

BIOLOGICAL CONTROL OF MIST FLOWER (*AGERATINA RIPARIA*, ASTERACEAE) IN NEW ZEALAND

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ABSTRACT

Mist flower (*Ageratina riparia*) is an invasive weed of natural, rural and urban areas in the North Island of New Zealand. A biological control programme is underway using two natural enemies of mist flower that successfully controlled the weed in Hawai'i: a white smut fungus (*Entyloma ageratinae*) and a gall fly (*Procecidochares alani*). Host range tests conducted overseas, and as part of this study, have shown both agents are highly specific to mist flower. The fungus was released in New Zealand in November 1998. Permanent plots have been established so that the impact of the biocontrol agents on the target weed can be monitored.

Keywords: Mist flower, biological control, pathogen, gall fly.

INTRODUCTION

Mist flower (*Ageratina riparia*: Asteraceae) is a shrubby, perennial herb that grows 0.3-2 m tall 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. The plant was introduced to Hawai'i in 1925 and by 1972 it infested about 52 000 ha of rangeland (Morin *et al.* 1997) making it unusable for farming (E. Killgore, pers. obs.). A highly successful biological control programme was implemented in Hawai'i. A white smut fungus (*Entyloma ageratinae*), a plume moth (*Oidaematophorus beneficus*) and a gall fly (*Procecidochares alani*) were introduced in the mid 1970s and ten years later, the infested range land in Hawai'i 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 (e.g., forest margins, stream edges, pastures and road sides) in both urban and rural environments across the northern half of the North Island. The weed forms dense mats of semi-woody stems that smother and displace indigenous vegetation and limit regeneration (Anon. 1999).

Analysis of the climate at Hawai'ian sites where mist flower was effectively controlled revealed that annual rainfall in northern New Zealand should be sufficient to support high activity of both agents (Morin *et al.* 1997). Temperatures should be adequate for half the year, and ideal in summer (Morin *et al.* 1997). It was decided to proceed with host range testing of the fungus and the gall fly, but to delay further work on the plume moth until it is known if there are situations where the other agents would not perform well (Morin *et al.* 1997).

Entyloma ageratinae (Ustilaginales: Basidiomycotina) is a biotrophic fungus that requires living plant material to grow and sporulate. In Hawai'i, mist flower plants that are heavily attacked by the fungus suffer severe necrosis of leaves, which as a result, fall prematurely. Where climatic conditions are suitable, the fungus also causes die-back of shoots and most plants at a site eventually become infected, leading to decline in weed cover over wide areas (Hill *et al.* 1997). Larvae of the mist flower gall fly, *Procecidochares alani* (Diptera: Tephritidae), feed inside mist flower plants thus causing the formation of stem galls. These galls retard stem elongation and reduce the weed's competitive ability (Matayoshi 1978; 1979, cited in Morin *et al.* 1997).

Following the success of the Hawai'ian biocontrol programme, the white smut fungus was released for the biological control of mist flower in South Africa and the mist flower gall fly was released against the same weed in Australia (Morin *et al.* 1997).

This paper reports the results of host range testing of *E. ageratinae* and *P. alani* conducted prior to their release overseas and, as part of this study, prior to their release in New Zealand. Also discussed are the release of the fungus in New Zealand and plans to monitor the impacts of both agents.

METHODS

Experimental determination of the host ranges of *E. ageratinae* and *P. alani*

Before a biological control agent is released, it is necessary to determine if it is likely to attack plants other than the target weed(s). Potential agents are tested against a representative range of plant species that are selected using internationally recognised criteria (Wapshere 1974). Fifty-five non-target plant species had been exposed to *E. ageratinae*, and 56 to *P. alani*, in host range tests conducted prior to this study (Morin *et al.* 1997).

Forty-seven additional plant species, of significance in New Zealand, were selected for host range testing as part of this study (21 were exposed to the fungus alone, 12 to the insect alone and 14 species were exposed to both, see Table 1). Representative plants for testing were chosen from: New Zealand native plants, especially in the Asteraceae; ornamental daisies (Asteraceae) grown in New Zealand; and plants known to host fungi or insects that are closely related to the proposed biocontrol agents. Most host range testing for this study was completed in a quarantine and containment facility in Hawai'i (Table 1). Test plants that were not obtainable in Hawai'i were mainly shipped from New Zealand as seeds or bare-rooted plants. Cuttings were sent of three plant species that needed to be exposed to the fungus but could not be re-established in Hawai'i (Table 1). Nine other species, that could not be induced to grow in Hawai'i, were exposed to the gall fly in an invertebrate quarantine and containment facility in New Zealand (Table 1).

Introducing and monitoring the biological control agents for mist flower

Permission to import and release *E. ageratinae* was granted by the Ministry of Agriculture, and the fungus arrived in New Zealand for the first time on 5 October 1998. Nine mist flower infestations were chosen as appropriate release sites for the fungus and the smut was subsequently distributed to these sites towards the end of 1998 (Table 2). 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 within 1 m of each other in the field, or three mist flower plants were infected with the fungus in the glasshouse, transferred to the field, and planted within 1 m of each other (Table 2).

All nine sites where *E. ageratinae* was released were visited 4-6 weeks later, to assess the establishment and local spread of the agent. The inoculated mist flower plants, the plants beside them, and the plants within 10-15m of them, were searched (each group searched by 1-3 people for 10-15 minutes, using a hand lens) for lesions characteristic of infection by *E. ageratinae*. Where characteristic lesions were found, a sample of the affected leaves was collected, placed in a plastic bag that was labelled with its approximate distance from the release point, and returned to the laboratory.

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

Family	Subfamily	Tribe	Subtribe	
Asteraceae	Asteroideae	Anthemideae Astereae	<i>Leptinella rotundata</i> ^{a c}	
			<i>Aster</i> sp. ^c	
			<i>Bellis perennis</i> ^{a c}	
			<i>Celmisia coriacea</i> ^c	
			<i>Celmisia lindsayi</i> ^c	
			<i>Celmisia major</i> ^b	
			<i>Lagenifera lanata</i> ^b	
			<i>Lagenifera petiolata</i> ^c	
			<i>Olearia paniculata</i> ^{a c}	
			<i>Pachystegia insignis</i> ^b	
			<i>Vittadinia australis</i> ^b	
			Calenduleae	<i>Calendula officinalis</i> ^a
				Eupatorieae
			<i>Ageratina altissima</i> ^c	
		<i>Ageratina riparia</i> ^{a b c}		
		<i>Ageratum</i> sp. ^{a c}		
		<i>Eupatorium rugosum</i> ^a		
		<i>Liatris</i> sp. ^a		
		Heliantheae	<i>Bidens ferulaefolia</i> ^c	
			<i>Cosmos</i> sp. ^c	
			<i>Dahlia</i> sp. ^c	
		Inuleae	<i>Anaphalis keriensis</i> ^{b c}	
			<i>Anaphalis rupestris</i> ^c	
			<i>Cassinia leptophylla</i> ^{b c}	
			<i>Craspedia uniflora</i> ^c	
			<i>Craspedia</i> sp. ^b	
			<i>Helichrysum</i> sp. ^c	
		Senecioneae	<i>Pseudognaphalium luteoalbum</i> ^c	
			<i>Brachyglottis repanda</i> ^{b c}	
			<i>Dolichoglottis scorzonerooides</i> ^c	
		Cichorioideae	Arctoteae	<i>Senecio lautus</i> ^{a c}
				<i>Arctotis</i> sp. ^b
			Cardueae	<i>Gazania</i> sp. ^a
<i>Centaurea</i> sp. ^{a c}				
Echomopeae	<i>Cirsium japonicum</i> ^c			
	<i>Echinops</i> sp. ^a			
Lactuceae	<i>Kirkianella novae-zelandiae</i> ^a			
	<i>Sonchus kirkii</i> ^{a c}			
Ericaceae			<i>Dracophyllum acerosum</i> ^c	
Onagraceae			<i>Fuchsia excorticata</i> ^c	
Pittosporaceae		<i>Pittosporum tenuifolium</i> ^{a c}		
Ranunculaceae		<i>Clematis paniculata</i> ^{a c}		
Rosaceae		<i>Prunus</i> sp. ^c		
		<i>Rubus argutus</i> ^c		
Rubiaceae		<i>Coprosma robusta</i> ^c		
Rutaceae		<i>Citrus</i> sp. ^c		
Scrophulariaceae		<i>Hebe stricta</i> ^{a c}		

^aPlant exposed to the gall fly in quarantine and containment in Hawai'i^bPlant exposed to the gall fly in quarantine and containment in New Zealand^cPlant exposed to the fungus in quarantine and containment in Hawai'i

In the laboratory, all of the collected leaves were examined under a stereo microscope with zoom lens (up to 60x magnification) within 24 hrs of collection. Any white spore-masses visible were transferred to a microscope slide, and the presence of *E. ageratinae* was only considered confirmed if characteristic conidia were observed using a compound microscope (up to 400x magnification). Leaves with characteristic lesions, but without white spore masses, were incubated on moist filter paper in sealed petri dishes at 18°C and re-examined periodically until either *E. ageratinae* spores were observed, or the tissue was completely brown. The release sites will be re-examined approximately 6 months and 1 year after release. If *P. alani* is released, a similar monitoring regime is likely.

In order to monitor the impact of *E. ageratinae* and *P. alani* on mist flower, a quantitative survey of the weed was undertaken in the Waitakere Ranges Regional Park. In October and November of 1998, 10 plots (each 500 m²) were established along 11 walking tracks within the park (total 110 plots). Each plot comprised a 50m length of track and the land within 5m of either side (unless visibility was blocked on one side, in which case the plot was shifted to encompass a proportionately larger area on the other side). Tracks were chosen randomly (except that inaccessible, and/or very long tracks were excluded) and the centre of each plot on a given track was located exactly 500 m from the centre of the former plot. This was achieved in the field using a calibrated counter that measures the amount of cotton that unravels from a "hip chain" as one walks (the loose end of the cotton is fastened at the starting point). The amount of mist flower present in each plot was determined by estimating the area (m²) of ground covered by the species. The mist flower cover, and the abundance of the two biocontrol agents, in these plots will be reassessed at yearly intervals.

In order to determine the distribution of mist flower on a regional scale, a questionnaire was devised asking for reports of weed infestations anywhere in New Zealand. Mist flower sites reported in returned surveys will be added to a database of previously recorded sites and this data will be used to draw a distribution map of the weed. Recipients were also asked to comment on weed density, and the habitat around the infestation. This questionnaire was posted to over 200 people throughout New Zealand (with emphasis on the North Island) in November 1998, and similar surveys in the future should allow changes in the weed's distribution to be monitored.

RESULTS

Experimental determination of the host ranges of *E. ageratinae* and *P. alani*

Results of host range tests conducted prior to this study, and prior to the release of *E. ageratinae* in Hawai'i and South Africa, and/or the release of *P. alani* in Hawai'i and Australia, showed that both agents were highly host specific (Morin *et al.* 1997). The only symptoms of infection by the fungus on a non-target plant were small (<1 mm) lesions on Mexican devil weed (*Ageratina adenophora*) (Morris 1989 cited in Morin *et al.* 1997). The only plant (apart from mist flower) that the gall fly laid eggs on in these tests was also Mexican devil weed (in choice tests) (Morin *et al.* 1997). The eggs on Devil weed hatched, but the resulting larvae did not develop and no galls were formed (Morin *et al.* 1997).

None of the 34 non-host species that were exposed experimentally to *E. ageratinae* as part of this programme (Table 1) developed any symptoms of infection except Mexican devil weed (*Ageratina adenophora*). Symptoms on Mexican devil weed were limited to small (<2 mm) lesions and sporulation did not occur.

P. alani only laid one egg on one (*Eupatorium rugosum*) of the 25 non-target plant species in our tests and was unable to form galls on anything but mist flower. Mist flower plants (of both Hawai'ian, and New Zealand origin) exposed to *E. ageratinae* and *P. alani* were found to be highly susceptible to both agents in our tests, and in those done previously (Morin *et al.* 1997).

Monitoring the impact of the biological control agents for mist flower

Spores of *E. ageratinae*, and lesions typical of infection, were found on inoculated plants, and neighbouring plants, at all nine sites where the fungus was released (Table 2). At eight of the New Zealand sites smut spores were not yet evident on plants further

than 200 mm away from the inoculated plants. At one site however, spores were found on leaves collected 3 - 10 m from the inoculated plants in the field.

TABLE 2: Release and subsequent spread of *E. ageratinae* at nine sites in the North Island of New Zealand.

Site No.	Location	Region	Date of Release	Date of 1st monitoring	Maximum distance spores found beyond inoculated plants (m)
1	Mt. Eden ¹	Auckland	19/11/98	17/12/98	0.2
2	Waitakere Ranges ¹	Auckland	20/11/98	18/12/98	0.2
3	Waiheke Island ¹	Auckland	21/11/98	21/12/98	0.1
4	Puriri ²	Waikato	27/11/98	21/1/99	0.2
5	Omapere ²	Northland	9/12/98	22/1/99	0.1
6	Puketi ²	Northland	9/12/98	22/1/99	3-10
7	Ngunguru ¹	Northland	9/12/98	22/1/99	0.1
8	Paeroa ¹	Waikato	11/12/98	21/1/99	0.2
9	Brookby ²	Auckland	23/12/98	20/1/99	0.1

¹Plants inoculated in the field

²Plant inoculated in the glasshouse and transferred to the field

Of the 110 plots examined in the pre-release survey of the Waitakere Ranges, 20 (18.2%) were found to contain mist flower. The mist flower cover (in m²) in these 20 plots was: 0.5, 0.5, 1, 1, 1.5, 2, 2, 4, 5, 5, 6, 15, 16, 23, 25, 31, 38, 62, 90 and 180. The plots containing mist flower were found along seven of the 11 tracks surveyed (63.6%). There are 143 named walking tracks in the Waitakere Ranges Regional Park, and these have a combined length of ca. 258 km. A total of 508.5 m² of mist flower was found in the 54 300 m² within survey plots, indicating that the weed covered 0.94% of the area surveyed. If this mist flower coverage near surveyed tracks is typical of the whole park, then there are approximately 24 156 m² of mist flower within the 5 m either side of tracks in the whole park (a total area of 2 579 500 m²).

While a map of the distribution of mist flower in the North Island has not yet been completed, it is already clear that the weed is widespread in the Auckland and Northland regions. Infestations have been recorded as far north as Mangonui and the Cavalli Islands, and as far south as Raglan Harbour, west of Hamilton. Many outlying islands are infested including the Poor Knights, Little, and Great Barrier Islands.

DISCUSSION AND CONCLUSIONS

Of the 89 plant species from 34 plant families that have now been tested for susceptibility to *E. ageratinae*, only mist flower and, to a lesser extent, the Mexican devil weed, have developed disease symptoms (Morris 1991; Trujillo 1985). Mexican devil weed is the only species of *Ageratina* naturalised in New Zealand apart from mist flower, 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 laboratory tests and has not been recorded from Mexican devil weed in the field in Hawai'i, or South Africa, even where the plant grows beside infected mist flower (Hill *et al.* 1997). Thus, *E. ageratinae* is likely to be restricted to mist flower in the field and poses no threat to non-target plants in New Zealand.

In studies of the host range of *P. alani* conducted prior to its release in Hawai'i and Australia, the insect did not lay eggs on any of the 56 test plant species apart from Mexican devil weed (when it was in the same cage as mist flower) and did not form galls on any non-target species (Morin *et al.* 1997). The mist flower gall fly was also

unable to form galls on any of the 25 additional plant species tested as part of this study, and so can be considered highly host specific to *A. riparia*.

At all nine sites where *E. ageratinae* was released in New Zealand the fungus was found to have established and caused secondary infections within 4 - 6 weeks. 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 are very promising. If the gall fly is also able to establish and reproduce, 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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REFERENCES

- Anonymous, 1999. Mistflower *Ageratina riparia*. Auckland Regional Council Pest Facts Sheet No. 46. Auckland.
- Hapai, M.N., 1977. The biology and ecology of the hamakua pamakani gall fly, *Procecidochares alani* (Steyskal). Unpublished MSc thesis, University of Hawai'i, Honolulu, USA. 77 pp.
- Hill, R. L., Killgore, E. M., Sugiyama, L. S., Morin, L. and Fröhlich, J., 1997. Introduction of *Entyloma ageratinae* for Biological control of Mist Flower *Ageratina riparia*. Importation Impact Assessment (unpublished) LC9798/66. Auckland. 28p.
- Morin, L., Hill, R. L. and Matayoshi, S., 1997. Hawai'i's successful biological control strategy for mist flower (*Ageratina riparia*) - Can it be transferred to New Zealand? *Biocontrol News and Information* 18(3), 77N-88N.
- Morris, M. J., 1989. Report on a leaf pathogen, *Entyloma ageratinae*, as a biological control agent for the weed, *Ageratina riparia*, in South Africa. Plant Protection Research Institute report (unpublished) Stellenbosch, South Africa. 14 p.
- Morris, M. J., 1991. The use of plant pathogens for biological weed control in South Africa. *Agric., Ecosyst. Environ.* 37: 239-255.
- Trujillo, E. E., 1985. Biological control of Hamakua Pa-Makani with *Cercospora* sp. in Hawai'i. Pp. 661-671. In: Proceedings of VI International Symposium on Biological Control of Weeds. E.S. Delfosse (Ed.). 1984, Vancouver, Canada.
- Wapshere, A., 1974. A protocol for programmes for biological control of weeds. *Annals Applied Biol.* 77: 201-211.
- Webb, C.J., Sykes, W.R. and Garnock-Jones, P.J., 1988. Naturalised Pteridophytes, Gymnosperms, Dicotyledons. Christchurch, Botany Division, D.S.I.R. 1365 p.