Biological control of Navua sedge (*Cyperus aromaticus*) in Australia

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Summary Navua sedge (*Cyperus aromaticus*), a native of equatorial Africa, is an extremely aggressive perennial sedge, affecting beef, dairy, and sugarcane industries in the wet tropical regions of northern Queensland, Australia. Navua sedge is also a major weed of crops and pastures in Fiji and other South Pacific Islands. Navua sedge is unpalatable and forms dense stands that can replace palatable tropical pasture species. Current management options are mechanical and chemical, which are expensive and offer only short-term relief. Biological control is considered the most cost-effective and long-term management option. Navua sedge has been approved as a target for biological control in Australia, where a biological control program was initiated in 2017.

Native range surveys in Kenya, Nigeria and Tanzania found three promising biological control fungi; specifically, a smut fungus (*Cintractia kyllingae*) that infects flower heads and seeds; a rust fungus (*Uredo kyllingae-erectae*) that attacks leaves and stems; and an inflorescence-colonising ascomycete (*Curvularia tanzanica*). Field surveys have only recorded these fungi in association with Navua sedge. For effective biological control of Navua sedge, multiple agents that target different parts of the sedge may be needed to reduce seed production and minimise its impact and spread. These three fungal pathogens have been exported to CABI-UK, where host-specificity testing for *C. kyllingae* is in progress; and testing for *U. kyllingae-erectae* will commence soon. If proven to be host specific, the pathogens will be released in Australia. Current research in Australia has focused on the search for local pathogens on Navua sedge that may have potential as mycoherbicides. Several fungi of interest have been found in Australia, including species of *Curvularia*, *Epicoccum*, *Fusarium*, *Neopestalotiopsis*, *Nigrospora*, and *Phaeosphaeria*, as well as other fungal pathogens yet to be identified.

Keywords Navua sedge, *Cyperus aromaticus*, weed, biological control, Australia.

INTRODUCTION

Navua sedge, *Cyperus aromaticus* (Cyperaceae), is an extremely aggressive and invasive sedge of tropical environments. In the Australian wet tropics, Navua sedge is of concern to the beef, dairy, and sugarcane industries (Shi *et al*. 2021). Navua sedge forms dense strands, and strongly competes with crops and pastures for nutrients, light and moisture (Vitelli *et al.* 2010). Navua sedge provides little nutritional value for cattle and replaces palatable tropical pasture species (Vitelli *et al.* 2010). In Fiji, Navua sedge lowered the carrying capacity of pastures by up to 40 %, which led to reduced milk production (Karan 1975; Kerr *et al.* 1995).

Current management options are mechanical and chemical, which are expensive and offer only short-term solutions. Mechanical control methods require repeated applications and often increase dispersal of the seeds, and thus further contamination. Halosulfuron is the only approved herbicide for Navua sedge control (Vitelli *et al.* 2010, Vogler *et al*. 2015). This chemical kills the aerial part of Navua sedge while established underground rhizomes remain viable (Chadha *et al*. 2022). There is also an issue of herbicide residues and prolonged withholding periods for both beef and dairy cattle.

As biological control may be a cost-effective and long-term management option for Navua sedge, the invasive weed has been approved as a target for biological control in Australia. In 2017, a biological control program commenced that aims to (1) conduct native range surveys and identify prospective biological control agents, (2) conduct host-specificity tests for prioritised agents, and (3) seek the release of approved biological control agents in Australia.

MATERIALS AND METHODS

Native range studies Surveys were conducted in Kenya, Tanzania (May – June 2018, December 2019, and March 2021) and Nigeria (June 2019, March – November 2020, and June 2021), focusing on

locations based on herbaria records. Navua sedge was sampled for plant pathogens, phytophagous insects, and mites. Other co-occurring sedges were also sampled to ascertain the field host range of prospective agents associated with Navua sedge in the field. Prospective biological agents were prioritised based on disease/infestation severity and observed field-host range.

Test plants for host specificity The centrifugal phylogenetic method (Wapshere 1974) was used to compile a plant list for host-specificity testing (Taylor and Dhileepan 2021). Test-plant species were selected based on phylogenetic relationships, following the familial classification of the Angiosperm Phylogeny Group (mobot.org/MOBOT/research/APweb/). Within the phylogenetic framework, an emphasis was placed on native and economically important species with a biogeographic overlap with Navua sedge in Australia.

Host-specificity tests Multiple consignments of Navua sedge plants (as seeds and rhizomes) sourced from Queensland were exported to quarantine facilities at CABI, UK to establish *in planta* cultures of the respective pathogens and determine their life cycles. Plants of other species (as seeds and bare rooted plants) were also sent to CABI, UK for host-specificity testing. Specimens of flower smut and leaf and stem rust from multiple sites in Nigeria and Tanzania were exported to CABI, UK. The inoculations methodology for the flower smut and the leaf and stem rust were developed and spores of the fungal pathogens were stored in liquid nitrogen for future use. Flower smut and leaf rust strains from Nigeria and Tanzania were assessed on Australian Navua sedge populations to select the most virulent ones for host-range testing. Life-cycle studies and host-specificity testing of the smut and life-cycle studies for the rust fungus are currently in progress in quarantine at CABI, UK.

Australian native pathogens Surveys for pathogens on Navua sedge in Queensland were conducted in September 2020, April 2021, and March 2022. Samples of leaves and stems showing symptoms of disease (leaf spots, blight, necrosis, discoloration) were examined for potential pathogens. Fungal pathogens from the samples were cultured for identification by morphological and molecular analysis. Pathogenicity and host specificity of promising pathogens as prospective mycoherbicides are being screened in containment glasshouses.

RESULTS

Biological control agents Three prospective biological control agents for Navua sedge were determined from surveys in Africa. These were (i) the smut fungus *Cintractia kyllingae* (Basidiomycota, Anthracoideaceae) that infects the inflorescences and destroys florets and seeds, (ii) the rust fungus *Uredo kyllingae-erectae* (Basidiomycota, Pucciniaceae) that attacks leaves and stems, and (iii) the fungus, *Curvularia tanzanica* (Ascomycota, Pleosporaceae) that colonises inflorescences (Crous *et al.* 2021, Kruse *et al.* 2021, Dhileepan *et al*. 2022).

Test plants for host specificity There are about 125 native *Cyperus* spp. in Australia, as well as numerous naturalised species. Based on centrifugal phylogenetic methods, the test list for Navua sedge agents includes 38 species, of which 21 are *Cyperus* spp. and 13 are from other genera of sedges (Cyperaceae). Four representatives from other families have been included as outlier species, including rice and sugar cane (Taylor and Dhileepan 2021).

Smut fungus *Cintractia kyllingae* This smut fungus was found in Kenya, Tanzania, and Nigeria. It infects some or all of the florets of the inflorescence on Navua sedge (Figure 1). In the field *C. kyllingae* was not found on other co-occurring sedges, which indicates that the pathogen may be host specific. Field samples of four smut strains from Nigeria and two smut strains from Tanzania were screened for viability and virulence on Navua sedge plants sourced from Australia, Nigeria, and Tanzania. Inoculation studies confirmed that the Australian Navua sedge is susceptible to the smut strains from both Nigeria and Tanzania. Inoculation studies also showed that young flower heads of Navua sedge plants were highly susceptible to infection by *C. kyllingae*, with many seeds being destroyed. A strain of the smut from Tanzania that was found to be particularly virulent towards Australian Navua sedge was selected for host-specificity tests that are currently in progress.

Rust fungus *Uredo kyllingae-erectae* This rust fungus infects the leaves and stems of Navua sedge (Figure 2) in Nigeria and Tanzania. Life-cycle studies and its evaluation as a potential biological control agent for Navua sedge in Australia have commenced under quarantine conditions at CABI, UK. To date, preliminary inoculation studies using four Nigerian strains and two Tanzanian strains have

produced symptoms of rust infection on Navua sedge plants collected from their respective countries, but not on Australian sourced plants. Other Nigerian stains are being evaluated to identify a rust strain(s) that is (are) pathogenic to the Australian population(s) of Navua sedge.

Figure 1. Inflorescences of Navua sedge. Healthy (left) and infected by the smut fungus *Cintractia kyllingae* (right).

Figure 2. Leaf and stem rust, *Uredo kyllingae-erectae*, on Navua sedge in Nigeria.

Flower blight *Curvularia tanzanica* Flower blight (Figure 3) was found only in Tanzania, sometimes together with the smut *C. kyllingae*. *Curvularia tanzanica* was not found in Kenya and Nigeria. *Curvularia tanzanica* colonised the inflorescences of Navua sedge and was superficially very similar in appearance to the flower smut. The flower blight samples were exported to CABI, UK, for isolation of the pathogen and its culture was stored in liquid nitrogen for future use.

Figure 3. *Curvularia tanzanica* on blighted inflorescence of Navua sedge in Tanzania.

Australian native pathogens Surveys for endemic or established fungal pathogens on Navua sedge conducted at 12 sites in northern Queensland yielded 41 fungal isolates. Molecular phylogenetic methods were used to identify the isolates as culturable ascomycetes in the genera *Curvularia* (5 isolates), *Epicoccum* (3), *Fusarium* (6), *Neopestalotiopsis* (4), *Nigrospora* (16) and *Phaeosphaeria* (1). Six isolates have yet to be identified to the rank of genus. These isolates were cryopreserved in the Queensland Plant Pathology Herbarium. An isolate of *Curvularia asiatica* and three isolates of unidentified *Curvularia* species, were spray-inoculated onto seedlings of Navua sedge in glasshouse pathogenicity tests. Only minor leaf spotting was observed, and therefore these fungi were not considered further as potential biological control agents. Testing of the remaining pathogens for pathogenicity is in progress.

DISCUSSION

Native range studies in equatorial Africa have identified three fungi as potential biological control agents, specifically, a flower smut (*C. kyllingae*), a leaf and stem rust (*U. kyllingae-erectae*) and a flower blight (*C. tanzanica*). For effective biological control, multiple agents that target different parts of Navua sedge are needed. We have prioritised two pathogens that target foliar and reproductive parts of Navua sedge for further investigation. The flower smut attacks the inflorescence destroying the seeds and thereby reducing the seed bank in the soil; and the rust targets leaves and stems thereby reducing the plant vigour. Smit and rust fungi are obligate biotrophic pathogens that have previously been exploited successfully as weed biological control agents (Hershenhorn *et al.* 2016). To date, phytophagous insects or mites feeding on Navua sedge have not been observed in the native range of

Navua sedge nor has an agent been found that attacks underground parts of the sedge (roots and rhizomes).

The three fungal pathogens, *C. kyllingae*, *C. tanzanica*, and *U. kyllingae-erectae*, have been exported to CABI, UK for further research. Among them, the inflorescence smut (*C. kyllingae*) and the leaf and stem rust (*Uredo kyllingae-erectae*) have been prioritised for life cycle and host-specificity studies. Host-specificity tests for the flower smut as well as the selection of virulent strains for the leaf and stem rust are currently in progress. If one or more of the pathogens are shown to be specific to Navua sedge, an application seeking approval for their release in Australia will be submitted.

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