Restoring biodiversity in NSW through biocontrol of mistflower

Final report for stakeholder distribution

Louise Morin, Canberra, ACT

31 January 2013

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Contents

Acknowledgments........................................................................................................................................... ii

1 Summary...................................................................................................................................................... 1

2 Background to and objectives of the project.............................................................................................. 2
  2.1 Background............................................................................................................................................. 2
  2.2 Objectives............................................................................................................................................... 3
  2.3 Achievements against objectives.......................................................................................................... 3

3 Outputs....................................................................................................................................................... 5

4 Outcomes.................................................................................................................................................... 6
  4.1 Innovative method developed............................................................................................................... 6
  4.2 Technical or scientific conferences..................................................................................................... 6
  4.3 Presentations of project at events involving stakeholders................................................................. 6
  4.4 Individuals engaged............................................................................................................................. 6
  4.5 Publications developed........................................................................................................................ 6
  4.6 Trust funded staff involved.................................................................................................................. 7
  4.7 Volunteers involved............................................................................................................................... 7
  4.8 Researchers involved........................................................................................................................... 7
  4.9 Partnerships with community and government.................................................................................. 8
  4.10 Individuals potentially reached.......................................................................................................... 8

5 Methodology/Approach................................................................................................................................. 9
  5.1 Monitoring sites...................................................................................................................................... 9
  5.2 Host-specificity tests............................................................................................................................ 10
  5.3 Release of the fungus............................................................................................................................ 10

6 Issues, Changes, Opportunities................................................................................................................. 11

References.................................................................................................................................................. 12

Appendix 1 Maps of surveyed locations ...................................................................................................... 1
Appendix 2 Results of host-specificity tests.................................................................................................. 4
Appendix 3 Location of monitoring sites....................................................................................................... 6
Appendix 4 Baseline data collected at each monitoring site ........................................................................ 9
Appendix 5 Comparison between baseline data and data collected at the monitoring sites 12-18 months later ........................................................................................................................................ 13
Appendix 6 Photographic examples of initial impact of the white-smut fungus on mistflower and on the recovery of other plant species (where appropriate) ........................................................................................................... 15
Appendix 7 Location of sites where the fungus was deliberately released .................................................. 17
Appendix 8 Location of sites on the NSW South Coast where the fungus was found following natural dispersal from release sites .................................................................................................................. 18
Appendix 9 Example of stakeholder feedback on the project...................................................................... 21
Acknowledgments

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Finally, we would like to thank CSIRO and the NSW Environmental Trust for providing financial support to this project. We also thank the Lake Baroon Catchment Care Group and the Sunshine Coast Regional Council for additional seed funding to support activities in Queensland.
1 Summary

Mistflower (Ageratina riparia: Asteraceae) is a perennial herbaceous alien plant that invades wet habitats, particularly riparian areas and moist cliff faces in eastern Australia. On 21 October 2010, the white-smut fungus Entyloma ageratinæ, 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 NSW North Coast 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 closely-related plant species to mistflower within the Eupatorieæ tribe, including two Australian native Adenostemma species, revealed that only the invasive plant crofton weed (Ageratina adenophora) developed some disease symptoms, albeit to a much lesser extent than mistflower. 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 were made in May 2011. Monitoring sites were revisited in May to July 2012 and a measurable adverse impact of the fungus on mistflower was recorded. There was more than 60% decrease in percentage cover of mistflower across sites, with a corresponding increase, by also more than 60%, of the percentage cover of other plant species. Within a very short timeframe, the project demonstrated that the white-smut fungus has great potential as a highly effective and self-sustaining control method for mistflower across its range.
Background to and objectives of the project

2.1 Background

2.1.1 MISTFLOWER

Mistflower (Ageratina riparia = Eupatorium riparium: Asteraceae), native to the Caribbean, is a perennial herbaceous 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. Each seed has a pappus, which, along with its small seed size, allows the plant to colonise upstream and upslope habitats on the prevailing winds. In late-spring, the flowering stalks die back to the stolon, particularly during dry periods, and new shoots begin to grow in early-summer.

Mistflower was introduced to Australia (NSW) in 1875 as an ornamental garden plant and was first recorded in Queensland in the 1930s. 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).

Unlike many other invasive plants, mistflower has the ability to spread along riparian corridors into pristine catchment headwaters and negatively impact biotic diversity in ecologically important areas such as World Heritage Areas and other environmentally sensitive areas. Mistflower negatively affects populations of a number of threatened and endangered species in NSW including plants; Brachyscome ascendens, Daphnandra sp., C Illawarra, Doryanthes palmeri, Euphrasia bella, Irenepehus trypherus, Rapanea sp., Sarcochilus hartmannii and a native bird, Dasyomis brachypterus (Coutts-Smith and Downey 2006).

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.

2.1.2 BIOLOGICAL CONTROL

Major mistflower infestations in Hawaii were controlled by a fungal pathogen, the white-smut fungus Entyloma ageratina (hereafter referred as the fungus), 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) (Morris 1991, 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).

Extensive testing undertaken overseas (as part of biocontrol programs in Hawaii, South Africa and New Zealand) indicated that the fungus is highly host specific (Morin et al. 1997, Fröhlich et al. 2000). Crofton weed (Ageratina adenophora), which belongs to the same genus of mistflower, was the only non-target species that developed disease symptoms (Morris 1991, Fröhlich et al. 2000). Lesions produced however, were much smaller than on mistflower and did not support formation of any spores.

On 21 October 2010, the fungus was found near Lamington National Park, Queensland and the pathway of introduction is unknown. At the time, we had just heard that our project proposal to the Trust had been successful. In the original project objectives, we were planning to do the required testing to obtain
permission from the authorities to introduce this fungus into Australia. In light of this incursion, the objectives of our original proposal were modified, agreed by the Trust and incorporated into the contract.

2.2 Objectives

The project objectives were to:

1) perform a survey in the vicinity of the area where the fungus has been detected to determine the extent of its current distribution;

2) determine if the fungus will pose a threat to representative species in the Eupatorieae tribe present in Australia;

3) collect baseline data on mistflower and associated plant communities (and other species if any are found to be susceptible during testing) at sites where the fungus is not yet present to determine subsequent effects of the fungus on mistflower abundance and plant community composition;

4) providing the fungus proves sufficiently host specific and following consultation with the Biosecurity section of I&I NSW and NSW DECCW, mass-rear and perform releases at non-infected sites in NSW.

2.3 Achievements against objectives

**Objective 1**: Complete surveys to determine the extent of the current distribution of the fungus [Achieved]

We undertook many surveys from October 2010 to July 2011 to delimit the distribution of the fungus. We also produced an identification guide for the fungus that we sent to the Mistflower Stakeholder Group (30 stakeholders from NSW and Queensland), asking them to circulate widely and to report back if they saw any signs of the fungus in their localities. Maps of surveyed locations indicating where the fungus has been found and where it was absent are presented in Appendix 1.

**Objective 2**: Test the host range of the fungus against other plants in the Eupatorieae tribe [Achieved]

Of of the 17 non-target plant species within the Eupatorieae tribe tested, only crofton weed (Ageratina adenophora), which belongs to the same genus of mistflower, developed some disease symptoms, albeit to a much lesser extent than on mistflower (Appendix 2). Spores were produced only on a few of the lesions. All other species, including the two Australia native species, Adenostemma lavenia and Adenostemma macrophyllum, were found to be immune to the disease.

**Objective 3**: Collect baseline data on mistflower and associated plant communities to determine effect of the fungus [Achieved]

A Scientific License to undertake research in National Parks in NSW was obtained prior to the initiation of this objective. Monitoring transects were established at eight sites in NSW and three in Queensland and baseline plant community data collected between 21 December 2010 and 21 July 2011. Locations and summary of data collected are presented in Appendices 3 and 4. Following collection of the baseline plant community data, the fungus was released at the five monitoring sites on the NSW Central and South Coasts where it was not present. Sites were revisited in May to July 2012 to assess the percentage cover of mistflower and other vegetation and a measurable adverse impact of the fungus was recorded (Appendices 5 and 6). There was more than 60% decrease in percentage cover of mistflower across sites, with a corresponding increase, by also more than 60%, of the percentage cover of other plant species.

**Objective 4**: Redistribute fungus to currently uninfected sites [Achieved]

Once it was established that the fungus was more widespread than originally thought and present in NSW (see Obj. 1), we obtained confirmation from the Biosecurity section of NSW Department of Primary Industry that there were no intra state restrictions to the movement of this fungus in NSW. On the basis that the fungus was spreading rapidly and of previous studies overseas that demonstrated its high specificity towards mistflower, we went ahead with a series of strategic releases to non-infected mistflower...
sites (including monitoring sites) on the NSW Central and South Coasts in autumn 2011. Maps of the location of release sites are presented in Appendix 7.
3 Outputs

The most important outputs achieved during the project are:

- The setting-up of 11 monitoring sites across the distribution of mistflower and the gathering of baseline data on plant communities as well as a quantitative assessment of the initial impact of the fungus on mistflower and the indirect effect on the recovery of other plant species.

- Our releases of the fungus at strategic locations in the southern distribution of mistflower (from Barrington Tops to Nowra) have led to an impressive natural spread of the fungus, which meant that no other released were required.

4 Outcomes

4.1 Innovative method developed

The white-smut fungus has already demonstrated its potential to deliver a new, highly effective and self-sustaining control method for mistflower (see Appendices 5 and 6). Our project demonstrated that spores of the fungus can naturally disperse over long distances and readily initiate new infections that develop to cause severe damage on mistflower (Appendix 8).

4.2 Technical or scientific conferences

Dr Louise Morin delivered oral presentations on the project at the 18th Australasian Weeds Conference held on 8-11 Oct 2012 in Melbourne and at the meeting of the NSW Biocontrol Task Force held on 15 Nov 2012 in Grafton.

4.3 Presentations of project at events involving stakeholders


4.4 Individuals engaged

Dr Shon Schooler established in the lead up to this project an informal group of stakeholders (the ‘Mistflower Stakeholder Group’) that comprises 30 people from various agencies. Permission was obtained from all property owners / land managers of the 11 sites where we have established monitoring transects (and released the fungus where absent) and of an additional 4 sites where we have released the fungus in NSW. At least another 10 people (mostly government agencies, including Councils and National Parks) have assisted us in locating sites for releases and/or surveyed their localities for sign of the fungus.

4.5 Publications developed

Newsletter articles:

1- Schooler SS (Summer 2011) Mistflower (Ageratina riparia). Nambucca Valley Landcare Newsletter, p. 3.

2- Schooler SS (Summer 2010/11) Monitoring mistflower and the smut fungus. North Coast WEED READ, no. 20, NSW North Coast Weeds Advisory Committee.


5- Morin L (Autumn 2012) Biological control of mistflower. Gecko, issue 51, p. 6, Blue Mountains City Council Bushcare Program.


7- Morin L (Spring 2012) Fungi offer biological control hope for mistflower and crofton weed – Mistflower, watch out! Bush Matters, no. 15, p. 14, Conservation Partners Program of the Office of Environment and Heritage NSW.

Scientific conference paper:


Scientific web-based factsheet:


Book chapter:


4.6 Trust funded staff involved

Two technical staff involved in the project were partially funded by the Trust: Ms Melissa Piper (25%); Mr Andrew White (10%). Dr Shon Schooler (5%), the Principal Investigator until his departure in mid-August 2011 and Dr Louise Morin, the other scientist involved in the project (5% until mid-August and thereafter 10% as new Principal Investigator) were in-kind contribution to the project by CSIRO.

4.7 Volunteers involved

A French student intern (Ms Clementine Le Naire) provided approximately 300 hours of volunteered work to assist the project from 13 September 2010 to 28 January 2011. We did not have involvement by other volunteers as such, notwithstanding the assistance provided by the people mentioned in the ‘Individuals engaged’ category above.

4.8 Researchers involved

Dr Shon Schooler (5%) was the Principal Investigator of the project until his departure in mid-August 2011. Dr Louise Morin, was the other researcher involved in the project (5% until mid-August and thereafter 10% as new Principal Investigator). Both researchers were in-kind contribution to the project by CSIRO.
4.9 Partnerships with community and government

We have ongoing involvement with the Lake Baroon Catchment Care Group and the Sunshine Coast Regional Council who have provided seed funding to support activities in Queensland. We also have support by the NSW National Parks and Wildlife Services through the involvement of rangers and permission to conduct research in nominated national parks.

4.10 Individuals potentially reached

In addition to all the individuals mentioned above, we estimate that there are many more than the projected 200 individuals that have heard about our project. In November 2011 we emailed the Mistflower Stakeholder Group asking them to circulate widely our request for reports of any sighting of mistflower infected by the fungus. This request was mentioned in the article published in publications no 1 to 3 above. As the project progressed several articles were published in a range of community group newsletter (see above) which would have reached a large number of people.
5 Methodology/Approach

A summary of activities undertaken can be found in section 2.3.

5.1 Monitoring sites

The following is a summary of the methods employed to set-up and gather plant community data at monitoring sites:

**Diversity transects sampling methods**

1) select site
2) set up 4 permanent 10m long transects through dense mistflower stands, mark endpoints with cattle tags (transects should be within 50m of each other)
3) sample 10 1m$^2$ plots on one side of transect (preferably on right side from starting end looking down transect)
4) record cover of each species, litter, bare dirt, and rock to nearest 1%
5) take canopy cover values over each plot in each transect
6) take soil moisture reading for each plot in each transect
7) record slope of transect
8) record aspect of transect
9) if applicable, record % stems infected by fungus in transect (randomly examine 50 stems)
10) take GPS readings of endpoints of each transect (averaged in GPS for 100% confidence)
11) take photo from each end of transect (download and label by site, transect (1-4), and end (A or B))
12) record elevation of site

**Biomass plots sampling methods**

1) randomly locate 1 plot (0.25m$^2$) within 4m of each end of the transect but not in transect (2 plots for each transect)
2) remove all mistflower aboveground biomass of all plants with roots in the plot and put into bag
3) label each with site, transect (1-4), and transect end (A or B)
4) place into paper bags and dry at 60deg C to constant weight
5) weigh to nearest 100th gram (0.01g)
5.2  Host-specificity tests

A culture of the white-smut fungus (ex Maleny, QLD) was maintained on mistflower plants through regular inoculations at CSIRO Ecosystem Sciences in Canberra. At 3 weeks after inoculation, infected leaves were placed (lower surface facing upwards) onto moist paper towel contained in large trays covered with transparent plastic bags to provide a high humidity environment to stimulate spore production. Trays were left on the bench in a controlled environment room (at 20°C, 12-h photoperiod) for 3-6 days. Leaves were then placed in a solution of distilled water with 0.05% Tween 80 and gently shaken to dislodge spores. The suspension was filtered through a layer of miracloth, its density determined using a haemocytometer and adjusted to $1 \times 10^5$ spores ml$^{-1}$ with water-Tween 80 solution. The suspension was sprayed onto test plants (four or five replicates per species) using a hand-held sprayer. Mistflower plants were inoculated as a positive control in each trial. Inoculated plants were misted with water and placed in boxes enclosed in large transparent plastic bags in a controlled-environment room (20°C, 12-h photoperiod) for 48 hours. Plants were then removed from the boxes and placed on the bench of the controlled-environment room. Three and five weeks after inoculation, all plants were examined individually for disease symptoms. Each taxon was tested in two separate trials to account for any possible variation in time.

5.3  Release of the fungus

The fungus was released in the field by placing 3-10 infected, potted mistflower plants with sporulating lesions among natural mistflower infestations at a site. It was preferable to release when prevailing conditions were wet and cool on the days of and after the release.
6 Issues, Changes, Opportunities

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 2010 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.

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.

Within a very short timeframe, our project demonstrated that the white-smut fungus has great potential as a highly effective and self-sustaining control method for mistflower across its range. The fungus is highly efficient at naturally dispersing itself and therefore we do not believe that concerted efforts to redistribute it are required, except maybe for remote mistflower-infested sites that do not naturally become infected in the next few years.

Biological control is the only realistic option for managing some of our most troublesome environmental weeds that are threatening the environment. This project is a good example of the benefits that can be derived from the implementation of a biological control program. The outputs/outcomes achieved are testimony that this project was an excellent investment of Trust funds.

An example of stakeholder feedback on the project is provided in Appendix 9.
References


Appendix 1 Maps of surveyed locations

Maps of surveyed locations where the fungus has been found on mistflower (red symbol) and where it was absent (blue symbol) prior to any deliberate releases undertaken.
Closed-up of regions

South East Queensland & NSW North Coast
Appendix 2 Results of host-specificity tests

Results of host-specificity tests with the white-smut fungus on plant species in the Eupatoriae tribe (to which mistflower belongs) that are present in Australia.

<table>
<thead>
<tr>
<th>Species</th>
<th>Native (N) / Exotic (E)</th>
<th>Development of disease symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ageratina riparia (mistflower) *</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>Ageratina adenophora (crofton weed) *</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>Ageratina altissima (syn Eupatorium rugosum)</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Adenostemma lavenia</td>
<td>N</td>
<td>No</td>
</tr>
<tr>
<td>Adenostemma macrophyllum</td>
<td>N</td>
<td>No</td>
</tr>
<tr>
<td>Ageratum conyzoides subsp. conyzoides</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Ageratum houstonianum *</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Bartlettina sordida (syn Eupatorium megalophyllum, E. atrorubens)</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Chromolaena odorata *</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Conoclinium coelestinum (syn Eupatorium coelestinum)</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Eupatorium cannabinum</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Eupatorium purpureum var. purpureum</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Gymnocoronis spilanthoides</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Liatris spicata</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Mikania micrantha</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Praxelis clematidea</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Stevia ovata</td>
<td>E</td>
<td>No</td>
</tr>
<tr>
<td>Stevia rebaudiana</td>
<td>E</td>
<td>No</td>
</tr>
</tbody>
</table>

* Tested in previous projects overseas.
Disease symptoms that developed on crofton weed (left) and mistflower (right) at 5 weeks after inoculation with the white-smut fungus.
Appendix 3 Location of monitoring sites

Red symbol = fungus present at time of set-up; blue symbol = fungus absent at time of set-up.

<table>
<thead>
<tr>
<th>Site</th>
<th>State</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornfoot</td>
<td>QLD</td>
<td>A</td>
</tr>
<tr>
<td>Mount Mee</td>
<td>QLD</td>
<td>B</td>
</tr>
<tr>
<td>Teakle</td>
<td>QLD</td>
<td>C</td>
</tr>
<tr>
<td>Toonumber</td>
<td>NSW</td>
<td>D</td>
</tr>
<tr>
<td>Woods Camp</td>
<td>NSW</td>
<td>E</td>
</tr>
<tr>
<td>Darkwood</td>
<td>NSW</td>
<td>F</td>
</tr>
<tr>
<td>Barrington Tops</td>
<td>NSW</td>
<td>G</td>
</tr>
<tr>
<td>Windy Gully</td>
<td>NSW</td>
<td>H</td>
</tr>
<tr>
<td>Clover Hill Rd</td>
<td>NSW</td>
<td>I</td>
</tr>
<tr>
<td>Minimurra RF</td>
<td>NSW</td>
<td>J</td>
</tr>
<tr>
<td>Kangaroo Valley</td>
<td>NSW</td>
<td>K</td>
</tr>
</tbody>
</table>
Close-up of regions
Appendix 4 Baseline data collected at each monitoring site

**Cornfoot**

- Number of species vs. Mistflower cover (%)
- Other species cover (%) vs. Mistflower cover (%)
  - Equation: $y = -0.127x + 14.59$
  - $R^2 = 0.1554$

**Mount Mee**

- Number of species vs. Mistflower cover (%)
- Other species cover (%) vs. Mistflower cover (%)
  - Equation: $y = -0.2766x + 24.954$
  - $R^2 = 0.1231$

**Teakle**

- Number of species vs. Mistflower cover (%)
- Other species cover (%) vs. Mistflower cover (%)
  - Equation: $y = -0.4226x + 32.3$
  - $R^2 = 0.133$
Toonumbar

Woods Camp

Darkwood
**Barrington tops**

![Graph](image1)

\[ y = -0.2281x + 25.229 \]

\[ R^2 = 0.1721 \]

**Windy Gully**

![Graph](image2)

\[ y = -0.2219x + 32.265 \]

\[ R^2 = 0.1382 \]

**Clover Hill Rd**

![Graph](image3)

\[ y = -0.2219x + 32.265 \]

\[ R^2 = 0.1382 \]
Minimurra RF

Kangaroo Valley

\[ y = -0.3453x + 58.222 \]

\[ R^2 = 0.0724 \]
Appendix 5 Comparison between baseline data and data collected at the monitoring sites 12-18 months later

Initial impact of the white-smut fungus on *mistflower* percentage cover and biomass. See Appendix 3 for detail on sites comprised in 'Northern' and 'Southern' sites. Blue column = 2010-11; Yellow column = 2012. Percentage reductions are shown next to arrows.
Initial impact of the white-smut fungus on other plant species percentage cover and number of different plant species (other than mistflower) per m². See Appendix 3 for detail on sites comprised in ‘Northern’ and ‘Southern’ sites. Blue column = 2010-11; Yellow column = 2012. Percentage increases are shown next to arrows.
Appendix 6 Photographic examples of initial impact of the white-smut fungus on mistflower and on the recovery of other plant species (where appropriate)

Kangaroo Valley – transect no 1

3 May 2011

28 May 2012
Windy Gully (Mt Kembla) – transect no 4

20 April 2011
28 May 2012
Appendix 7 Location of sites where the fungus was deliberately released
Appendix 8 Location of sites on the NSW South Coast where the fungus was found following natural dispersal from release sites

Survey carried out on the NSW South Coast in early October 2011, 5 months after deliberate releases of the fungus was made at strategic sites (Appendix 7). Red symbol = fungus present; Blue symbol = fungus absent.
Symptoms of the smut before leaf death

Examples of severe defoliation

Royal NP, Upper causeway
Minamurra
Mt Kembla
Bugong Creek
Foxground Road

Cambewarra

Kangaroo Valley
Appendix 9 Example of stakeholder feedback on the project

From: Les Mitchell and Janet Bundey [mailto:janles@fastrac.net.au]
Sent: Tuesday, 14 August 2012 5:30 PM
To: Morin, Louise (CES, Black Mountain); Piper, Melissa (CES, Black Mountain)
Cc: Phil Craven; Kevin Mills; Alastair & Molly Stevenson; Tess Heighes; Tessh@shoal.net.au; Greg Thompson; Greg Thompson; Garry Daly; janet mayer
Subject: Impact of Smut Fungus at 'Budderoo' Upper Kangaroo Valley

Hi Louise and Melissa,

I've recently returned from 10 weeks in Nth Qld and have been checking out several areas on our property where I have been monitoring the ongoing effects of the smut fungus on mistflower. The attached photographs were taken from a photopoint that I established along a walking track on this property in mid 2010. As you can see from the July 2010 photo it was a very dense infestation of mistflower prior to your release of the smut fungus at the transects which are 600 metres from this site. I think you'll agree that the changes in mistflower cover since 2010 and particularly in the past 12 months have been dramatic.

I've also checked out the 4 transects and the mistflower has declined even more since your visit in May. That general slope on the downhill side of the road is so changed since the release of the fungus, with a very healthy cover now of native ferns, sedges and *Stellaria flaccida* far outcompeting the mistflower.

Are you planning another visit and resurvey of release sites? Hope you are both well.

Cheers
Les Mitchell
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