Biological control of Chilean needle grass (*Nassella neesiana*, Poaceae) in Australasia. Application to Release.

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Summary: *Nassella neesiana* (Chilean needle grass) is a significant agricultural and environmental weed in Australia and New Zealand and the two countries have worked together for ca. ten years to find a suitable biological control agent to use against it. The only suitable agent found in the home range of the weed (South America), and the subject of this paper, is the rust fungus *Uromyces pencanus* (Anderson *et al.* 2010). Most of the pathogens that have been used for classical biocontrol of weeds world-wide have been rusts, and they have never caused unpredicted non-target damage in the field (Barton 2004). *Uromyces pencanus* has been observed killing infected leaves, and reducing seed production, of *N. neesiana* in the field in Argentina (Anderson *et al.* 2006). The rust is particularly damaging in dry weather. Laboratory experiments have confirmed the rust can reduce the growth of infected plants (Giordano *et al.* 2009).

A rust may complete its life cycle on only one host, or it can form some spore types on one host and other types on another (not closely related) host (Kirk, *et al.* 2008). *Uromyces pencanus* has been reported in the literature to form three types of spores on *N. neesiana*: urediniospores, teliospores and aeciospores (Arthur 1925). We have previously shown that aeciospores often found on *N. neesiana* belong to the life cycle of another rust (*Puccinia graminella*) (Anderson *et.* 2010). Teliospores of *U. pencanus* appear to have lost the ability to produce basidiospores and therefore, the nature of its life cycle can not be categorically proven. It is believed to cycle as urediniospores on its grass host (Anderson *et al.* 2010).

Mycoparasites that exist in Australia are similar to those that exist in Argentina, so there is no reason to believe they will significantly hamper the activity of the rust here. It is extremely unlikely that the introduction of *U. pencanus* would lead to adverse impacts on native rusts through hybridization.

Extensive host range testing has been conducted with a single strain of *U. pencanus* (UP 27). It was applied to 79 taxa including 14 populations of *N. neesiana*, 2 populations of the weed serrated tussock and 7 cultivars of wheat (in total, 60 species were tested). UP 27 was shown to be highly host specific, however it did form pustules on two *Austrostipa* species, *A. compressa* and *A. macalpinei.* On these species, spore formation was 30 and 10 times less respectively than on the target plant. When the *U. pencanus* spores collected from *Austrostipa compressa* were applied to *N. neesiana* plants, no infection resulted. Climatic isolation from current and future spread of *N. neesiana* (Gallagher and Duursma 2012) makes it unlikely that Western Australian populations of *A. compressa* or *A. macalpinei* will ever be in close association with *N. neesiana* making it unlikely they would ever come into contact with spore concentrations capable of infection. The ephemeral biology of these *Austrostipa* species (Baker *et al.* 2005) further reduces the likelihood of *U. pencanus* attack on these species.

In members of all tested species including accessions of *N. neesiana* that did not become infected with *U. pencanus*, one or more defence mechanisms were found to occur in response to the rust. These included: abnormal spore germination; formation of distorted appresoria and/or incorrect appresorium positioning (non-recognition reaction); inhibition of growth shortly after penetration; thickening of host cell walls by build-up of callose-like materials in response to the presence of -or contact with- fungal hyphae and /or haustorium mother cells; collapse and/or necrosis of cells in invaded areas; encasement of haustoria by deposition of callose-like material; and senescence of host cells in response to haustorium formation. These mechanisms restrained fungal development within the host tissues and account for the failure of the rust to produce pustules on these species.

Development of intercellular mycelium and a few haustoria was observed within leaves of *Austrostipa eremophila*, *A. breviglumis*, *A. mollis*,

A. nitida, A. nullanulla, A. platychaeta, A. stuposa and *Piptatherum miliaceum.* However, the rust did not develop further to form uredinia on these species. Since it is the formation of uredinia (and the resulting disruption to the leaf epidermis) that is the main cause of damage to the target weed these plants would be unlikely to be significantly adversely affected by the rust if they were to encounter it in the field.

Strain UP 27 will form pustules on 7 out of 9 *N. neesiana* populations collected from Australia. Therefore, other strains of *U. pencanus* in Argentina may be required in the future to control *N. neesiana* infestations. If so, there is no reason to believe these other strains would have a broader host range than UP 27. If *U. pencanus* were introduced to Australia, there is no reason to believe its host range would broaden over time through evolution.

To conclude: The introduction of *U. pencanus* to Australia is unlikely to cause any significant negative impact on native or otherwise valued plants or fungi but host testing does show some risk to two closely related *Austrostipa* species. The massive environmental and agricultural impacts being caused by *N. neesiana* to Australian grasslands outweighs the relatively small risk that there may be some off target damage to two *Austrostipa* species. The introduction and release of *U. pencanus* for biological control of *N. neesiana* was approved in New Zealand in June 2011. We would recommend its introduction and release on *N. neesiana* in Australia as well.

Keywords: Biological control, rust fungi, Chilean needle grass, *Uromyces pencanus*

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