand. Climate data from sites in Hawai'i where mist flower was effectively biologically controlled were compared with climate data from areas in New Zealand where mist flower is a problem. This comparison revealed that annual rainfall in northern New Zealand should be sufficient to support the biological control agents and temperatures should be adequate for half the year, and ideal in summer. Consequently, Landcare Research decided to proceed with plans to import two biological control agents, the fungus and the gall fly, from Hawai'i. It was decided to delay further work on the plume moth until it is known if there are situations where the other agents will not perform well (Morin *et al.* 1997).

Entyloma ageratinae (Ustilaginales: Basidiomycotina) sporulates on the underside of green, living leaves 7-10 days after infecting a mist flower plant. The effected leaves quickly become brown and necrotic and the fungus, which has been reported to grow slowly in culture (Baretto and Evans 1988), appears to have both biotrophic and necrotrophic stages. Necrotic leaves fall prematurely and where climatic conditions are suitable, the fungus also causes dieback of shoots. Most plants at a site eventually become infected, leading to a decline in weed cover over wide areas. Larvae of the mist flower gall fly, *Procecidochares alani* (Diptera: Tephritidae), feed inside mist flower plants and cause the formation of stem galls. These galls retard stem elongation and reduce the competitive ability of the weed (Matayoshi 1979, cited in Morin *et al.* 1997).

Extensive host specificity tests were conducted on *E. ageratinae* and *P. alani* prior to their introduction to Hawai'i. After further tests were completed, *E. ageratinae* was released for the biological control of mist flower in South Africa and *P. alani* was released against the same weed in Australia (Morin *et al.* 1997). Consequently, 55 non-target plant species had been exposed to *E. ageratinae*, and 56 to *P. alani*, prior to the present study (Morin *et al.* 1997).

This paper reports the results of additional host specificity testing of *E. ageratinae* and *P. alani* conducted as a prerequisite to introducing these biological control agents to New Zealand. Also discussed are the release of the fungus and plans to monitor the impacts of both agents in New Zealand.

Materials and Methods

Host-specificity testing of *E. ageratinae* and *P. alani*.

Forty-seven plant species of significance in New Zealand were selected for host speci-

Table 1.
Plants of significance to New Zealand exposed experimentally to
E. ageratinae* and/or *P. alani

Family	Subfamily	Tribe	Species	a	b	c
Asteraceae	Asteroideae	Anthemideae	<i>Leptinella rotundata</i> (Cheeseman) D.G.Lloyd and C.J.Webb	†	†	
		Astereae	<i>Aster</i> sp. <i>Bellis perennis</i> L. <i>Celmisia coriacea</i> (G.Forst.) Hook.f. <i>Celmisia lindsayi</i> Hook.f. <i>Celmisia major</i> Cheeseman <i>Lagenifera lanata</i> A.Cunn. <i>Lagenifera petiolata</i> Hook.f. <i>Olearia paniculata</i> (J.R.Forst. and G.Forst.) Druce <i>Pachystegia insignis</i> (Hook.f.) Cheeseman <i>Vittadinia australis</i> A.Rich.	†	†	†
	Calenduleae	<i>Calendula officinalis</i> L.	†			
	Eupatorieae	<i>Ageratina adenophora</i> (Sprengel) R. King and H. Robinson <i>Ageratina altissima</i> (L.) Spach <i>Ageratina riparia</i> (Regel) R.King and H. Robinson <i>Ageratum</i> sp. <i>Eupatorium rugosum</i> Houtt. <i>Liatris</i> sp.	†	†	†	
	Heliantheae	<i>Bidens ferulaefolia</i> (Jacq.)DC. <i>Cosmos</i> sp. <i>Dahlia</i> sp.			†	
	Inuleae	<i>Anaphalis keriensis</i> (A.Cunn.) C.J.Webb <i>Anaphalis rupestris</i> C.J.Webb <i>Cassinia leptophylla</i> (G.Forst.)R.Br. <i>Craspedia uniflora</i> G. Forst. <i>Craspedia</i> sp. <i>Helichrysum</i> sp. <i>Pseudognaphalium luteoalbum</i> (L.) Hilliard and B.L. Burt	†	†	†	
	Senecioneae	<i>Brachyglottis repanda</i> J.R.Forst. and G.Forst <i>Dolichoglottis scorzonoides</i> (Hook.f.) B.Nord. <i>Senecio lautus</i> Willd.	†	†	†	
	Cichorioideae	Arctoteae	<i>Arctotis</i> sp. <i>Gazania</i> sp.			†
	Cardueae	<i>Centaurea</i> sp. <i>Cirsium japonicum</i> DC.	†	†	†	
	Echomopeae	<i>Echinops</i> sp.	†			
	Lactuceae	<i>Kirkianella novae-zelandiae</i> (Hook.f.) Allan <i>Sonchus kirkii</i> Hamlin	†	†	†	
	Ericaceae	<i>Dracophyllum acerosum</i> Berggr.			†	
	Onagraceae	<i>Fuchsia excorticata</i> (J.R.Forst. and G.Forst) L.f.			†	
	Pittosporaceae	<i>Pittosporum tenuifolium</i> Sol. ex Gaertn.	†	†		
	Ranunculaceae	<i>Clematis paniculata</i> J.F.Gmel.	†	†		
	Rosaceae	<i>Prunus</i> sp. <i>Rubus argutus</i> Link			†	
	Rubiaceae	<i>Coprosma robusta</i> Raoul			†	
	Rutaceae	<i>Citrus</i> sp.			†	
	Scrophulariaceae	<i>Hebe stricta</i> (Benth.) L.B.Moore in Allan	†	†		

^a Plant exposed to the gall fly in quarantine and containment in Hawai'i

^b Plant exposed to the gall fly in quarantine and containment in New Zealand

^c Plant exposed to the fungus in quarantine and containment in Hawai'i

ficity testing, using internationally recognised criteria (Wapshere 1989). Twenty-one species were exposed to the fungus alone, 12 to the insect alone, and 14 species were exposed to both agents (separately) (Table 1). Test plants included native plants in the Asteraceae, commercially grown members of the Asteraceae, and plants known to host fungi or insects that are closely related to the proposed biological control agents.

All host specificity testing of *E. ageratinae* was conducted at the Biological Control Facilities of the Hawai'i Department of Agriculture in Honolulu, Hawai'i. To establish a supply of inoculum, asexual spores (conidia) of the fungus were collected from diseased mist flower plants in the field and inoculated onto healthy mist flower plants in a glass house. Spores were collected by brushing the leaves with a fine sable brush that was repeatedly dipped into a small beaker of sterile distilled water. For host specificity tests, the concentration of spores in the beaker was adjusted to 1×10^5 spores per ml and the inoculum was sprayed or brushed onto both surfaces of the leaves of the test plants. After inoculation, plants were incubated at 17–23°C and 100% humidity for 48 h, then transferred to the glass house for a 60 day observation period. All test plants were closely examined for any signs of infection (leaf spots/sporulation) over a period of 30 days. In most cases, 4 plants of each species were used in each test, and the test was repeated for each species.

Host specificity testing of *P. alani* was conducted in Hawai'i and in New Zealand. In Hawai'i, a colony of *P. alani* was started using field-collected galls. Fly pupae were dissected from the galls and allowed to develop on moistened filter paper in uncovered Petri dishes, inside screened cages containing water and food (a slurry made up of one part protein hydrolysate, one part honey and three parts sugar). Emerging adults were collected and transferred to cages containing potted mist flower plants (grown from field-collected cuttings) for egg laying and larval development.

In Hawai'i, tests were conducted in an insectory under ambient temperature and a 12 h light/ 12 h dark cycle. Choice tests, with mist flower present, and no-choice tests, with mist flower plants absent, were both conducted simultaneously, using mesh-covered cages. For the choice tests, 1 mist flower plant was placed in a cage, along with 1 plant of each of 9 randomly selected and randomly positioned test plant species. For no-choice tests, 10 randomly selected test plants were placed in a 2nd cage. At the start of testing, 10 pairs of 2–3-day-old (adult) gall flies and 1 mist flower plant were added to each of the 2 cages, and water and food were provided for the insects. After 24 h, the mist flower plants were removed and replaced with a full complement of test plants, with a control in 1 cage (choice test) and without a control in the other cage (no-choice test). These were in turn replaced by new plants (with or without a control) the following day. This procedure was repeated on a daily basis, with the flies remaining in their respective cages for the duration of the test, until more than half the flies had died. Each test plant was exposed to *P. alani* in 5 tests, with and without controls. Choice and no-choice tests were alternated between cages.

All plants were then examined with a hand lens for the presence of eggs. The plants were then placed into outdoor cages and monitored for signs of egg hatching and/or gall formation. Stems were dissected if the presence of galls was suspected. The number of eggs laid, the number of these which hatched, and the number of galls formed were recorded.

Host specificity testing of *P. alani* in New Zealand was conducted in a room operating at 23°C with a 16 h light/ 8 h dark cycle. Two shipments of mist flower galls were

obtained from the Hawai'i Department of Agriculture and gall flies that emerged were kept secure in "receiving" cages, in a different quarantine room from that used for the host specificity tests.

As in Hawai'i, both choice and no-choice tests were conducted (although not simultaneously) using mesh-covered cages. For the choice tests, 2 mist flower plants were placed in each of 5 cages, along with 1 of each of the 9 plant species to be tested. Nine pairs of 2–3-day-old gall flies were added, and the flies remained in their respective cages for the duration of the test. Twenty days after the last pairs of flies were added to each cage, by which time all the adult flies were dead, all plants were examined with a hand lens, and a stereo microscope, for the presence of eggs or empty egg chorion. The number of galls on each plant was recorded. The same methodology was used for no-choice tests, except that only the 9 test plant species were placed in each of the 5 test cages and 10 pairs of 2–3-day-old gall flies were added to each of the cages. Also, a 6th cage, containing only two mist flower plants, was used as a control cage.

Introducing and monitoring the biological control agents for mist flower

Permission to import and release *E. ageratinae* was granted by the New Zealand Ministry of Agriculture, and the fungus was shipped from Hawai'i to New Zealand on 5 October 1998, on inoculated, detached mist flower leaves. Nine mist flower infestations had been selected as release sites and the pathogen was subsequently released at these sites towards the end of 1998. Two methods were used to release the fungus. Either a suspension of conidia in water (ca. 2×10^5 conidia/ml) was painted onto several mist flower plants at the site, or 3 infected mist flower plants from the glasshouse were transplanted to the site.

Release sites were visited 4–6 weeks later, to assess establishment and local spread of the fungus. Inoculated mist flower plants, the mist flower plants beside them, and mist flower plants within a 10–15m radius were searched for symptoms characteristic of infection by *E. ageratinae*. Where symptoms were found, a sample of the affected leaves was collected and returned to the laboratory where they were examined under higher magnification. The presence of *E. ageratinae* was considered confirmed only if characteristic conidia were observed using a compound microscope (up to 400 \times magnification). Leaves with characteristic lesions, but without spores, were incubated in a moist chamber and re-examined periodically until either *E. ageratinae* spores were observed, or the tissue was completely brown. Four of the release sites were re-examined, using the same methodology, 3–7 months after release, and as many as possible will be visited again 1 y after release.

In order to monitor the impact of *E. ageratinae* and *P. alani* on mist flower, a quantitative survey of the weed was undertaken in a Regional Parkland (Waitakere Ranges RP) near Auckland. The methodology for this survey, and results of the base-line survey conducted prior to the release of *E. ageratinae* in the area, are reported by Fröhlich *et al.* (1999).

Results

Host specificity testing of *E. ageratinae* and *P. alani*.

Of the 35 plant species that were exposed experimentally to *E. ageratinae* (Table 1) only mist flower and Mexican devil weed (*Ageratina adenophora* (Sprengel) R. King and H. Robinson) developed disease symptoms. Symptoms on Mexican devil weed were lim-

ited to small (<2 mm) lesions and sporulation did not occur.

The gall fly laid a single egg on one of the 25 non-target plant species tested (*Eupatorium rugosum* Houtt.) but did not form galls on any plant except mist flower. Mist flower plants of both Hawai'ian, and New Zealand origin were highly susceptible to both *E. ageratinae* and *P. alani* in all tests.

Monitoring the Impact of the Biological Control Agents for Mist Flower.

Four to 8 weeks after its release, spores of *E. ageratinae*, and lesions typical of infection, were found on inoculated plants and neighbouring plants at all nine release sites. At eight of the sites, spores of the smut were not yet evident on plants further than 0.2 m away from the inoculated plants, but at one site spores were found up to 10 m from the inoculated plants.

Four of the 9 sites were monitored again 3–7 mo after release. Characteristic symptoms and spores of *E. ageratinae* could not be found at 1 site, but were present at the other 3. At 2 sites near Auckland, on Waiheke Island and in the Waitakere Ranges Regional Parkland, mist flower plants within a 5 m radius of inoculated plants were found to be suffering severe disease symptoms, with up to 50% necrotic tissue. Lesions and spores of *E. ageratinae* were found on mist flower leaves up to 12 m from inoculated plants at the Waiheke Island site and up to 450 m from inoculated plants at the Waitakere Ranges RP site.

Discussion

The results of host specificity tests of *E. ageratinae* conducted for this study agree with the findings of similar tests performed prior to its release in Hawai'i and South Africa. The only symptoms of infection by the fungus on a non-target plant in tests conducted prior to this study were small (<1 mm) lesions on Mexican devil weed (Morris 1991), and this was also the only species which showed symptoms in our tests. Mexican devil weed is the only other species of *Ageratina* naturalised in New Zealand, and as its name suggests, it is also an aggressive weed (Webb *et al.* 1988). The smut fungus could not complete its life cycle on Mexican devil weed in our tests and has not been recorded from any plant other than mist flower in the field in Hawai'i, or South Africa (M.J. Morris and E.K. unpublished data). Thus, *E. ageratinae* is likely to be restricted to mist flower in the field and poses no risk to non-target plants in New Zealand.

Fifty-six plant species were exposed to *P. alani* in tests conducted prior to its release in Hawai'i and Australia. The insect only laid eggs on mist flower, and on Mexican devil weed in choice tests (Morin *et al.* 1997). The eggs on Mexican devil weed hatched, but the resulting larvae did not develop and no galls were formed (Morin *et al.* 1997). The gall fly was also unable to form galls on any of the 25 additional plant species tested as part of this study, although it laid a single egg on *Eupatorium rugosum*. Since it is through gall formation that *P. alani* inhibits the growth of mist flower, the insect is unlikely to have an adverse impact on non-target plants in New Zealand.

At all 9 sites where *E. ageratinae* was released in New Zealand the fungus was found to have established and caused secondary infections within 4–6 wk. Within 7 mo, it has caused substantial tissue death on mist flower plants at at least two sites. This rapid transfer of the pathogen between plants was not unexpected as *E. ageratinae* has a short life cycle and its wind dispersed spores readily germinate under optimum conditions (Morin *et al.* 1997). Similar results were recorded in Hawai'i where secondary infections were

observed at all release sites 20 days after inoculation (Trujillo 1985).

While it is too soon to determine the likely impact of *E. ageratinae* on mist flower in New Zealand, the ease of establishment and early signs of spread of the fungus at most sites are very promising. If permission is granted to import the gall fly, and it also establishes, then there is a good chance that the successful control of mist flower achieved in Hawai'i will be repeated in New Zealand.

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