SAFETY IN NEW ZEALAND WEED BIOCONTROL: A NATIONWIDE PATHOGEN SURVEY FOR IMPACTS ON NON-TARGET PLANTS

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ABSTRACT

The accuracy of host range testing in weed biocontrol programmes using plant pathogens has been questioned prompting a research programme into the field safety record of pathogens used for weed biocontrol in New Zealand. Nationwide disease surveys were conducted from 2000-2009, and focused on species closely related to target weeds and therefore potentially most at risk of attack. These surveys identified pathogens associated with any disease symptoms observed on non-target hosts. Disease damage attributable to biocontrol agents was observed on two non-target plants. Pustules of the blackberry rust, *Phragmidium violaceum*, were found on the endemic *Rubus* species, *R. cissoides* (bush lawyer, tataramoa) at one location. This result was predicted from host range safety tests conducted prior to its arrival in New Zealand. No non-target damage was observed in the remaining case studies, confirming to date that all biocontrol pathogens are highly host specific to their target weeds.

Keywords: biological control, disease, specificity, risk, environmental safety.

INTRODUCTION

Concerns about potential non-target impacts and risks associated with classical biological control of weeds (Howarth 1991; Simberloff & Stiling 1996) are resulting in tighter controls over the importation and release of biological control agents worldwide (Sheppard et al. 2003). In New Zealand, the Hazardous Substances and New Organisms (HSNO) Act (1996) administered by the Environmental Risk Management Authority (ERMA), requires a rigorous risk analysis for the potential introduction of biological control agents that considers the environmental risk of each proposed agent to attack non-target plant species (Fowler et al. 2000; Paynter et al. 2004). Host testing of all pathogens for weed biocontrol is currently based on the internationally accepted host range testing systems that help delineate the host-range for potential agents (Wapshere 1974; Briese & Walker 2002; Louda et al. 2003). Although biological control of invasive weeds is often perceived as environmentally safe, there have been reported cases of damage to non-target plants by some introduced invertebrate agents (e.g. Pemberton 1995; Louda et al. 1997, 2003). International reviews of weed biocontrol introductions have shown that of the almost 400 invertebrates and pathogens introduced as classical biocontrol agents for weeds, only 29 invertebrate species (7.25%) have been reported from non-target plants (Barton 2004). Furthermore, the few reviews of weed biocontrol safety that have specifically focused on pathogens have been unanimous in concluding

that they have proved extremely safe (Evans 2000; Goettel et al. 2001). A comprehensive global assessment by Barton (2004) determined there were no reports of deliberately introduced fungi unexpectedly attacking non-target plants after release. However, past investigations of non-target effects of weed biocontrol agents in New Zealand have been sporadic, with systematic surveys typically carried out only at a local scale if a report of suspected non-target damage was received (Paynter et al. 2004). No systematic field surveys of introduced (deliberate or otherwise) plant pathogens on non-target species had been undertaken in New Zealand. Evaluating weed biological control projects is essential to improve our ability to predict the impact and safety of future introductions and, therefore, underpin the continued use of biological control as a tool against damaging invasive weeds (Paynter et al. 2004).

The research reported here is the first systematic field survey of non-target plants undertaken in New Zealand. The aim was to specifically look for plant pathogens with biocontrol potential that had been introduced (deliberately or not).

MATERIALS AND METHODS

Surveys were conducted throughout New Zealand, and focused on plants that are closely related to the target weeds and are therefore most at risk of non-target attack (Barton 2004; Paynter et al. 2004). The objective was to search for any disease damage caused by the following five plant pathogens, all of which have been deliberately, accidentally or autonomously introduced to New Zealand.

1. The white smut fungus, *Entyloma ageratinae* Barreto & Evans was released in 1998 for the biocontrol of mist flower, *Ageratina riparia* (Regel) R.M.King & H.Rob (Barton et al. 2007). This pathogen was searched for on the most closely related host present in New Zealand, the invasive Mexican devil weed, *Ageratina adenophora* (Spreng.) R.M.King & H.Rob.

2. Strains of the violet bramble leaf or blackberry rust, *Phragmidium violaceum* (Schultz) G. Winter, were first officially released in Australia in 1991 for the biocontrol of blackberry *Rubus fruticosus* L. aggregate. Strains had already appeared there earlier in 1984 (Marks et al. 1984) due to illegal introduction(s) that consequently resulted in its arrival in New Zealand in 1990 (McKenzie 1998). The New Zealand introduction was potentially autonomous via its wind blown spores (Gomez et al. 2006). Evergreen blackberry (*Rubus laciniatus* Willd.), three commercially grown *Rubus* species, namely raspberries (*R. idaeus* L.), boysenberry (*R. ursinus x idaeus* Chamiasso & Schlenhtendal) and loganberry (*R. ursinus loganobaccus* Bailey), and three endemic *Rubus* species (bush lawyer, tataramoa), namely *R. cissoides* A. Cunn., *R. australis* G.Forst and *R. schmidelioides* A.Cunn., were surveyed for rust infection over three periods (Table 1), 2000-2001, 2004-2006 and 2008-2009, the latter at two sites.

3. *Puccinia hieracii* var. *piloselloidarum* (Probst) Jørst. was either illegally or accidentally introduced to New Zealand (McKenzie 1998), after originally being scoped as a potential biocontrol agent against mouse-eared hawkweed (*Hieracium pilosella* L.) (Jenkins 1995a,b). The three most common and related *Hieracium* species in New Zealand, field hawkweed (*H. caespitosum* Durmort), king devil hawkweed (*H. praealtum* Gochant) and tussock hawkweed (*H. lepidulum* (Stenstroem) Omang.), were checked for the rust.

4. In 2000, the rust *Puccinia myrsiphylli* G.Winter was released in Australia for the biocontrol of bridal creeper/smilax (*Asparagus asparagoides* (L.) W. Wight). By 2005 it was first recorded in New Zealand (Waipara et al. 2006) and by 2008 had spread over most of the invasive range of the weed (Harman et al. 2008). Another closely related and invasive weed, climbing asparagus (*Asparagus scandens* Thunb.), was surveyed for *P. myrsiphylli* infection. This case study was a belated inclusion due to the discovery of the rust after the other non-target surveys had been initiated.

5. Californian thistle rust, *Puccinia punctiformis* (Str.) Röhl, was first recorded in New Zealand as early as 1881 (Cunningham 1927). Subsequently it was used as an augmentative biocontrol agent against Californian thistle *Cirsium arvense* L. Scop., and a

recent comparative survey has shown that this pathogen occurs in New Zealand at levels similar to its native range of Europe (Cripps et al. 2009). Three related thistle species, marsh thistle (*Cirsium palustre* L. Scop.), Scotch thistle (*Cirsium vulgare* (Savi) Ten.) and nodding thistle (*Carduus nutans* L.), were checked for presence of *P. punctiformis*.

Where possible, field sites were selected where the agent, the target weed and the potential non-target species were all present. These sites maximise the potential for inoculum to 'spill over' onto any proximate and susceptible non-target species nearby. However, as all five pathogens can disperse via airborne spores, it was assumed that any substantial stand of the non-target plant was exposed to the agent, even if several kilometres away from potential inoculum sources. Details of field sites, year of inspection and number of plants inspected are provided in Table 1.

Any diseased plant materials (leaf, shoot, flower, fruit) were collected and the causative pathogens associated with the visible symptoms were identified either by direct examination or by cultural isolation. A dissecting microscope was used to search necrotic areas for the sporulation of rust/smud pustules. Methods outlined previously (Waipara et al. 2009) were used to isolate other pathogenic fungi from diseased tissues when required. Voucher specimens were deposited into the Landcare Research PDD herbarium at Auckland.

RESULTS

Pustules containing the diagnostic teliospores of *P. violaceum* were identified sporulating on the leaves of two individual plants of endemic bush lawyer (*R. cissoides*). Therefore, non-target attack by the rust was confirmed during the field surveys of *Rubus* plants (Table 2). Visible infection of *R. cissoides* was infrequent, with the rust being found on only 2 out of the 132 (1.5%) plants of that species examined during 2004-2006, and no infection being recorded in subsequent checks (2008-2009). Both infected plants were located at the same field site (Mt Maroanui, north of Taupo). The site contained many blackberry plants, heavily infected with the rust, growing directly below the infected *R. cissoides* plants. The close proximity between target and non-target plants potentially contributed to this result. Nevertheless, at 14 other North Island sites, infected blackberry plants were also found growing in close proximity to *R. cissoides*, but without signs of any cross infection. The two *R. cissoides* plants found with pustules of *P. violaceum* displayed only minor disease symptoms; there were only a few infected leaves and these leaves only had a small number of lesions attributable to the rust. To date, the strain/s of blackberry rust present in New Zealand are having a negligible impact on this native species (J. Barton & N. Waipara, unpubl. data).

While not a specified target weed for *P. violaceum*, the evergreen (cut leaf) blackberry, *R. laciniatus*, was also found to be infected by the rust in 2000-2002 and 2004-2006 (Table 2). This result further demonstrated the rust's broad host range and its potential to infect different *Rubus* species. None of the three commercially cultivated *Rubus* species were infected by *P. violaceum*, which was consistent with predictions drawn from earlier host tests (Bruzese & Hasan 1986b).

No non-target infection was found by the pathogens, *Entyloma ageratinae*, *Puccinia hieracii piloselloidarum*, *Puccinia myrsiphylli* and *Puccinia punctiformis* on any of the non-target plants examined, despite positive infection being observed on many of the host plants (Table 2).

The disease survey also identified four other rust pathogens and *Septoria rubi* damaging *Rubus* hosts (Table 3).

The host-specific fungus *Phaeoramularia eupatorii-odorati* (Yen) Liu & Guo was observed on 40 Mexican devil weed plants at all 7 field sites (Table 3). This leaf pathogen produces necrotic leaf lesions that can resemble damage by the mist flower smut *E. ageratinae*, and Barton (2004) noted several false alarms that the smut was attacking the wrong *Ageratina* species.

Other pathogens that resembled potential non-target attack were also identified in the surveys (Table 3). For example, the rust *Puccinia cnici* H. Mart. was recorded on

TABLE 1: Number of field sites (by region), assessment dates (year) and samples (N=number of plants) surveyed for disease on non-target species(NT) caused by plant pathogens that had been introduced for biocontrol of target weed species(T).

Pathogen	Plant	Region ¹	Sites	Year	(N)
<i>Entyloma ageratinae</i>	Mist flower ^(T)	1,2	7	2005	35
			3	2007	12
	Mexican devil weed ^(NT)	1,2	7	2005	35
			3	2007	12
	Total Ageratina spp.	2	7		94
<i>Phragmidium violaceum</i>	Blackberry ^(T)	2,4,5,7-14	22	2000-02	24 ²
		1-9	25	2004-06	188 ²
		2	2	2008-09	12 ²
	Evergreen blackberry ^(NT)	10, 14	2	2000-02	2
		5,8	2	2004-06	12 ²
	Raspberry ^(NT)	4,6	3	2004-06	30 ²
	Boysenberry ^(NT)	4,6	2	2004-06	20 ²
	Loganberry ^(NT)	4,6	2	2004-06	20 ²
	<i>R. australis</i> ^(NT)	1,3,7,8	5	2004-06	31
	<i>R. cissoides</i> ^(NT)	1-9	26	2004-06	132 ²
			2	2008-09	3
	<i>R. schmidelioides</i> ^(NT)	1,5-9	16	2004-06	69 ²
	Total Rubus spp.	14	35		543²
<i>Puccinia hieracii piloselloidarum</i>	Mouse-eared hawkweed ^(T)	7,11,12,14	10	2005	100
	Field hawkweed ^(NT)	11,14	3	2005	30
	King devil hawkweed ^(NT)	12,14	7	2005	70
	Tussock hawkweed ^(NT)	11,12,14	6	2005	60
	Total Hieracium spp.	4	26		260
<i>Puccinia myrsiphylli</i>	Bridal creeper ^(T)	1,2	5	2007-09	15
	Climbing asparagus ^(NT)	1,2	5	2007-09	27
	Total Asparagus spp.	2	5		42
<i>Puccinia punctiformis</i>	Californian thistle ^(T)	3,4	9	2001-04	172
		2-4,8,12,14	20	2007-09	55
	Marsh thistle ^(NT)	2	1	2007-09	4
	Scotch thistle ^(NT)	3,4	9	2001-04	29
		2-4,8,12,14	20	2007-09	25
	Nodding thistle ^(NT)	2,3	4	2007-09	16
	Total thistles	7	32		301

¹Key to region: 1=Northland, 2=Auckland, 3=Waikato//King Country, 4=Coromandel/ Bay of Plenty, 5=Gisborne, 6=Hawke's Bay, 7=Taupo, 8=Taranaki, 9=Wanganui/ Manawatu, 10=Wellington, 11=Nelson/Marlborough, 12=Canterbury, 13=West Coast, 14=Otago/Southland.

²Estimated minimum number. Where *Rubus* plants formed dense thickets the number of plants examined was recorded as 'greater than' this minimum figure.

TABLE 2: Occurrences of positive infection on target and non-target plant species caused by plant pathogens introduced for weed biocontrol. N=number of plants exhibiting infection by the pathogen/total number of plants examined.

Plant species	Year	Pathogen	N
Mist flower	2005	<i>Entyloma ageratinae</i>	35/35
	2007	<i>Entyloma ageratinae</i>	12/12
Blackberry	2000-02	<i>Phragmidium violaceum</i>	22/24
	2004-06	<i>Phragmidium violaceum</i>	116/188
	2008-09	<i>Phragmidium violaceum</i>	3/3
Evergreen blackberry	2000-02	<i>Phragmidium violaceum</i>	1/2
	2004-06	<i>Phragmidium violaceum</i>	1/12
<i>R. cissoides</i>	2004-06	<i>Phragmidium violaceum</i>	2/132
	2008-09	- (no non-target infection)	0/3
Mouse-eared hawkweed	2005	<i>Puccinia hieracii piloselloidarum</i>	27/100
Bridal creeper	2007-09	<i>Puccinia myrsiphylli</i>	15/15
Californian thistle	2001-04	<i>Puccinia punctiformis</i>	153/172
	2007-09	<i>Puccinia punctiformis</i>	19/55
	2007-09	<i>Puccinia punctiformis</i>	19/55

Scotch thistle and produced superficially similar symptoms to that of *P. punctiformis* on Californian thistle. All other pathogens are previously reported primary and secondary pathogens on their respective hosts (Pennycook 1989). These included *Alternaria* spp., *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Phomopsis* sp. and two powdery mildews (*Erysiphe cichoracearum* and *Oidium* sp.). Most were associated with very minor leaf/shoot necroses and are cosmopolitan species in New Zealand (Pennycook 1989).

DISCUSSION

Of the five pathogens surveyed, non-target damage was observed only in the *Rubus* case study. *Phragmidium violaceum* was not deliberately introduced to New Zealand for blackberry biocontrol and the strict host specificity protocols required by ERMA for the release of all biocontrol agents would have most likely precluded its official release here. Field infection of the blackberry rust on *R. cissoides* was predicted from previous host range testing when the pathogen was first being considered as a potential biocontrol agent for blackberry in Australia (Bruzzese & Hasan 1996a). Laboratory inoculations of native Australian and New Zealand *Rubus* species showed that *R. gunnianus* from Tasmania, *R. schmidelioides* and *R. cissoides* from New Zealand were all moderately susceptible to *P. violaceum* (Bruzzese & Hasan 1996a). These studies concluded that damage to *R. schmidelioides* and *R. cissoides* could be expected if the rust reached New Zealand, but was unlikely to affect other endemic and commercial *Rubus* species (Bruzzese & Hasan 1996a,b). In contrast to the Australian tests, the present field surveys did not detect any non-target damage on *R. schmidelioides*. This may indicate environmental conditions in New Zealand are rarely, or not at all, conducive to infection by the rust, but future checks of this species are recommended given the Australian test results. The infection of evergreen blackberry by *P. violaceum* has previously been reported (Osterbauer et al. 2005), but is not considered deleterious given that this species is also invasive in New Zealand.

Despite its clear lack of host specificity, proponents of the blackberry biocontrol programme in Australia argued successfully that the rust should be released because “although the effect of the rust on populations of the susceptible species of Australian

TABLE 3: Other pathogens observed on host plants. N = number of plants exhibiting infection/total number of plants examined.

Plant species	Year	Pathogen	N
Mexican devil weed	2005	<i>Phaeoramularia eupatorii-odorati</i>	29/35
	2007	<i>Phaeoramularia eupatorii-odorati</i>	11/12
Blackberry	2000-02	<i>Kuehneola uredinis</i>	1/24
	2004-06	<i>Kuehneola uredinis</i>	83/188
	2008-09	<i>Septoria rubi</i>	1/3
Evergreen blackberry	2000-02	<i>Kuehneola uredinis</i>	1/2
	2004-06	<i>Kuehneola uredinis</i>	10/12
Raspberry	2004-06	<i>Phragmidium rubi-idaei</i>	30/30
Loganberry	2004-06	<i>Kuehneola uredinis</i>	10/20
<i>R. cissoides</i>	2004-06	<i>Kuehneola uredinis</i>	3/132
	2004-06	<i>Hamaspora australis</i>	8/132
<i>R. schmidelioides</i>	2004-06	<i>Kuehneola uredinis</i>	8/69
	2004-06	<i>Hamaspora australis</i>	23/69
	2004-06	<i>Uredo</i> sp.	14/69
Mouse-eared hawkweed	2005	<i>Erysiphe cichoracearum</i>	9/100
Field hawkweed	2005	<i>Phomopsis</i> sp.	1/30
King devil hawkweed	2005	<i>Erysiphe cichoracearum</i>	1/70
Tussock hawkweed	2005	<i>Ramularia</i> sp.	1/60
Bridal creeper	2007-09	<i>Colletotrichum gloeosporioides</i>	9/15
Climbing asparagus	2007-09	<i>Colletotrichum gloeosporioides</i>	8/27
Californian thistle	2001-04	<i>Phoma exigua</i>	31/172
	2007-09	<i>Phoma exigua</i>	9/55
	2007-09	<i>Alternaria</i> spp.	5/55
	2007-09	<i>Colletotrichum</i> sp.	2/55
Marsh thistle	2007-09	<i>Botrytis cinerea</i>	1/4
Scotch thistle	2001-04	<i>Puccinia cnici</i>	19/29
	2001-04	<i>Oidium</i> sp.	3/29
	2001-04	<i>Alternaria</i> sp.	7/29
	2007-09	<i>Botrytis cinerea</i>	1/25
Nodding thistle	2007-09	<i>Phoma exigua</i>	2/16
	2007-09	<i>Colletotrichum</i> sp.	1/16

and New Zealand native *Rubus* is not known, these are not economic plants, nor are they listed as endangered species" (Barton 2005). Rightly or wrongly a decision was made in Australia that any collateral damage was an acceptable outcome for their management of blackberry. Unfortunately this decision was made on an incorrect assumption that all susceptible endemic species, such as *R. schmidelioides*, were not endangered. However, in many parts of New Zealand this species is now absent or rare and regionally threatened (Stanley et al. 2005). This case study emphasises the importance of close Australasian collaboration when highly mobile biocontrol agents are being considered.

Additional strains of *P. violaceum* were officially permitted for release in Australia in 1991 and 2004 (Gomez et al. 2006). It is therefore recommended that ongoing checks of New Zealand's susceptible native *Rubus* species are made to ensure these novel

Australian strains are not contributing to any non-target damage or further regional decline of these plants.

No symptoms of non-target attack by the remaining four pathogens were observed on any of the species inspected. Previous host range testing demonstrated all of these agents damaged their respective target weed but did not infect any non-target species (Jenkins 1998; Fröhlich et al. 1999; Kleinjan et al. 2004). The present field inspections further confirmed the monospecific status of all four of these pathogens in New Zealand.

The first release of a fungus for classical biocontrol of a weed occurred in 1971, when *Puccinia chondrillina* was introduced into Australia for the control of skeletonweed (*Chondrilla juncea*) (Julien & Griffiths 1998). To date, 26 species of fungi, originating from 15 different countries, have been used as classical biocontrol agents against over 26 species of weeds in seven countries (Barton 2004). No reports were uncovered of deliberately introduced fungi unexpectedly attacking non-target plants after release (Barton 2004). The present study also confirms the low risk status for use of approved pathogens for weed biocontrol. Of the five case studies, only the mist flower smut has been deliberately introduced to New Zealand, where it has remained monospecific whilst proving a highly effective agent against its target plant (Barton et al. 2007). A parallel survey of the 14 invertebrate case studies by Paynter et al. (2004), also reported that the overall reliability of host-range testing in past weed biocontrol programmes for New Zealand has been high.

Predictions of field host-range in classical weed biocontrol programmes undertaken in New Zealand have been based on a combination of thorough host-range testing, knowledge of the taxonomy of the agent, the target weed and non-target species, and knowledge of the ecology of the agent in its native range. This has ensured that the deliberate introduction of exotic pathogens has been an environmentally safe and sustainable method for weed control.

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