An update on progress towards biological control of *Nassella neesiana* in Australia and New Zealand

Freda E. Anderson[1], Andrea C. Flemmer[1], Paula V. Hansen[1], David A. McLaren[2,3] and Jane Barton[4]
[1] CERZOS-UNS, Camino La Carrindanga Km 7, 8000, Bahía Blanca, Argentina
[2] Department of Primary Industries Frankston, PO Box 48, Frankston, Victoria 3199, Australia
[3] CRC for Australian Weed Management
[4] Contractor to Landcare Research, Private Bag 92170, Auckland, New Zealand
Email: David.McLaren@dpi.vic.gov.au

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. Three rust species have been selected for further study: *Uromyces pencanus, Puccinia graminella* and *Puccinia nassellae*. All three have been observed causing severe damage to *N. neesiana* in the field and are believed to be quite host specific. Attempts to elucidate their life-cycles experimentally have failed to-date and this is discussed. *Uromyces pencanus* is the most promising of the three because reliable methods have been developed for culturing and storing inoculum and applying it to plants. This paper outlines progress made towards assessing its host specificity and determining its life cycle and also discusses recent findings on *P. graminella*.

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

INTRODUCTION

Nassella neesiana (Trin. & Rupr.) Barkworth (Chilean needle grass) is a tussock forming grass from South America that is a declared noxious weed across Australia and a Weed of National Significance there (Thorpe and Lynch 2000). The plant is also a recognised weed in three regions of New Zealand (Auckland, Hawke's Bay and Marlborough). A biological control project was initiated in 1999 to investigate pathogens for control of *Nassella trichotoma* (Nees) Hack. ex Arechav. (serrated tussock) and *N. neesiana* (Anderson *et al.* 2006). Due to their virulence and host specificity, researchers have prioritised the rusts *Puccinia nassellae* Arth. & Holw., *Puccinia graminella* Diet. & Holw. and *Uromyces pencanus* Arth. & Holw. for the biological control of *N. neesiana.* This paper gives a short update on investigations into the life cycles and host specificities of *U. pencanus* and *P. graminella.*

MATERIALS AND METHODS

Uromyces pencanus

Host specificity testing A host specificity test list of 63 plant species in the family Poaceae, which grow in Australia and New Zealand, has been developed using the order of taxonomic relatedness of the test plants to the target weed, *N. neesiana.* A *U. pencanus* isolate, Up 27, was selected for these tests on the basis of 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. Dry urediniospores 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 100% relative humidity (RH) for 48 h, after which they were kept under the same conditions but at 70% RH for four 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.

Life cycle Teliospores have repeatedly failed to germinate under a range of treatments applied to try and induce basidiospore formation. (Anderson *et al.* 2006). Therefore at all field sites where rust infected *N. neesiana* plants have been recorded a search is underway for potential alternate hosts. All evidence collected to date on the life cycle is discussed.

Puccinia graminella

Culture and mass rearing technique Two geographically distant isolates of *P. graminella* are being examined. Plants naturally infected with each of the isolates were transplanted into pots and brought back to the laboratory as inoculum sources and spores from

these were used to inoculate *N. neesiana* plants from different accessions as indicated in Table 2. Batches of 4–15 plants were inoculated at one time, with batch size dependent on availability of suitable aeciospores. Whole aecia were clipped off and transferred from infected to healthy leaves under the stereomicroscope with a pair of fine forceps and then evenly distributed with a paintbrush. Inoculated plants were kept at 100% RH (but not sprayed) for 48 hours, at 15°C and under a 12 h photoperiod, and later incubated under the same conditions except that RH was reduced to 70%. Plants were inspected for aecia for up to a month after inoculation after which they were discarded if none developed. Infected plants were kept as spore sources for further inoculations. On the basis of previous unpublished results, spores from closed, one-month-old aecia growing on green leaves were used as inoculum to re-infect new plants.

RESULTS

Uromyces pencanus

Host specificity testing Details of results are presented in Table 1. Most 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 from both accessions from New Zealand that were tested, did not become infected. There were no pustules formed on any of the other tested species. Small yellow specks were formed on a few species. Plants belonging to *N. neesiana* were not examined at the microscopic level because the leaf anatomy did not allow observation of post penetration events. The infection process in *N. neesiana* is being studied in detail by another technique, which involves cutting fine leaf sections using a rotary microtome, and results of this study will be published elsewhere. This technique may also be needed to assess other species where the traditional clearing–staining technique does not give satisfactory results.

Life cycle Only uredinia and telia have been recorded on *N. neesiana* in the field. A series of experiments designed to induce the production of basidiospores have failed (unpublished data) which suggests that telia may have lost the capacity to germinate. No consistent association with any aecia-infected alternate host has been recorded in the field after many field trips covering thousands of kilometres during the last four years.

Puccinia graminella

Culture and mass rearing technique It has not been possible to establish a pure culture of this rust. Although satisfactory inoculation protocols have been developed, it would appear that there is qualitative resistance between this rust and some *N. neesiana* accessions (unpublished data). The fact that many of the inoculated plants exhibited resistant genotypes to the tested isolates made it impossible to mass produce spores. Results of inoculation tests with the two tested isolates are presented in Table 2.

DISCUSSION

Results presented here on host specificity testing of *U. pencanus* with isolate Up 27 are only partial, so it is not possible to assess the specificity of this agent fully at this stage. Notwithstanding, results are quite promising, as *U. pencanus* pustules only formed on *N. neesiana.* Some yellow spots did form on other species but microscopic studies showed that these

Table 1. Macro (A) and microsymptoms (B) recorded on species within Poaceae inoculated with *U. pencanus*.

Species	A	B
N. trichotoma (North Canterbury, NZ)	–	1
N. neesiana (ACT)[A]	Pustules	NE
N. neesiana (Edgar Rd, Vic)[A]	Pustules	NE
N. neesiana (Truganina, Vic)[A]	Pustules	NE
N. neesiana (Ballarat, Vic)[A]	–	NE
N. neesiana (Mulwaree Ponds, NSW)[A]	Pustules	NE
*N. neesiana (*Bacchus Marsh, Vic)[A]	Pustules	NE
*N. neesiana (*Laverton, Vic)[A]	Pustules	NE
N. neesiana (Hawke's Bay, NZ)	–	NE
N. neesiana (Auckland, NZ)	–	NE
Nassella hyalina	Yellow spots	1, 2, 3, 4
Austrostipa aristiglumis	–	1, 2, 3, 4
Avena sativa	–	1, 2, 4
Phalaris aquatica	Yellow spots	1,2,5
Lolium perenne	–	1,2,3,5
Festuca arundinacea	–	1,2,3,5
Bromus catharticus	Yellow spots	1,2,3, 5
Hordeum vulgare	Yellow spots	1,2,5
Triticum aestivum	Yellow spots	1,2,3,4
Secale cereale	–	1,2,3,5
Zea mays	–	1,2,4
Sorghum halepense	–	1,2,5

[A]Material from Australia. 1 – normal spore germination; 2 – normal appresorium formation; 3 – abnormal appresorium formation, appresoria not on stomata; 4 – no sign of penetration observed; 5 – abnormal penetration, hyphae growth ceased; NE – not examined.

Table 2. Inoculations with *P. graminella*.

Plant accession	Rust isolate	No. plants inoculated	% infection
64	PG64	191	16
161	PG161	10	40
45	PG161	151	29
27	PG161	130	28.5

Location of sites. 64: 31°54.45'S, 64°31.39'W (province of Córdoba); **161**: 38°05.47'S, 61°56.49'W, **45**: 38°21.93'S, 62°16.89'W; **27**: 38°39.96'S, 62°14.07'W (province of Buenos Aires).

resulted from abnormal hyphae penetration that soon ceased. Results presented on the life cycle are in contrast with previously published descriptions (Arthur 1925, Greene and Cummins 1958), where aecia are also mentioned. We believe the aecia they described most probably belonged to the life cycle of *Puccinia graminella* as we have often observed these infecting *N. neesiana* with *U. pencanus.* This will be discussed further elsewhere. A hypothetical life cycle is proposed in which this rust cycles as urediniospores and either persists as latent infections in its grass host or becomes locally extinct during unfavourable conditions. While abundant telia are produced they seem to have become redundant. New searches are currently being carried out to try and identify an isolate of *U. pencanus* that will infect those *N. neesiana* accessions not susceptible to Up27.

Results of inoculation experiments with spores of *P. graminella* are disappointing. Two isolates have been tested so far with poor infection results, although PG 161 behaved somewhat better than PG 64 (Table 2). Results not presented here in detail strongly suggest the existence of qualitative resistance within this pathosystem. Experiments are being planned to demonstrate this experimentally. It has been observed that heavily infected plants tend to produce less seed than healthy ones. Consequently, it is speculated that we collected more seed from resistant than susceptible *N. neesiana* genotypes from site 64 (Table 2) and that this explains the low levels of infection obtained on plants grown from that seed. In the case of accession 161, seed was collected late in the season and most failed to germinate so that there were very few plants available from this site for inoculation. Plants from the closest available sites were therefore also tested and some from sites 27 and 45 were found to be susceptible. Results could have been very different if plants from seed collected at the same site and from susceptible genotypes had been used in all experiments as indicated by the much higher rates of success (40 %) obtained with our few plants from site 161 (Table 2). Further experimentation with these and other *P. graminella* isolates is needed to properly assess the potential of this rust as a biological control agent for *N. neesiana* in Australia and New Zealand.

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 is thanked for providing laboratory and glasshouse facilities in Bahía Blanca, Argentina.

REFERENCES

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).

Arthur, J.C. (1925). The grass rusts of South America; based on the Holway collections. *Proceedings of the American Philosophical Society* 64, 131-223.

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.

Greene, H.C. and Cummins, G.B. (1958). A synopsis of the uredinales which parasitize grasses of the genera *Stipa* and *Nassella*. *Mycologia* 50, 6-35.

Thorpe, J.R. and Lynch, R. (2000). 'The determination of weeds of national significance'. (National Weeds Strategy Executive Committee, Launceston).