Spread, specificity and initial impact of the white-smut fungus *Entyloma ageratinae* on mistflower in Australia

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Summary Mistflower (*Ageratina riparia*) is primarily an environmental weed that invades wet habitats in eastern Australia. On 21 October 2010, the white-smut fungus *Entyloma ageratinae*, previously introduced to Hawaii, South Africa and New Zealand for the biological control of mistflower, was found near Lamington National Park, Queensland. Field surveys confirmed that the fungus was widespread in south east Queensland and north coast NSW and present in the Coffs Harbour region, mid-north Coast NSW. It was not found further south in NSW. Host-specificity testing of the fungus on plant species closely related to mistflower that had not been tested before, including the two Australian native *Adenostemma* spp., was undertaken. Out of the 16 non-target plant species tested so far, only crofton weed (*Ageratina adenophora*), which belongs to the same genus as mistflower, developed some disease symptoms, albeit to a much lesser extent. Monitoring transects were established at eight sites in NSW and three in Queensland, and baseline vegetation data were collected to enable quantitative assessment of the impact of the fungus in the future. A series of strategic releases of the fungus to non-infected mistflower sites in NSW (Blue Mountains, South Coast and Barrington Tops regions) were made in May 2011. Major defoliation of mistflower was observed 5–6 months later at the release sites. Visits to other mistflower-infested sites in these regions revealed that the fungus was spreading rapidly and causing severe defoliation.

Keywords Biological control, plant pathogen, smut, environmental weed, impact.

INTRODUCTION

Mistflower (*Ageratina riparia* (Regel) R.M.King & H.Rob.: Asteraceae) is a perennial herbaceous alien plant that invades wet habitats, particularly riparian areas and moist cliff faces. In subtropical habitats it grows through most of the year, setting abundant white composite flowers in late-winter. It is currently present in eastern Australia from Nowra, NSW to Cairns, Queensland. In Australia and elsewhere, it is primarily a problem in mid-high elevation rainforest areas where it creates a canopy over headwater streams and displaces native riparian plant species (Barton *et al.* 2007). It can also be a problem in wet meadows where it reduces forage quality for livestock (Trujillo 2005).

Current management is restricted to hand-pulling and herbicides, which can have adverse effects on non-target species. Physical removal is labour intensive and mistflower often infests areas that are difficult to access (headwater riparian corridors and cliff faces). Herbicide application is increasingly expensive, often destroys desirable vegetation along with mistflower (creating disturbed habitat for mistflower seed germination), and is known to harm fish and amphibian populations.

Major mistflower infestations in Hawaii were controlled by a fungal pathogen, the white-smut fungus *Entyloma ageratina*, imported from Jamaica, in the 1970s (Trujillo 2005). More recently, the same fungus was introduced to South Africa (in 1989) and New Zealand (in 1998) (Morin *et al.* 1997, Barton *et al.* 2007). A field study in New Zealand demonstrated effective control of mistflower by the fungus and the subsequent increase in plant diversity (Barton *et al.* 2007). On 21 October 2010, the mistflower white-smut fungus was found near Lamington National Park, Queensland (Schooler *et al.* 2012). The pathway of introduction is unknown.

This paper reports on research performed since the fungus was recorded in Australia. It includes:

1) delimitation surveys in the months following the first report of the fungus
2) host-specificity testing of species not investigated in previous research overseas
3) the establishment of monitoring plots in NSW and Queensland and collection of baseline plant community data
4) releases of the fungus in areas where it was not present.

MATERIALS AND METHODS

Surveys Field surveys in south east Queensland and NSW were conducted from October 2010 to July 2011. Up to 275 haphazardly selected mistflower

stems were examined per site for signs of infection by the fungus. An identification guide for the disease symptoms caused by the fungus was also produced and sent to 30 land managers in regions where mistflower is present in NSW and Queensland, asking them to circulate widely and to report back if they saw any signs of the fungus in their localities.

Host-specificity tests Seeds or plants of 16 species in the Eupatoriae tribe (to which mistflower belongs), including the two Australian native *Adenostemma* spp., were obtained and grown in the glasshouse. Each species was tested in two separate trials. Plants (four or five replicates per species) were sprayed with a suspension of (1×10^5) spores per ml of a solution of 0.05% Tween 80 in water using a hand-held sprayer. Mistflower plants were inoculated as a positive control. Inoculated plants were misted with water and placed in boxes enclosed in large transparent plastic bags in a controlled-environment room (at 20°C, 12 hour photoperiod) for 48 hours. Plants were then removed from the boxes and placed on the bench of the controlled-environment room (conditions as above). Three and five weeks after inoculation, all plants were examined individually for disease symptoms.

Monitoring sites Eight sites in NSW and three in Queensland located across mistflower range and not at risk of flooding were selected. At each of the sites, four permanent 10 m long transects were established through dense mistflower stands. Fifty stems were haphazardly selected along each transect and assessed for presence of the fungus. Percentage cover of mistflower and other species was visually estimated in ten 1 m^2 plots along one side of each transect. The dry weight of mistflower above-ground biomass from a 0.25 m^2 plot within 4 m of each end of each transect was also measured.

Releases The fungus was released in the field in the areas where it was not present in NSW by placing 3–10 infected, potted mistflower plants with sporulating lesions among the natural mistflower infestation at a site. Sites were revisited 5–6 months after the release to assess the fungus establishment.

RESULTS

Surveys Field surveys confirmed that the fungus was present at 69 sites in south east Queensland plus north and mid-north coast, NSW (Figure 1). It was not found at the 34 sites surveyed further south in NSW. It was also not found at sites surveyed in Carnarvon Gorge and Atherton, Queensland (not shown on map).

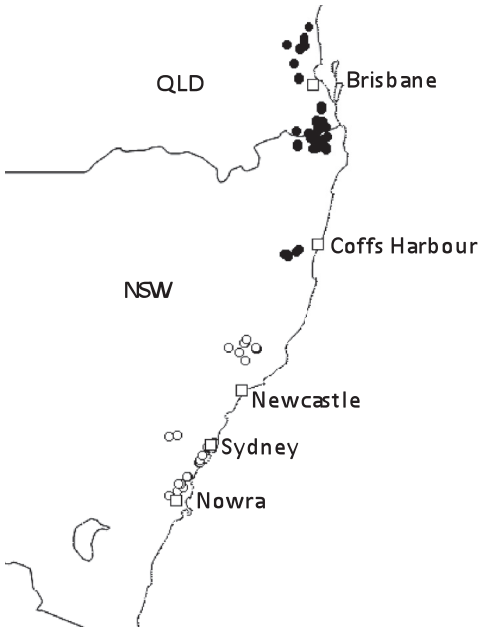

Figure 1. Surveyed locations where the fungus was found on mistflower (full circle) and where it was absent (open circle) prior to deliberate releases undertaken.

Host-specificity tests Out of the 16 non-target plant species tested so far, only crofton weed (*Ageratina adenophora* (Spreng.) R.M.King & H.Rob.), which belongs to the same genus of istflower, developed some disease symptoms, albeit to a much lesser extent than on mistflower. Spores were produced only on a few of the lesions. All other species, including the two Australian native species, *Adenostemma lavenia* (L.) Kuntze and *Adenostemma macrophyllum* (Blume) DC. Candolle, were found to be immune to the disease.

Monitoring sites Baseline plant community data were collected at the monitoring sites between December 2010 and July 2011. Overall, there was a negative relationship between the percentage cover of mistflower and that of other plant species cover (Figure 2). Thirty-one to one hundred percent of stems were infected by the fungus at sites where it was already present at the time of the assessment.

Releases Eight strategic releases of the fungus to non-infected mistflower sites in NSW were made in May 2011. These included the five monitoring sites on the central and south coast NSW, where the fungus was not present at the time baseline plant community data

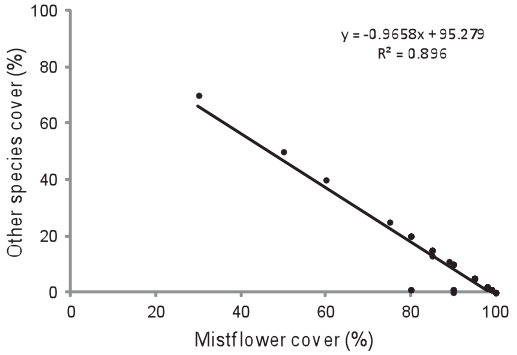

Figure 2. Example of baseline data collected at one of the monitoring sites (Darkwood, NSW).

were collected. Five to six months later, major defoliation of mistflower caused by the fungus was observed at all release sites revisited on the NSW south coast. Visit of other mistflower-infested sites in the region revealed that the fungus was already widespread and causing severe damage.

DISCUSSION

The widespread distribution of the white-smut fungus, extending from south east Queensland to the mid-north coast NSW, observed in our surveys was unexpected. This indicated that the fungus had possibly been in Australia for a while prior to the first record in Lamington National Park in October 2011 and had had the opportunity to naturally spread to other mistflower-infected sites. Nonetheless, we cannot rule out the possibility of human-mediated movement of infected material across this wide region soon after its accidental or deliberate illegal introduction to Australia.

Previous extensive testing of the fungus undertaken overseas, as part of biocontrol programs in Hawaii, South Africa and New Zealand, indicated that it is highly host specific (Morin *et al.* 1997, Schooler *et al.* 2012). Our results further confirmed this and demonstrated that the fungus does not pose a threat to the two Australian native *Adenostemma* spp., which had not been previously tested.

Baseline plant community data collected at monitoring sites show that mistflower severely limits the area occupied by other plant species. Direct competition for resources and light may play an important role, but allelopathy may also be involved. Leachate from decaying leaves of mistflower has been reported to have an allelopathic effect by restricting growth of other species (Rai and Tripathi 1982).

The fungus has already demonstrated its potential to deliver a highly effective and self-sustaining control method for mistflower. The severity of mistflower defoliation observed 5 months after the release of the fungus at sites on the south coast of NSW was astonishing. Further, the presence of the fungus at other mistflower-infested sites in this region indicated that natural long-distance dispersal of spores *via* wind most likely occurred. The wet winter conditions in 2011 combined with some periods of high winds would have contributed to this spread. This initial severe defoliation of mistflower however, did not kill plants outright and regrowth from roots and stems were observed by collaborators in late spring-early summer. Signs of infection on new shoots were also detected soon after emergence.

All monitoring sites will be revisited in June 2012 to reassess percent cover of mistflower and other species and above-ground biomass in small quadrats near monitoring transects to determine if the fungus is having an immediate impact.

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