Investigations into biological control of Chilean needle grass (*Nassella neesiana*) in Australia and New Zealand

Freda A. Anderson[1], Lucrecia Gallego[1], Gabriel Roth[1], Eduardo Botto[2], David McLaren[3,4] and Jane Barton[5]
[1] CERZOS-UNS, Camino La Carrindanga Km 7, 8000, Bahía Blanca, Argentina
[2] Insectario de Investigaciones para Lucha Biológica, IMYZA-CNIA, INTA. C.C. 25, (1712) Castelar, Buenos Aires, Argentina
[3] Department of Primary Industries, Victorian AgriBiosciences Centre, Bundoora, Vic 3083, Australia
[4] La Trobe University, Bundoora, Vic 3086, Australia
[5] Contractor to Landcare Research, Private Bag 92170, Auckland, New Zealand
Corresponding author: anderson@criba.edu.ar

Summary *Nassella neesiana* (Chilean needle grass, Poaceae) is a Weed of National Significance in Australia and a declared pest plant in parts of New Zealand. Field observations and laboratory experiments have been undertaken in Argentina to identify fungal pathogens suitable as biocontrol agents. *Uromyces pencanus* has been prioritised for host specificity testing as it has been observed causing severe damage to *N. neesiana* in the field; it is relatively easy to mass rear and has been shown to be very host specific. Conducting a complex biological control project in a foreign country is both costly and time consuming. Progress towards completion of host specificity testing and issues encountered in conducting this project in the country of origin, Argentina, are discussed.

Keywords *Nassella neesiana***,** biological control, rusts, grasses, host specificity.

INTRODUCTION

Nassella neesiana (Trin. & Rupr.) Barkworth (Chilean needle grass) (Poaceae) is a South American tussock-forming grass that is a Weed of National Significance in Australia (Thorp and Lynch 2000). It is also a recognised weed in the Auckland, Hawke's Bay and Marlborough regions of New Zealand (MAF 2009). A biological control project was initiated to identify potential pathogens for control of *N. neesiana* and *N. trichotoma* (Nees) Hack. ex Arechav. (serrated tussock) in 1999. Potential biological pathogens for *N. trichotoma* were either not host specific, not virulent or were too difficult to mass rear. As a consequence, this project is now concentrating on biological control of *N. neesiana* and due to its virulence and host specificity, the rust *Uromyces pencanus* Arth. & Holw. (Anderson *et al.* 2010) is a priority potential agent. This paper gives an update on progress towards host specificity testing of *U. pencanus* (Up) and its potential introduction into Australia and New Zealand as a biological control agent.

MATERIALS AND METHODS

Host specificity testing was initially planned to occur within quarantine facilities in Australia. However, a review by the Australian Quarantine and Inspection Service (AQIS) regulations during 2003 determined that air borne pathogens could only be introduced and assessed within quarantine facilities having a Physical Containment level 4 (PC4) status. No biological control quarantine facilities had such a high containment status in Australia at this time. As a consequence, the host specificity testing had to be conducted within the country of origin, Argentina. A host specificity test list of 63 plant species has been developed providing a range of significant Australian native and commercially important grass species selected according to their taxonomic relatedness to the target weed, *N. neesiana.* One of the two screened isolates of the rust fungus (Up 27) was selected from a field site at Bahia Blanca in Argentina on the basis of its virulence against Australian accessions of *N. neesiana* (Anderson *et al.* 2006). Batches of 4–5 species were screened at one time, four plants per species with a total of 8 plants (unless otherwise stated) being tested for each test species. Dry urediniospores (harvested from infected plants by means of a cyclone collector and stored at −70°C until use) mixed in talcum powder (ratio 1:30) were brushed onto the adaxial side of leaves, two per plant, which were later sprayed with water. Accessions of *N. neesiana* from the Australian Capital Territory (ACT) were included in each test as positive controls. Inoculated plants were maintained at 18–20°C under a 12 h photoperiod and 0% relative humidity (RH) for 48 h, after which they were kept under the same conditions but at 70% RH for 4 weeks, double the latent period for infection and sporulation on the positive controls. All inoculated plants were then inspected for external symptoms of infection and samples taken for internal microscopic examination. The samples were stained-cleared using a

modification of the Bruzzeze and Hasan (1983) method. Each species was screened twice.

RESULTS

Details of results of host specificity testing are presented in Table 1. Eight out of nine Australian accessions of *N. neesiana* proved to be susceptible to isolate Up 27, with development of normal uredinia on infected leaves. *Nassella neesiana* from Ballarat (Australia) and two accessions from New Zealand (Hawke's Bay and Auckland) did not become infected. An accession of *N. neesiana* from Marlborough, New Zealand, was susceptible to *U. pencanus* (Up 27). Rust infection produced premature senescence of infected leaves. There were no pustules formed on any of the other host species tested. Macroscopic small dark spots were observed on leaves of *Austrostipa breviglumis* (J.M.Black) S.W.L.Jacobs & J.Everett and *A. eromophila* (Reader) S.W.L.Jacobs & J.Everett. Microscopic examination of these species revealed that hyphae penetration had occurred and that haustoria had developed but no further development of spore growth occurred. Small yellow leaf blemishes were observed on several species (Table 1) but no pustules developed in any of the non-target plants.

DISCUSSION

Conducting an extensive biological control project in the country of origin, Argentina, including agent exploration, assessment of agent biology, development of mass-rearing techniques and assessment of agent host specificity testing within a foreign country, has presented many challenges. Conducting the host specificity testing has been an expensive and protracted process. Understandably, Argentinean authorities didn't wish to have the threat of Australian and New Zealand native grass species escaping from low level containment facilities at the project base station located at CERZOS in Bahia Blanca, Argentina. After extensive investigations, a quarantine facility was identified in IMYZA-INTA, Castelar, Buenos Aires, Argentina, that could safely contain the species proposed for host specificity testing. Obtaining permission was a prolonged process requiring development of a detailed importation protocol including a phytosanitory certification that the seed was free of a long list of insect, weed and nematode pests. Permission for importation had to be obtained from the Argentinean Ministry of Economy and Production and the Ministry of Social Development, while the shipments were supervised and assessed by SENASA (equivalent of AQIS in

Table 1. Host specificity testing results of Poaceae species inoculated with *Uromyces pencanus*.

Species	Macroscopic symptoms [% occurrence]	Microscopic symptoms[1] (Examined from samples collected from one or two plants per species)
Nassella neesiana [ACT] #	Pustules [75%]	1, 2, 8
N. neesiana [Goulburn, NSW] #	Pustules [100%]	NE
N. neesiana [Fitzroy flats, NSW] #	Pustules [100%]	1, 2, 8
N. neesiana [Edgars Rd, Vic] #	Pustules [50%]	NE
N. neesiana [Truganina, Vic] #	Pustules [62.5%]	NE
N. neesiana [Ballarat, Vic] #	None [0%]	NE
N. neesiana [Bacchus Marsh, Vic] #	Pustules [12.5%]	NE
N. neesiana [Laverton, Vic] #	Pustules [50%]	NE
N. neesiana [Clifton Springs, Qld] #	Pustules [62.5%]	1, 2, 8
N. neesiana [Hawke's Bay, NZ] #	None [0%]	1, 2, 5
N. neesiana [Auckland, NZ] #	None [0%]	1, 2, (3), (5), 6, 7
N. neesiana [Marlborough, NZ] #	Pustules [100%]	1, 2, 8
Nassella trichotoma [North Canterbury, NZ] #	None [0%]	1, 4
N. trichotoma [Dalgety, NSW] #	Yellow leaf spots [62.5%]	1, 2, 4
Nassella hyalina #	Yellow leaf spots [37.5%]	1, 2, (3), 4
Nassella tenuissima #	None [0%]	1, 2, (3), 4
Achnatherum caudatum #	None [0%]	1, 2, (3), 5, (6)
Piptochaetium montevidense #	Yellow leaf spots [25%]	1, 2, 3, (6)
Piptatherum miliaceum #	Yellow leaf spots [20%, 5 plants tested]	(1), 2, 7, (8)

Table 1 continued on next page/…

Table 1. Continued from previous page.

Species	Macroscopic symptoms [% occurrence]	Microscopic symptoms[1] (Examined from samples collected from one or two plants per species)
Austrostipa aristiglumis	None [0%]	1, 2, 3, 5
Austrostipa scabra	None [0%]	1, 2, (3), 5, (6), (7?)
Austrostipa bigeniculata	None [0%]	1, 2, 3, (5), 7
Austrostipa breviglumis	Dark leaf spots [25%]	1, 2, 3, 5, 7, (8?)
Austrostipa eremophila	Dark leaf spots [17%, 6 plants tested]	1, 2, 3, 5, 7, (8)
Austrostipa mollis	None [0%, 1 plant tested]	1, 2, (3), (7)
Austrostipa verticillata	None [0%, 2 plants tested]	(1), (2), (7)
Avena sativa # ◆	None [0%]	(1), (2), 4
Phalaris aquatica #	Yellow leaf spots [25%]	1, 2, 5, (6), (7)
Lolium perenne #	None [0%]	1, 2, 3, 5
Festuca arundinacea #	None [0%]	1, 2, 3, 5, (7)
Bromus catharticus #	Yellow leaf spots [62.5%]	1, 2, 3, 5
Hordeum vulgare #, ◆	Yellow leaf spots [62.5%]	1, 2, 5
Triticum aestivum unknown cv. #, ◆	Yellow leaf spots [37.5%]	1, 2, 3, 4
T. aestivum cv. ACA 303 #, ◆	None [0%]	1, 2, 3, 5, 6, 7
T. aestivum cv. Liquén #, ◆	Yellow leaf spots [37.5%]	1, 2, 3, 5, 6, 7
T. aestivum cv. Arriero #, ◆	None [0%]	1, 2, 3, 5, 6, 7
T. aestivum cv. Sureño #, ◆	None [0%]	1, 2, 3, 5, 6, 7
T. aestivum cv. Malevo #, ◆	Yellow leaf spots [25%]	1, 2, (3), 5, 6, 7
T. aestivum cv. Guapo #, ◆	Yellow leaf spots [37.5%]	1, 2, 3, 5, 6, 7
Secale cereale #, ◆	None [0%]	1, 2, 3, 5, 6
Phyllostachys aurea #	None [0%]	1, (2), (3), 4
Phragmites australis	None [0%]	1, 2, 3, 4
Chloris gayana #	None [0%]	1, (2), 3, 4
Cynodon dactylon #	None [0%]	1, 2, 4
Sporobolus rigens #	None [0%]	1, (2), 4
Aristida pallens #	Yellow leaf spots [25%]	1, (2), 4
Pennisetum clandesinum #	None [0%]	1, 2, 5, 6, 7
Zea mays #,◆	None [0%]	1, 2, 3, 5, 6, 7
Sorghum halepense #,	None [0%]	1, 2, 5
Brachypodium distachyon #	None [0%]	1, 1a, (2), (3), 7
Oryza sativa #, ◆	None [0%]	1, 2, 3, 5, (6)
Eragrostis curvula #	None [0%]	1, (2), (3), 4
Cymbopogon citratus #	None [0%]	(1)
Poa ligularis #	None [0%]	1, 2, 3, (5)
Elymus scabrifolius #	Yellow leaf spots [62.5%]	1, (2), 3, (6), (7)
Bothriochloa springfieldii #	None [0%]	(1), (2), (5), (6)
Paspalum dilatatum #	Yellow leaf spots [12.5%]	1, (2), 5, 6, (7)
Dicanthium aristatum #	None [0%]	1, (2), 3, 5, 6, 7

[1] Abbreviations used: 1 = normal spore germination; 1a = abnormal spore germination; 2 = normal appresoria; 3 = abnormal appresoria or non-stomatic appresoria; 4 = penetration not observed; 5 = penetration, two to four infection hyphae formed from substomatal vesicle, growth cessation; 6 = penetration + contact with plants cells, growth cessation; 7 = penetration + contact with plants cells + thickening of cells wall, growth cessation; 8 = haustoria, abundant intercellular mycelia. () = observation was infrequent; ? = a doubtful observation; # = exotic species to Australia; ◆ = crop; NE = not examined.

Australia). Additional issues have been recently encountered with poor germination or dormancy issues of some of the Australian and New Zealand species being tested.

Host specificity testing results using *U. pencanus* isolate 'Up 27' are promising in that to date no pustules have developed on any test species other than the target species *N. neesiana.* There has been some development of the rust within the leaves of *Austrostipa eremophila*, *A. breviglumis* and *Piptatherum miliaceum* where a few haustoria and some development of intercellular mycelium were observed. However, resistance mechanisms (thickening of cell walls upon hyphal contact) were also observed within sections of the same samples, suggesting that the rust will not persist within these species. Some yellow leaf spots did form on several other species but microscopic studies showed that these resulted from abnormal hyphae penetration that soon ceased. Negotiations are currently underway for a third shipment of fresh host specificity seeds into Argentina from Australia and New Zealand for those species proving difficult to germinate and it is anticipated that testing should be completed by August 2010. The Victorian Department of Primary Industries is currently building a new BioSciences Research Centre facility in collaboration with La Trobe University that will have a quarantine building enabling testing of air borne pathogens, and this should increase Australia's capacity to fast track complex biological control projects without the substantial costs and delays encountered with the current biological control project undertaken in Argentina. New Zealand Landcare Research has had preliminary discussions with the New Zealand Environmental Risk Management Authority (ERMA) and they have agreed that there are enough host specificity data to begin development of an application for release of *U. pencanus* for control of *N. neesiana* in New Zealand during 2010–11. If successful and host specificity testing can be concluded by August 2010, it is anticipated that an application for release of *U. pencanus* in Australia will be made during late 2010 or early 2011.

ACKNOWLEDGMENTS

The authors acknowledge the support provided by the Australian Commonwealth Government through the Defeating the Weed Menace program and for supplying the resources enabling the project to proceed. CERZOS-CONICET and IMYZA-INTA are thanked for providing laboratory and glasshouse facilities in Bahía Blanca and Buenos Aires, Argentina.

REFERENCES

Anderson, F.E., Barton, J. and McLaren, D.A. (2010). Studies on *Uromyces pencanus*, a prospective biological control agent for *Nassella neesiana* (Poaceae) in Australia and New Zealand, *Australasian Plant Pathology* 39 (1), 69-78.

Anderson, F.E., Diaz, M.L. and McLaren, D.A. (2006). Current status of research on potential biological control agents for *Nassella neesiana* and *Nassella trichotoma* (Poaceae) in Australia. Proceedings of the 15th Australian Weeds Conference, eds C. Preston, J.H. Watts and N.D. Crossman, pp. 591-4. (Weed Management Society of South Australia, Adelaide.)

Bruzzeze, E. and Hasan, S. (1983). A whole leaf clearing and staining technique for host specificity studies of rust fungi. *Plant Pathology* 32, 335-8.

MAF (2009). Ministry for Agriculture and Forestry, Biosecurity New Zealand. Map display – regional pest management. *Nassella neesiana*. Available at http://www.biosecurityperformance.maf.govt.nz/public/Map.aspx

Thorp, J.R. and Lynch, R. (2000). 'The determination of Weeds of National Significance', pp. 1-234. (National Weeds Strategy Executive Committee, Launceston).