A rust pathogen affecting the invasive Navua sedge (*Cyperus aromaticus*) in Australia – A fortuitous biocontrol agent?

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Summary Navua sedge (*Cyperus aromaticus*), a perennial invasive sedge affecting beef, dairy and sugarcane industries in the Queensland wet tropics, is a target for biocontrol in Australia. Host specificity tests for a floret-infecting smut fungus *Cintractia kyllingae* and a leaf and stem infecting rust fungus *Puccinia kyllingae-erectae*, both native to Africa, are in progress at CABI, UK. In 2023, a rust fungus on leaves and stems of Navua sedge was reported from a grazing property in Topaz, northern Queensland. The rust was morphologically and genetically identical to *P. kyllingae-erectae* from Africa. *Puccinia kyllingae-erectae* was found to be widespread in the Atherton Tablelands, and in the coastal lowlands, from Innisfail in the south to Cape Tribulation in the north. *Puccinia kyllingae-erectae* killed 80–95% of the above ground foliage, although Navua sedge resprouts from underground rhizomes. The rust was not found on pasture species or other native sedge species indicating that it is host specific. Future studies will focus on determining the distribution, impact and host range of *P. kyllingae-erectae* in the field.

Keywords Rust fungus, *Puccinia kyllingae-erectae*, *Uredo kyllingae-erectae*, Navua sedge, *Cyperus aromaticus*, biological control.

INTRODUCTION

Navua sedge, *Cyperus aromaticus* (Ridley) Mattf. & Kukenth (Cyperaceae) is an aggressive invasive perennial sedge that impacts the beef, dairy and sugarcane industries in the Queensland wet tropics (Shi *et al*. 2021; Dhileepan *et al*. 2022a, 2022b). Navua sedge is also a major weed of pastures and crops in many Pacific Island countries, including Fiji and French Polynesia (CABI 2024). Navua sedge forms dense stands, replacing palatable tropical pasture species that results in reduced beef cattle and milk production (Shi *et al*. 2021).

Two genetically distinct populations of Navua sedge occur in Queensland - a predominant population south of Port Douglas (southern population), and a population north of Daintree (northern population) with a restricted distribution (Dhileepan *et al*. 2023).

Current management options for Navua sedge are mechanical and chemical (Vitelli *et al*. 2010, Vogler *et al*. 2015, Chadha *et al*. 2022), which are expensive and have been shown to offer only short-term relief. Biocontrol offers a cost-effective and long-term management option for Navua sedge. Native range surveys in African countries of Kenya, Nigeria and Tanzania have led to the discovery and sample collection of a smut fungus, *Cintractia kyllingae*, that infects Navua sedge florets and destroys its seeds; the survey also led to the identification of a rust fungus, *Puccinia kyllingae-erectae* (synonym: *Uredo kyllingae-erectae*), that attacks leaves and stems. Thus, these fungi are prospective biocontrol agents (Dhileepan *et al*. 2022a, Kurose et al., 2023, Tan and Shivas 2024). Host-specificity testing for a virulent strain of *C. kyllingae* from Tanzania is in progress at CABI, UK (Dhileepan *et al*. 2022b). Host specificity tests for *P. kyllingae-erectae* sourced from Madagascar was initiated at CABI, UK in 2024.

In this study we report the detection and distribution of *P. kyllingae-erectae* on Navua sedge in the wet tropics of Queensland, Australia. The impact of *P. kyllingae-erectae* on Navua sedge in

Australia is assessed and its potential for biocontrol discussed.

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MATERIALS AND METHODS

Surveys: Surveys were conducted in the Atherton Tablelands and areas along the coast, from Ingham in the south to Cape Tribulation in the north, during the months of August, September and November 2023, and again in February, March and May 2024. Surveys have focused on the incidence of *P. kyllingae-erectae* on Navua sedge, on co-occurring native sedges and pasture species. Both the predominant southern and the restricted northern populations of Navua sedge were surveyed for rust incidence and rust infected plant materials were collected.

Rust identification: Molecular examination based on comparison of ITS and 28S rDNA sequence data of specimens of the rust from Africa (Nigeria, Tanzania and Madagascar) and Queensland, Australia were conducted at the Queensland Department of Agriculture and Fisheries (QDAF) and at CABI, UK.

Impact assessment: The impact of *P. kyllingae-erectae* on Navua sedge was monitored at three trial sites - a cattle grazing property at Malanda, Atherton Tablelands (-17.457650°; 145.594167°), a sugarcane farm at Gordonvale (-17.204805°; 145.911950°) a coastal town in Far North Queensland, and along a roadside near the coastal town of Ingham (-18.605662°; 146.184518°) in North Queensland. Six 0.25 m^2 plots at each site were monitored at quarterly intervals. The trial allowed the quantification of the impact of *P. kyllingae-erectae* on the mortality of above ground tillers before and after rust detection. Univariate analysis of variance (using SPSS-IBM ver. 17 software) was used to compare the impact of the rust on tiller (shoot) mortality between sites and between seasons, as well as their interactions. The post-hoc test (LSD) was used to compare the means.

Field host range: Sites with *P. kyllingae-erectae* on Navua sedge were surveyed for the presence of rust on co-occurring pasture species as well as on native sedges. Specimens of these non-target plant species with the respective rust species were collected and identified using morphological and genetic studies.

RESULTS

A rust pathogen affecting Navua sedge (Fig. 1) was first collected on 28 August 2023 at a private grazing property at Topaz, northern Queensland. A specimen of the pathogen was lodged at the Queensland Plant Pathology Herbarium (BRIP 76099). As per the regulatory requirement, the Consultative Committee on Emergency Plant Pests (CCEPP) was notified of the detection through Queensland Government's Incidence Response and Preparedness (CPHM Qld) unit.

The rust was morphologically identical to *P. kyllingae-erectae* from West Africa (Yen, 1973). Molecular analysis has confirmed that the rust on Navua sedge in Queensland is genetically the same as *P. kyllingae-erectae* from Africa.

Figure 1. Navua sedge infected by the rust *Puccinia kyllingae-erectae* in Topaz, Atherton Tablelands, Queensland.

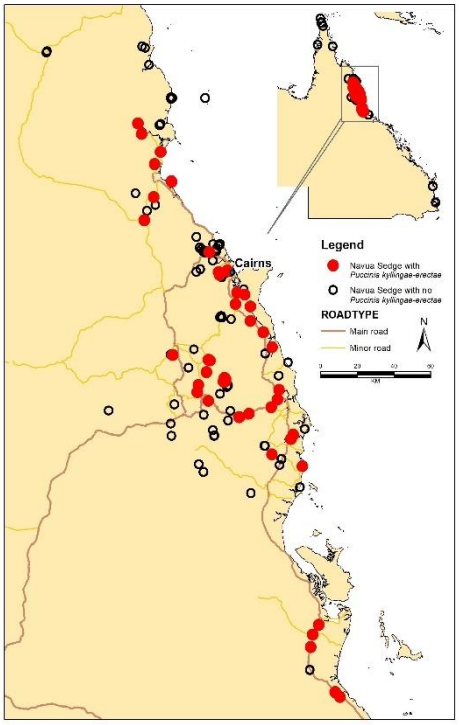

Figure 2. *Puccinia kyllinga-erecta* incidence (●) in the wet tropical regions of Queensland. The empty circles (○) represent sites where the rust has not been reported yet.

A survey in September 2023 confirmed that *P. kyllingae-erectae* was widespread on several grazing properties in the Topaz area. In follow-up surveys in November 2023, February 2024, March 2024 and May 2024, widespread occurrence of *P. kyllingae-erectae* was seen in the Atherton Tablelands, and along the coastal lowlands, from Innisfail in the south to Cape Tribulation in the north (Fig. 2). Rust incidence was widespread on the two genetically distinct southern and northern populations of Navua sedge.

Puccinia kyllingae-erectae was found at the Malanda and Gordonvale trial sites from late September 2023 onwards, although the rust was not found at the Ingham trial site. The rust killed about 90% of the tillers (above-ground foliage) at the Malanda and Gordonvale sites (Fig. 3). In contrast, at the Ingham site with no rust incidence, less than 3% of the tillers (above-ground foliage) died (Fig. 3).

Puccinia kyllingae-erectae was not found on tropical pasture grasses (*Brachiaria decumbens*, *Brachiaria mutica*, *Setaria sphacelata*) nor on Australian native sedges (*Cyperus iria*, *Cyperus exaltatus Cyperus polystachyos*) that co-occurred with rust-infected Navua sedge. The only known host of *P. kyllingae-erectae* is Navua sedge.

DISCUSSION

Since the initial detection of *P. kyllingae-erectae* in 2023, our surveys have shown that the rust is widespread across the Atherton Tablelands and along the coastal lowlands- from South Johnston in the south to Cape Tribulation in the north. Natural spread of the rust appeared to be primarily by the wind-carried urediniospores.

In the Atherton Tablelands, *P. kyllingae-erectae* persisted all year round. In the coastal lowlands (South Johnston to Cairns) the incidence of the rust was lower in summer (December 2023 to March 2024), signaling the influence of climatic factors. The rust increased in abundance in coastal lowlands from early autumn (April 2023). Future studies will focus on the seasonality of the rust in highland and coastal lowland regions of Queensland's wet tropics.

The rust caused significant above-ground tiller mortality in Navua sedge. However, infected plants resprouted from underground rhizomes. It is anticipated that re-infection by the rust over many years, may reduce the underground rhizome biomass and result in long-term management.

The impact of obligate plant pathogens as biocontrol agents for weeds in their introduced ranges is well documented. Incidence of specialist plant pathogens not deliberately introduced, but fortuitously acting as biocontrol agents for weeds in their introduced ranges, is also well known (e.g. King et al., 2017; Vitelli et al., 2017; Wood & den Breeÿen, 2021). In view of the detection of *P. kyllingae-erectae* in Queensland, a decision was made to discontinue host specificity testing of the rust at CABI, UK. Future studies will focus on determining the distribution and impact of *P. kyllingae-erectae* on Navua sedge; and on risk assessment of the rust on non-target plant species, involving host specificity tests in the glasshouse and field host range observations in Queensland.

Navua sedge is perennial with abundant subterranean rhizome. Multiple biocontrol agents

that target different parts of Navua sedge may best manage the weed populations. Host-specificity testing for a virulent strain of *C. kyllingae* from Tanzania is in progress at CABI, UK (Dhileepan et al., 2022b). If approved for field release, the flower-infecting smut will complement the leaf and stem-infecting rust (*P. kyllingae-erectae*).

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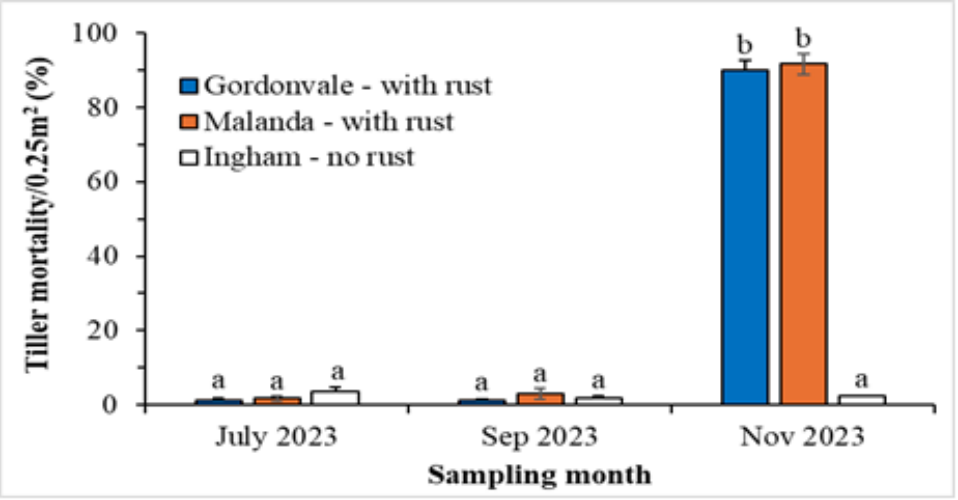

Figure 3. Above-ground tiller mortality (%) of Navua sedge before and after the outbreak of *Uredo kyllingae-erectae* at sites with rust (Gordonvale and Malanda) in comparison to a site (Ingham) without the rust. Two-way ANOVA: Site, $F_{2,45} = 411.9$; Season, $F_{2,45} = 1612.9$, $P < 0.001$; Site x Season, $F_{4,45} = 436.3$, $P < 0.001$. Values with the same alphabets are not significantly different (LSD test, $P > 0.05$).

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