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

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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 (Thorp and Lynch 2000). The plant is also a recognised pest 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 prioritized biological control of *N. neesiana* using the rusts *Puccinia nassellae* Arth. & Holw., *Puccinia graminella* Diet. & Holw. and *Uromyces pencanus* Arth. & Holw. 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. *Nassella neesiana* accessions from the Australian Capital Territory (ACT) were included in each test as positive controls. Inoculated plants were maintained at 18-20°C under a 12hr photoperiod and 100% relative humidity (RH) for 48hrs, 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 experimentally inducement treatments (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 both 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 below. Batches of 4 to 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 12hr 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.

Table 1. Location of field sites mentioned in text

Site ID	Latitude	Longitude	Province
NT 27	38 39.96	62 14.07	Buenos Aires
NT 45	38 21.93	62 16.89	Buenos Aires
NT 64	31 54.45	64 31.39	Córdoba
NN 16	38 05.47	61 56.49	Buenos Aires

RESULTS

Uromyces pencanus

Host specificity testing. Details of results are presented in Table 2. 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 from this 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 Km during the last four years.

Table 2. 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) *	Pustules	NE
N. neesiana (Edgar Rd) *	Pustules	NE
N. neesiana (Trugamina) *	Pustules	NE
N. neesiana (Ballarat) *	---	NE
N. neesiana (Mulwaree Ponds) *	Pustules	NE
N. neesiana (Bacchus Marsh) *	Pustules	NE
N. neesiana (Laverton) *	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	EU
Lolium perenne	---	EU
Festuca arundinacea	---	EU
Bromus catharticus	Yellow spots	EU
Hordeum vulgare	Yellow spots	1,2,5
Triticum aestivum	Yellow spots	1,2,3,4
Secale cereale	---	EU
Zea mays	---	1,2,4
Sorghum halepense	---	EU

*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; NE - not examined; EU- examination underway

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 3.

Table 3. Inoculations with *P. graminella*

Plant accession	Spore source	N° infected/ inoculated plants	% infection
NT64	PG64	30/191	16
NN16	PG16	4/10	40
NT 45	PG16	44/151	29
NT 27	PG16	37/130	28.5

DISCUSSION

Results presented here on host specificity testing of *U. pencanus* with isolate Up 27 are only partial, so it is not possible to fully assess the specificity of this agent at this stage. Notwithstanding, results are quite promising, as in most cases no penetration of the fungus into the tissues of the non-host tested species was recorded. Penetration was only observed in *Hordeum vulgare*, where growth of the abnormal penetration hyphae 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 unfavorable conditions. While abundant telia are produced these 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 16 behaved somewhat better than PG 64 (Table 3). 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. It is speculated that consequently we collected more seed from resistant than susceptible *N. neesiana* genotypes from site NT 64 (Table 3) and that this explains the low levels of infection obtained on plants grown from that seed. In the case of accession NN 16, 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 NT 27 and NT 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 NN 16 (Table 3). 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.

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