# Studies on *Puccinia coronata* var. *coronata* and other recently observed rust fungi in Minnesota

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA

BY

Nicholas John Greatens

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Drs. Pablo Olivera Firpo and Yue Jin, co-advisors

July 2023

## Acknowledgments

This project was only possible with the help of many people who have graciously offered their assistance and support throughout my time as a PhD student.

First, thank you to my advisors, Drs. Pablo Olivera Firpo and Yue Jin for their support. Their patient and careful contributions to this work have been invaluable and have made me a much better scientist and writer. It has been a pleasure to conduct my graduate work under their supervision, and thanks to them, I will spend a lifetime noticing the rust fungi on the plants all around me. Thank you also to my committee members, Dr. Robert Blanchette and Dr. Neil Anderson for their helpful advice, their knowledge, and their curiosity about this project.

As a researcher and later as a graduate worker, I have been a part of the plant pathology community at the University of Minnesota and the USDA-ARS Cereal Disease Lab more than six years. My many coworkers and lab mates have contributed significantly to my education and, more importantly, have made my work much more rewarding and enjoyable: Thank you to Julia Mitchell, Kim Nguyen, Sam Stoxen, Nick Klejeski, Sam Gale, Melissa Prenevost, Nik Prenevost, Josh Kielsmeier-Cook, Dr. Jyoti Sharma, Dr. Radhika Bartaula, Nisha Paneru Regmi, Worku Bulbula, Endale Abera, Karl Miller, Kane Farmer, Jillian Neece, Klara Peterson, Calista Hughes, Maya Sarkar, Gabe Zilmer, Ben van Raalte, and all of my other colleagues, past and present, at the CDL.

The Department of Plant Pathology has been an edifying and pleasant place to pursue a graduate education, and I will always be thankful to all the

people who have called it home during my time here. Thank you to Dr. Jim Bradeen, Susan Kingsbury, Diane Hennan, to my first plant pathology instructors as an undergraduate, Drs. Brett Arenz and Ruth Dill-Macky, to my fellow graduate students, and to the many other professors, researchers, and administrative staff.

Nick Klejeski, Dr. Les Szabo, Karl Miller, Dr. Eric Watkins, Dr. Yue Jin, and Dr. Pablo Olivera Firpo are co-authors on published and accepted manuscripts below. Dr. Gail Celio and the University Imaging Centers are responsible for the excellent scanning electron micrographs. Drs. Dave Garvin, John Vogel, Robert Blanchette, Neil Anderson and Alan Smith, and the USDA National Plant Germplasm System provided plant material. Many passionate community scientists and amateur mycologists on iNaturalist.org sent samples of rust fungi, significantly improving our first publication from the project.

The Minnesota Invasive Terrestrial Plants and Pests Center (MITPPC) and the Environment and Natural Resources Trust Fund provided financial support for this project. I thank Dr. Robert Venette, Heather Koop, Dr. Amy Morey, and the other staff of MITPPC for their guidance, care, and enthusiasm throughout the project.

Lastly, I thank Dr. Tankut Atuk, my loving partner, who will defend his dissertation five days before I defend mine. He ultimately talked me into going to graduate school, I suppose making him responsible for this as much as anybody. Thank you for everything, and especially for the dogs.

## Dedication

I dedicate this dissertation to Tankut Atuk, and to my many friends and family members who have supported me throughout this work. I hope I haven't bored you too much, going on about rust fungi.

## Table of Contents

List of Tables ..... v

List of Figures ..... vii

Introduction ..... 1

Chapter one: *Puccinia coronata* var. *coronata*, a crown rust pathogen of two highly invasive species, is detected across the Midwest and Northeastern United States ..... 20

Chapter Two: Aecial and telial host specificity of *Puccinia coronata* var. *coronata,* a Eurasian crown rust fungus of two highly invasive wetland species in North America ..... 48

Chapter Three: Effects of *Puccinia coronata* var. *coronata* on reed canarygrass and glossy buckthorn in greenhouse assays ..... 72

Chapter Four: Host specificity of *Puccinia digitaticoronata*, a new crown rust fungus of Kentucky bluegrass in North America ..... 91

Chapter Five: First reports of of *Puccinia glechomatis* infecting creeping charlie in Minnesota and of a rust fungus (*Puccinia* sp.) infecting lemongrass in Minnesota ..... 119

Bibliography ..... 127

Appendix: Supplementary tables ..... 147

## List of Tables

**Table 1.1** Collection information with GenBank and herbarium accession numbers of *Puccinia coronata* var. *coronata* samples..... 39

**Table 2.1** Summary of infection types observed in cereals in response to *Puccinia coronata* var. *coronata*..... 65

**Table 2.2** Summary of infection types observed in other grasses in response to *Puccinia coronata* var. *coronata*..... 66

**Table 2.3** Rust development in Rhamnaceae and Elaeagnaceae inoculated with *Puccinia coronata* var. coronata..... 69

**Table 3.1** Effects of *Puccinia coronata* var. *coronata* on biomass of glossy buckthorn seedlings grown together in in pots..... 85

**Table 4.1** Collection information with GenBank and herbarium accession numbers of *Puccinia digitaticoronata* samples..... 109

**Table 4.2** Summary of infection types in cereal crops inoculated with *Puccinia digitaticoronata*..... 110

**Table 4.3** Summary of infection types in other grass species inoculated with *Puccinia* digitaticoronata..... 111

**Table 4.4** Infection types in Rhamnaceae and Elaeagnaceae in response to *Puccinia* digitaticoronata..... 114

**Supplementary Table 1.1** Primers used in chapter one..... 148

**Supplementary Table 1.2** Coverage of consensus sequences of *Puccinia coronata* var. *coronata* samples evaluated in this study..... 149

**Supplementary Table 1.3** ITS variants and coverage of four *Puccinia coronata* var. *coronata* isolates..... 150

**Supplementary Table 1.4** Results of local BLAST searches for *Puccinia coronata* var. *coronata* sequences generated in this study..... 151
**Supplementary Table 2.1** Infection types observed in cereal crop species in response to *Puccinia coronata* var. *coronata*.....Online only*
**Supplementary Table 2.2** Infection types observed in other grass species in response to *Puccinia coronata* var. *coronata*.....Online only*
**Supplementary Table 2.3** Origins and rust development of Rhamnaceae and Elaeagnaceae inoculated with *Puccinia coronata* var. *coronata* and rust development..... 156
**Supplementary Table 4.1** Coverage of consensus sequences of *Puccinia digitaticoronata* samples sequenced with Oxford Nanopore..... 158
**Supplementary Table 4.2** Additional information for samples used in phylogenetic analyses……. Online only*
**Supplementary Table 4.3** Infection types observed in 370 accessions of cereal crop species in response to *Puccinia digitaticoronata*……Online only*
**Supplementary Table 4.4** Infection types observed in other grass species in response to *Puccinia digitaticoronata*…….. Online only*

*Due to the large size of the table, data were excluded from the main document but are available online at the University of Minnesota Digital Conservancy as supplementary material.

## List of Figures

**Figure 1.1** *Puccinia coronata* var. *coronata* and its effects on glossy buckthorn and reed canarygrass..... 42

**Figure 1.2** Microscopic images of *Puccinia coronata* var. *coronata* spores and spore structures..... 43

**Figure 1.3** Phylogenetic analysis of β-*tubulin*, ITS, and *RPB2* consensus sequences from four single pustule isolates of *Puccinia coronata* var. *coronata*..... 44

**Figure 1.4** Confirmed reports of *Puccinia coronata* var. *coronata* in the United States from this study with reports of reed canarygrass and glossy buckthorn on EDDMapS..... 45

**Figure 1.5** Reports of rust on glossy buckthorn on iNaturalist..... 46

**Figure 1.6** The results of surveys for *Puccinia coronata* var. *coronata* on glossy buckthorn and reed canarygrass in Minnesota..... 47

**Figure 2.1** Grasses and buckthorns inoculated with *Puccinia coronata* var. *coronata*..... 70

**Figure 3.1** Effects of *Puccinia coronata* var. *coronata* on seedlings of glossy buckthorn grown in pots..... 86

**Figure 3.2** Glossy buckthorn seedlings eight weeks after inoculation by *Puccinia coronata* var. *coronata*..... 87

**Figure 3.3** Effects of *Puccinia coronata* var. *coronata* on glossy buckthorn plants grown from seed in cone-tainers..... 88

**Figure 3.4** Effects of rust crown disease caused by *Puccinia coronata* var. *coronata* on reed canarygrass in a greenhouse trial..... 89

**Figure 3.5** Photographs of the effects of *Puccinia coronata* var. *coronata* on reed canarygrass..... 90
**Figure 4.1** Phylogenetic analyses of *Puccinia digitaticoronata* samples and related crown rust fungi..... 115
**Figure 4.2** Range of infection types in gramineous species inoculated with *Puccinia digitaticoronata* isolate 21MN_Pdc01-1..... 117
**Figure 5.1** *Puccinia glechomatis* on creeping Charlie..... 125
**Figure 5.2** Images of lemongrass rust..... 126

# Introduction

This project was first inspired by the observation of a prolific rust fungus on glossy buckthorn (*Frangula alnus*) around Central Park, Roseville, MN in the spring of 2017. Unlike common buckthorn (*Rhamnus carthartica*), glossy buckthorn had been relatively free of rust in previous years of observation. Uredinial infection on reed canarygrass (*Phalaris arundinacea*) near infected glossy buckthorn soon followed, leading to the initial hypothesis that the rust cycled between these two hosts. This hypothesis was confirmed by the successful inoculation of reed canarygrass with aeciospores from the rust on glossy buckthorn. Further sequence analysis would identify this crown rust fungus as *Puccinia coronata* var. *coronata* (*Pcc*). Curiously, this new pathogen appeared to have a desirable effect locally, strongly affecting only two highly invasive species.

In a research project that began in 2019 and was funded in 2020 by the Minnesota Invasive Terrestrial Plants and Pests Center (MITPPC), we pursued three goals: 1) to determine the distribution of *Pcc* in Minnesota and North America; 2) to assess its host specificity on potential buckthorn and grass hosts; and 3) to evaluate its potential as an augmentative biological control agent of one or both of its invasive hosts. Based on preliminary observations, we were optimistic about the effect of the rust, but *Pcc* is a close relative of major pathogens of cereals and turfgrass species, making a new introduction of concern. While our project was partly motivated by the prospect of a biological control agent, we were first concerned with establishing the effects of a new pathogen on native species, cultivated cereal crops, and turfgrasses, an essential component of any study on potential biological control agents. Chapters one

through three of this dissertation are based on the three research goals of this project.

Chapter four describes the results of a similarly designed study around another crown rust fungus recently observed in North America, *Puccinia digitaticoronata*. Crown rust disease has recently emerged as a disease of Kentucky bluegrass, a common turfgrass, but earlier reports by Beirn et al. (2011) and Liu and Hambleton (2013) had indicated that the causal agent was in the clade that includes the oat crown rust pathogen as well as the pathogens of perennial ryegrass and tall fescue. Isolates of each were collected locally with the intention of comparing their host specificities. Since Kentucky bluegrass, perennial ryegrass, and tall fescue often grow together, overlapping host ranges would have epidemiological significance. To our surprise, the isolates we collected from Kentucky bluegrass in Minnesota were identified by morphological analysis and ITS sequencing to be a second crown rust fungus of Kentucky bluegrass, *Puccinia digitaticoronata*, described by Ji et al. (2022) in northeast China. Following the protocols described in chapters one and two, we investigate its pathogenicity on buckthorns, cereal crop species, turfgrasses, and other wild grass species.

Chapter five combines the results of two small projects published as plant disease notes: first reports of *Puccinia glechomatis*, a rust of creeping charlie (*Glechoma hederacea*) in Minnesota and of a *Puccinia sp.* causing rust of lemongrass in Minnesota.

Chapters one, two, four, and five, have been published or accepted for publication in the journal *Plant Disease* either as research articles or as plant disease notes.

### Invasive species

Invasive species are one of the top drivers of biodiversity loss worldwide, along with climate change, habitat loss and fragmentation, and overexploitation of resources (Bellard et al. 2013; Wilcove et al. 1998). Threats are closely linked. Minnesota plant communities may suffer fragmentation, climate change, and displacement by invasive plant species at once. Invasive species are often advantaged by changing environmental conditions, and they can also drive ecosystem level changes (Dukes and Mooney, 2004).

While many exotic organisms naturalize with limited impact on native biota, some may cause extraordinary ecological impacts and economic harm (Holmes et al. 2009). For example, the introduction of the white pine blister rust pathogen (*Cronartium ribicola*) from east Asia in the late 19$^{th}$ century devastated plantations and natural populations of white pine in North America and continues to limit production to the present day (Hunt 2003). Another rust pathogen, *Austropuccinia psidii*, originally from Central and South America and known as a pathogen of guava (*Psidium guajava*) and other Myrtaceae has recently spread to Australia and New Zealand, among other regions (Fernandez Winzer et al. 2019; references within). In Australia, where forests are dominated by myrtle family plants such as *Eucalyptus* and *Melaleuca* spp., *A. psidii* is abundant, affecting a broad range of species, and in the long term, likely to significantly alter forest composition in some ecosystems (Pegg et al. 2018). Disturbances, whether due to climate change or the introduction of invasive pathogens, advantage other weedy, often alien, plant species (Thuiller et al. 2007). *Austropuccinia psidii* has been reported facilitating invasion by *Lantana*

(Carnegie et al. 2016; Pegg et al. 2018), a common tropical weed cultivated as an ornamental plant.

Invasive plant species may also cause significant changes to natural and managed ecosystems, affecting, for example, plant species richness and community composition, ecosystem productivity, nutrient cycling, soil organic matter, and fire regimes (Angeloni et al. 2006; Barney et al. 2013), with effects variable between species and ecological contexts. While many invasive species are exotic, or alien, for example originating on another continent, some native species function similarly to exotic invasive species, overcoming natural obstacles and dominating an ecosystem (Valéry et al. 2008). For this work, out of convenience these aggressive native species are considered invasive.

Many interrelated hypotheses have been developed to explain and predict biological invasion. Some relate to traits of the organism and others to features of the ecosystem, or its invasibility (Catford et al. 2009). Broadly, Catford et al. (2009) group these hypotheses as explaining invasion through propagule pressure, biotic interactions, and abiotic interactions. While a critical survey of the many hypotheses is outside the scope of this introduction, some are useful to understand the *Puccinia coronata* var. *coronata*-glossy buckthorn-reed canarygrass pathosystem in Minnesota and North America.

### Crown rust fungi

The crown rust fungi (*Puccinia* series *Coronata*) are some of the most common rust fungi in Minnesota and eastern North America. Many crown rust fungi are macrocyclic and heteroecious, usually cycling between a buckthorn host, the aecial host, and a grass host, the telial host. While today the devastating

oat crown rust pathogen (Nazareno et al. 2018) is by far the most well-known of the crown rust fungi, the original description of *Puccinia coronata* Corda (1837) is on a *Calamagrostis* sp., likely *C. arundinacea* or *C. villosa* (Urban, 1967), and the holotype specimen is now the holotype of *P. coronata* var. *coronata* Liu and Hambleton. The taxonomy of the crown rust fungi has been in flux nearly since the description of the species with the vast taxonomic literature published in numerous languages. Interested readers are directed to Urban and Marková (1993), Brown (1937), Melhus et al. (1922), and especially to Liu and Hambleton (2013), for a more thorough discussion of its history. Here it is summarized, with emphasis on *Puccinia coronata* var. *coronata*, or *Pcc*, the subject of chapters one through three of this dissertation.

In the 1890s, Klebahn (1894) split *P. coronata* into two species, *P. coronata,* composed of the crown rust fungi that use glossy buckthorn (*Frangula alnus*; syn. *Rhamnus frangula*) as the aecial host, and *P. coronifera,* crown rust fungi that use common buckthorn (*Rhamnus cathartica*) as the aecial host. Each species was further subdivided into *formae speciales: P. coronata* included the *formae speciales phalaridis* and *calamagrostidis*, among others; and *P. coronifera* included the formae speciales *festucae*, *lolii,* and *avenae,* the oat crown rust pathogen, among others. (See Urban and Marková, 1993, summarizing original sources; Erikson, 1898; Liu and Hambleton, 2013). Soon, North American crown rust fungi were investigated that did not conform to this dichotomy (Melhus et al., 1922; Dietz et al. 1926, Fraser and Ledingham, 1933), and later rust taxonomists (Cummins, 1971; Urban and Markova, 1993) favored broader variety level groupings over *formae speciales* (Liu and Hambleton, 2013). While the *forma specialis* concept is still in use in cereal rust pathology, it is acknowledged that, contrary to the name,

for some rust fungi, a single *forma specialis* may affect many grass species or genera (Anikster, 1984; Eshed and Dinoor, 1980).

With the advent of molecular phylogenetics, species delineations in cereal rust fungi have changed significantly, with the stripe rust fungi, crown rust fungi, and leaf rust fungi now split into many new taxa based on morphology, life cycle traits, host specificity, and especially molecular characters (Hambleton et al., 2019; Liu and Hambleton, 2010; Liu et al. 2013; Liu and Hambleton, 2013; Szabo, 2006). The crown rust fungi (*P. coronata s.l.*) are now formally a described in a series, *Puccinia* series *Coronata* Liu and Hambleton, which includes at least seventeen distinct species (Hambleton et al., 2019; Ji et al. 2022; Liu and Hambleton, 2010). New species continue to be described (Ji et al. 2022), and several lineages are acknowledged to be unresolved, in need of further attention (Liu and Hambleton, 2013). In some cases, following earlier taxonomists, sub-species level taxa are retained, sometimes making formal names of cereal rust fungi confusing even to experts in rust pathology. For example, *Puccinia coronata sensu stricto* is subdivided into two varieties: *P. coronata* var. *avenae* and *P. coronata* var. *coronata*. *Puccinia coronata* var. *avenae* is further subdivided into *P. c.* var. *avenae* forma specialis (f. sp.) *avenae* and *P. c.* var. *avenae* f. sp. *graminicola. Puccinia coronata* var. *avenae* f. sp. *avenae* includes the oat crown rust pathogen, but also phenotypically unique forms affecting ryegrass, fescue, and bluegrass (Beirn et al. 2011; Erikson, 1898; Liu and Hambleton, 2013).

In parts of eastern North America, where invasive buckthorn hosts (*R. cathartica* and *F. alnus*) are abundant, crown rust fungi are similarly abundant. For example, in a short walk on the St. Paul campus of the University of Minnesota, you will find: *P. coronata var. coronata* on reed canarygrass, on the

edge of the woods west of the horticulture display garden; three forms of *P. coronata* var. *avenae* f. sp. *avenae* on cultivated oat in research plots and underfoot on the common turfgrass species tall fescue (*Schedonorus phoenix*) and perennial ryegrass (*Lolium perenne*); *P. coronati-agrostidis,* on creeping bentgrass (*Agrostis stolonifera*), another common turfgrass; *P. coronati-brevispora* on the invasive smooth brome (*Bromus inermis*) lining the hillsides of Cleveland Avenue; *P. coronati-hordei* on weedy quackgrass (*Elymus repens*) along fences and building edges and on occasionally on barley grown for research; and *P. digitaticoronata* on Kentucky bluegrass, grown in research plots and all over as a turfgrass.

One hundred years ago, likely fewer crown rust fungi would have been encountered on a walk across campus. Some rust fungi are highly mobile, and often establish in new ranges as invasive pathogens, as discussed below. Through sexual or somatic hybridization, rust fungi may recombine, sometimes expanding their host range and successfully reproducing on previously resistant species or populations (Anikster, 1984). When rust is observed on a new host, it may be due to a new introduction of a species or pathotype or due to the expansion of the host range, for example by the acquisition of virulence on an important resistance gene, as is well documented in cereal rust fungi (Carson, 2008). As such, it is complicated to prove an introduction, although analysis of available herbarium specimens will help to establish historic species ranges. In any case, numerous crown rust fungi have certainly *emerged* as pathogens of widespread grass and buckthorn species.

*Puccinia coronati-hordei* was first reported naturally infecting barley in 1992 (Jin and Steffenson, 1999), although likely similar populations naturally occurring on rye were reported on as early as 1952 (Peturson, 1954; Liu and

Hambleton, 2013). *Puccinia coronati-brevispora,* today ubiquitous on smooth brome, was reported first in Wisconsin in 1995 (Delgado et al. 2001). *Puccinia coronata* var. *avenae* f. sp. *avenae* likely emerged as a common pathogen of bluegrass in the 2000s, although detection may have been delayed due to confusion of crown rust fungi with other rusts (Beirn et al. 2010). *Puccinia coronata* (*sensu lato*) var. *gibberosa*, previously known in Europe, was recorded for the first time in North America in 2011 on the ornamental grass *Helictotrichon sempervirens* (Demers et al. 2016). With this work, we report *Puccinia coronata* var. *coronata,* previously known in Europe, as widespread across the Midwest and Northeastern U.S on glossy buckthorn and reed canarygrass. It was first formally reported in Connecticut in 2016 (Kenaley et al. 2017), but had been first collected in 2013 near Ottawa, Canada (Greatens et al. 2023). *Puccinia digitaticoronata* was recently described (Ji et al. 2022) and possibly originates in East Asia, where close relatives are documented. It is now established in Minnesota, North Dakota, and Wisconsin, where it likely alternates between common buckthorn and Kentucky bluegrass, two of the most common plant species in the region (Chapter Four).

### Glossy buckthorn

Glossy buckthorn is a shrub or small tree, growing to about seven meters high. It can be distinguished from common buckthorn (*Rhamnus cathartica*) by its smooth leaf margins, perfect flowers, and berries that turn from green to red to black as they mature. Glossy buckthorn originates from Europe, western Asia, and parts of Africa along the Mediterranean coast (Zecchin et al. 2016). It was historically used as a source of medicine and dye, and when processed into charcoal, as a component of gunpowder (Zecchin et al. 2016).

In North America, the earliest dated herbarium specimen was collected in 1879 in northern New Jersey, and before 1900, eleven other specimens were collected near New York City and two near London, Ontario (Aiello-Lammens, 2014). Over the next one hundred years, its range would expand substantially as it gained popularity as an ornamental tree (Aiello-Lammens, 2014). Presently, its naturalized range stretches east from the Canadian Maritime provinces west across the Northeast and Midwest to the Great Plains (EDDMapS, 2023). The berries of glossy buckthorn are readily consumed by numerous mammals and birds, which aid in its dispersal (Catling and Porebeski, 1994), such as from residential landscape plantings to nearby wetlands and forests. Glossy buckthorn is regulated under noxious weed laws in ten states, including Minnesota, although it is often sold even where its sale and cultivation are prohibited, including at multiple sites in Wisconsin and Illinois (Beuary et al. 2021).

In North America, glossy buckthorn is highly invasive in bogs and fens (Fiedler and Landis, 2012) as well as mesic and upland forests (Fagan and Peart, 2004; Stokdyk and Herrman, 2016). In wetlands in Ontario and Michigan, glossy buckthorn has been linked to decreased plant species diversity and lower coefficients of conservatism (i.e. generally weedier plants associated with anthropogenic disturbance) (Fiedler and Landis, 2012; Frappier et al. 2003; Sinclair and Catling, 1999). In eastern North America, it is a persistent weed in forestry plantations, limiting recruitment of valuable tree species (Fagan and Peart, 2004).

Some evidence suggests glossy buckthorn may also alter abiotic factors in its environment and function as an ecosystem engineer. In upland environments, glossy buckthorn invasion has been linked to increased nitrate levels (Fagan and

Peart, 2004; Huebner et al. 2009; Stokdyk and Herrman, 2016), and in a Michigan prairie fen Fiedler and Landis (2012) find higher, but not statistically significant differences in nitrate and ammonium levels between invaded and uninvaded sites. Causation, however, is difficult to prove given the many factors that influence nutrient cycling (Mueller et al. 2017). In fens, Fiedler and Landis (2012) found in invaded areas a lower occurrence of hummocks, or small organic matter rich mounds. They observed that glossy buckthorn often colonizes hummocks, which provide an ideal microhabitat, and as they grow larger, transpire, reducing the water table and accelerating decomposition (Fiedler and Landis, 2012). Through alteration of carbon and nutrient cycling processes, glossy buckthorn may have a significant effect on native ecosystems.

Prior to the introduction of *Pcc*, glossy buckthorn was not commonly infected by crown rust fungi in North America, with few herbarium collections or reports (Greatens et al, 2023). In contrast, in Europe, glossy buckthorn was often reported infected by *Pcc* (Urban and Marková, 1993). Recent widespread occurrence of *Pcc* suggests that successful glossy buckthorn invasion in North America may in part be explained by enemy release, or the decreased regulation by enemies such as pests and parasites in an organism's expanded range (Keane and Crawley, 2002).

Glossy buckthorn is managed through controlled burns, where appropriate, and with application of herbicides to the stem (MN Dept. of Ag. Accessed May 2023). With severe infestations, management of glossy buckthorn is costly and labor intensive, and regional treatment plans are recommended for successful management (MN Dept. of Ag., 2023). Early detection and treatment are critical to reduce impact to native ecosystems.

### Reed canarygrass

Reed canarygrass (*Phalaris arundinacea*) is a large, rhizomatous, cool-season, perennial grass species with a circumboreal distribution (Barkworth, 1993+). It colonizes wet areas (Barkworth, 1993+), such as wetlands, wet meadows, riparian areas, and lakeshores and forms dense monotypic stands. A variegated form, *P. arundinacea* var. 'Picta' is a common ornamental grass, and many cultivars have been developed as forages, such as the low alkaloid 'Palaton' and 'Venture' cultivars (Anderson et al. 2021). In Europe, reed canarygrass has been investigated for use as a source of cellulosic ethanol (Wrobel et al. 2007), but it has not been widely adopted. In addition, reed canarygrass was historically used by some Indigenous people in North America for many purposes including weaving and thatching (Anderson 2019). Reed canarygrass has since been introduced to other temperate areas worldwide, including parts of South America, Australia, and New Zealand (USDA FEIS, 2023).

In temperate regions of North America (EDDMapS, 2023), reed canarygrass is widespread and is a major invasive species (Galatowitsch et al. 1999). It is a prolific weed in wetlands (Mulhouse and Galatowitsch, 2003) and wetland restorations, where it grows rapidly, shading and outcompeting many other plants, limiting species diversity (Adams and Galatowitsch, 2005). In part due to its aggressive or invasive growth pattern, reed canarygrass was for many years erroneously considered an exotic species in North America. While some European cultivars were introduced as forages (Anderson, 2019; Lavergne and Molofsky, 2004), there is little evidence that they have become invasive or

introgressed with native populations (Anderson et al. 2021). Recent genetic studies based on SSRs (Jakubowski et al. 2013), the ITS region (Graper et al. 2021), and genome wide SNP markers (Noyszewski et al. 2021**)**, have strongly shown that historic North American herbarium specimens and present populations are distinct from sampled European populations. Within both Europe and North America, reed canarygrass populations are highly diverse, with more diversity among populations than between them (Anderson et al. 2021; Noyszewski et al. 2021). Furthermore, use of reed canarygrass in Indigenous cultures and place names makes clear that reed canarygrass predated European colonization of North America (Anderson, 2019; sources within).

Despite its geographic origin, reed canarygrass is, in ecological function and impact, a highly invasive species. Invasion was likely aided by human-mediated spread of forage cultivars throughout the Midwest and Canada in the mid-20$^{th}$ century, increasing propagule pressure in natural ecosystems (Anderson 2019; Lavoie et al. 2005). In Minnesota, the spread of forage cultivars was concomitant with major land use changes, including the development and draining of many wetlands for agriculture and urban development, significantly fragmenting wetland habitat and altering the hydrology. Habitat fragmentation (Tilman, 1994) and disturbance more broadly (Marvier et al. 2004) favor the spread of weedy species with high colonization ability. Reed canarygrass is prolific and morphologically plastic, highly adaptive to changes in water levels (Galatowitsch et al. 1999). In mesocosm studies, simulated flooding, sedimentation, and nutrient addition interact to promote reed canarygrass growth (Kercher et al. 2006). While under low nitrogen conditions, reed canarygrass competitive ability is reduced relative to *Carex* spp., common

wetland components, it is strongly competitive under high nitrogen conditions (Perry et al. 2004), such as in wetlands polluted by agricultural nitrate runoff (Green and Galatowitsch, 2001). Eutrophication of terrestrial ecosystems is a growing problem worldwide (Clark et al. 2017), and globally, plant available nitrogen has doubled since pre-industrial levels, with major implications for plant community composition (Vitousek et al. 1997) and likely reed canarygrass invasion.

Management of reed canarygrass is complex and requires a multiyear, multifaceted approach for its success (Adams and Galatowitsch, 2006; Lavergne and Molofksy, 2006). Depending on the plant community, scale of invasion, and ecological context, various options should be considered for management, including burning, mowing, haying, excavation, grazing, planting, and herbicide use (WI reed canarygrass management working group, 2009). Reduced N-inputs and carbon enrichment may reduce reed canarygrass competitive ability (Perry et al. 2004)

### Biological control agents and "enemy reunion"

Biological control is prized as a low input, cost effective management option in invasive species control. Biological control is the control of a weed, pest, or pathogen through other living organisms, such as parasites, parasitoids, and predators. Classical biological control agents are generally imported from the native range of a target species, introduced, and then allowed to spread naturally. Augmentative biological control agents, or bioherbicides, are regularly released to increase populations of the control agent (Morin, 2020). Biological

control can be supplemented with other methods of management, including chemical control and mechanical removal of target organisms.

Rust fungi are frequent candidates for the classical biological control of weeds given their host specificity (Barton, 2012) and dispersal ability. According to Morin (2020), 36 fungal plant pathogens, including 24 rust pathogens, in 18 countries have been authorized as classical biological control agents. In the mainland United States, three rust fungi have been authorized for release, all microcyclic rusts of Asteraceae (Morin, 2020; Winston et al. 2014), and one other species, *Uromyces pisi* f. sp. *europaei*, was released for control of common gorse (*Ulex europaeus*) in Hawaii (Morin, 2020; Winston et al. 2014). *Uromyces pisi* is heteroecious, but alternates with another invasive species in Hawaii, *Euphorbia cyparissias* (Morin, 2020). The success of some biological control agents is often taken as evidence of the enemy release hypothesis, although, as noted by Colautti et al. (2004), all classical biological control agents are also nonindigenous species, and in their exotic ranges may likewise benefit from different biotic interactions, including enemy release.

Rust fungi are less commonly used as augmentative biological control agents, and Winston et al. (2014) list only two examples, *Puccinia canaliculata,* a rust of yellow nutsedge (*Cyperus esculentus*) and *Puccinia thlaspeos*, a rust of dyer's woad (*Isatis tinctoria*). In controlled preliminary trials, *Puccinia canaliculata* was successfully used for control of yellow nutsedge, significantly reducing tuber production both alone and in combination with herbicides (Phatak et al. 1983). However, due to resistance in host plants, susceptibility of cultivated sunflower (*Helianthus annuus*), and complications in production it was not successful (Winston et al. 2014). *Puccinia thlaspeos* was never commercialized but was

augmented by researchers (Winston et al. 2014). Unlike most other rust fungi investigated as biological controls, *P. thlaspeos*, an autoecious, microcylic rust, was manually spread through teliospores embedded in infected plant tissue (Thompson and Kropp, 2003). Neither species is currently in production as a bioherbicide (Morin, 2020).

While some natural enemies are deliberately introduced as classical or augmentative biological control agents to control invasive hosts, others are introduced by accident or expand their ranges by natural means. The Julien catalog of biological control agents of weeds (Winston et al. 2014), a comprehensive reference text for biological control agents, includes a large section on "agents found in exotic ranges where deliberate release [is] not recorded." For example, in Hawaii, myrtle rust infects invasive guava plants (Uchida et al. 2007). In Germany, a North American goldenrod rust (*Coleosporium solidaginis*), was recently observed on the invasive North American giant goldenrod (*Solidago gigantea*) (Beenken et al. 2017), and in Austria the closely related North American *C. montanum* rust is now found on two naturalized North American aster species, *Symphyotrichum lanceolatum* and *S. novae-angliae* (Voglmayr et al. 2020). In many countries including China, India, Kenya, and South Africa, the American Parthenium weed (*Parthenium hysterophorus*) has been observed diseased by the American rust pathogen *Puccinia abrupta* var. *partheniicola* (Maharjan et al. 2020 and references therein), a pathogen that was also released deliberately in Australia (Morin, 2020; Parker et al. 1994). Numerous other examples exist with insects and other fungal taxa (Winston et al. 2014).

In reference to this latter case, Newcombe et al. (2009) and Newcombe and Dugan (2010) propose "enemy reunion" or "pathogen reunion" as a corollary to the enemy release hypothesis: If plants, upon introduction to a new region, are advantaged by leaving enemies in their native ranges, when enemies eventually are reunited with hosts, presumably this advantage is diminished. As examples, they offer various classical biological control agents, crop pathogens such as the Asian soybean rust pathogen *Phakospora pachyrizi* on soy in the Americas, and pathogens on invasive plants in natural ecosystems, such as the European tansy rust pathogen (*Puccinia taniceti*) on common tansy (*Tanacetum vulgare*) in Idaho. Notably, as calculated by Newcombe and Dugan (2010), a vast majority of recent pathogen introductions to the United Kingdom from 1970-2004, often pathogens of ornamental plants, may be considered examples of enemy reunion (Jones and Baker 2007).

Enemy reunion is likely an apt description for several pathosystems investigated at length here. *Puccinia coronata* var. *coronata* was eventually reunited with glossy buckthorn in North America 150 or more years following its host's introduction. *Puccinia digitaticoronata,* as described in chapter four, was reunited with various East Asian buckthorn species. And as described a in chapter five, *Puccinia glechomatis,* of Eurasian origin, has been observed on introduced, Eurasian creeping charlie (*Glechoma hederacea*) in Minnesota, as in much of eastern North America (Böllman and Scholler, 2006).

In some cases, however, introduced pathogens of weeds may also affect native species, making the net benefit dependent on the local plant community. In Germany, the goldenrod rust also affects the European native *Solidago virgaurea* (Beenken et al. 2017), and in Hawaii, myrtle rust affects the 'Ohi'a tree,

an endemic tree of major local ecological importance (Uchida et al. 2007). Susceptible populations of invasive species may even facilitate enemy spread and population growth, affecting susceptible native species. This is clearly the case with glossy buckthorn, which enables disease on reed canarygrass in Minnesota, although not unwelcome in this instance.

The introduction of novel plants and pathogens into new regions is a feature of modernity. Given the geological and biological significance of climate change, an extinction crisis, and increased spread of organisms across natural boundaries, some scholars have proposed a new geologic era: variously, the Anthropocene (see Rull, 2017), the Capitalocene (e.g. Moore, 2017), or to Charles Mann (2011), placing emphasis on biological and cultural mixing between continents, the Homogenocene.

Some species may never establish or persist only ephemerally. Some will naturalize without issue. Others will become major invasive species and cause severe ecological or economic impact in natural and managed ecosystems. At worst, invasive species may interact positively with each other, in what Simberloff and von Holle (1999) call "invasional meltdown". Yet, other introduced species may counteract the impacts of invasive species, a negative feedback loop. Some of these species are investigated extensively and authorized as classical biological agents, but some, less studied, are introduced by accident or expand their ranges naturally, but to generally positive effect. These species are nevertheless exemplars of Mann's "Homogenocene" but demonstrate the varied and contingent effects of exotic and invasive species.

# Chapter One

## *Puccinia coronata* var. *coronata*, a crown rust pathogen of two highly invasive species, is detected across the Midwest and Northeastern United States

This chapter was published in a similar form as:

Greatens, N., Klejeski, N., Szabo, L.J., Jin, Y. and Olivera Firpo, P.D. 2023. *Puccinia coronata* var. *coronata*, a crown rust pathogen of two highly invasive species, is detected across the Midwest and Northeastern United States. Plant Dis. In press. 10.1094/PDIS-07-22-1711-RE

## Summary

*Puccinia coronata* var. *coronata* (*Pcc*) causes crown rust disease of glossy buckthorn (*Frangula alnus*) and reed canarygrass (*Phalaris arundinacea*), two highly invasive plant species in North America. *Pcc* is closely related to major pathogens of cereals, turfgrasses, and forage grasses. It occurs throughout Europe but was first recorded in North America in 2013. Where its hosts co-occur, such as in wetlands in the Twin Cities metro area in Minnesota, we have observed *Pcc* causing significant infection that results in defoliation and fruit loss in glossy buckthorns and premature leaf senescence in reed canarygrass. In this research, we mapped the distribution of this likely recently introduced rust fungus and provide a description of disease signs and symptoms and pathogen morphology. Samples were acquired by two primary means: by surveys in Minnesota and by correspondence with users of iNaturalist.org, a social network for nature enthusiasts and community scientists. With an Oxford Nanopore MinION, we sequenced two to four loci from twenty-two samples across thirteen states and identified samples by phylogenetic analysis and sequence similarity. Notably, four pure isolates appear to have intragenomic variation of the ITS region. We find that *Pcc* is present throughout the range of glossy buckthorn in the eastern United States. In Minnesota, *Pcc* is not common outside the range of glossy buckthorn, however, despite the presence of susceptible grass hosts.

## Introduction

Glossy buckthorn (*Frangula alnus*) and reed canarygrass (*Phalaris arundinacea*) are two highly invasive plant species in North America. Glossy buckthorn is a shrub or small tree of Eurasian and North African origin that, in North America, ranges from New England and the Maritime Provinces west to the Great Plains (EDDMapS, 2021). It is especially invasive in fens, bogs (Fiedler and Landis, 2012), and wet meadows where it can form dense monotypic stands. In eastern North America, it invades forestry plantations as well (Burnham and Lee, 2010; Hamelin et al., 2016). Reed canarygrass is a tall, cool-season grass distributed across temperate Eurasia and North America and introduced in temperate regions of the Southern Hemisphere (Barkworth, 1993+). It is sometimes grown as a forage crop (Carlson et al., 1996). Recent studies have shown that many Minnesotan populations are native (Noyszewski et al., 2021), thus presenting novel challenges for regulation and management (Anderson et al., 2021). Reed canarygrass nevertheless remains a major issue for wetland management and restorations, where it suppresses the growth of other more highly valued species (Green and Galatowitsch, 2001).

In 2016, in Storrs, Connecticut, rust aecia were observed on glossy buckthorn, and a first report was published by Kenaley et al. (2017) identifying the species as *Puccinia coronata* var. *coronata sensu stricto* [*s.s*] (*Pcc*), a rust pathogen well known in Europe (Urban and Marková, 1993), but not previously reported in the United States. Three years earlier, in Gatineau, Quebec, another sample of rust on glossy buckthorn was collected and later determined to be *Pcc* (Sarah Hambleton, personal communication), the first known occurrence in

Canada and North America, although unpublished at the time of the first report. In May of 2017, severe rust infections were observed on glossy buckthorn at several locations around St. Paul, Minnesota. Rust aecia occurred on leaves, young twigs, flowers, and fruits, leading to defoliation and reduced seed production. In mid-June, uredinia were observed on reed canarygrass plants nearby. Subsequent greenhouse inoculations confirmed that the rust cycles between these two plants (this study). Similar infections have been observed in Minnesota each spring since 2017. In wetlands around the Twin Cities, reed canarygrass and glossy buckthorn grow side by side in abundance and create an ideal environment for *Pcc*.

*Pcc* is a variety of *Puccinia coronata s.s.,* a species within the broader *Puccinia* series *Coronata* (Liu and Hambleton, 2013), a large complex of rust fungi that cause crown rust diseases on cereal crops and hundreds of other species of grasses. For example, *Puccinia coronata* var. *avenae* f. sp. *avenae* causes oat crown rust, a devastating disease of oats worldwide (Nazareno et al., 2018), and *P. coronati-hordei* is an emerging rust pathogen of barley (Jin and Steffenson, 1999). In North America, crown rust is also common in natural ecosystems where many native and introduced species of grasses and buckthorns are affected by various crown rust fungi. Exotic-invasive grass species such as smooth brome (*Bromus inermis*), quackgrass (*Elymus repens*), and velvet grass (*Holcus lanatus*) are often highly infected by *P. coronati-brevispora*, *P. coronati-hordei*, and *P. coronata* var. *avenae* f. sp. *graminicola*, respectively (Delgado et al., 2001; Jin and Steffenson, 1999; personal observation). Common buckthorn (*Rhamnus cathartica*), among North America's most destructive invasive plants, is an aecial host of many *P.* series *Coronata* spp. The abundance of buckthorn and grass hosts makes *Puccinia*

series *Coronata* spp. among the most common and well-known rust fungi in eastern North America.

Given the likely recent introduction of *Pcc* in North America, we investigated the distribution of the rust fungus across Minnesota and the United States through surveys and by correspondence with users of iNaturalist.org, an online platform for nature enthusiasts. We sequenced two to four loci of 22 samples from 13 states an produced a description of disease signs and symptoms based on microscopic examination of North American samples and observation of disease development in the Twin Cities metro area and in greenhouse inoculations.

## Materials and Methods

### Disease description and spore morphology

Since spring of 2018, we have made regular visits to sites around the Twin Cities metro area where reed canarygrass and glossy buckthorn co-occur (Acorn Park, Reservoir Woods, Central Park East, and the St. Paul campus of the University of Minnesota) and have documented rust development and symptoms and signs on both plant species. In the laboratory, glossy buckthorn plants were inoculated with telia on reed canarygrass following Jin and Steffenson (1999), and reed canarygrass was inoculated with aeciospores and urediniospores as described below. Spores from all five stages of the fungal life cycle were mounted in 50 % lactic acid in water and examined on a Zeiss standard light microscope equipped with an Excelis HD-Accuscope camera. Additional images were generated with a *Hitachi* S3500N scanning electron

microscope at the University of Minnesota University Imaging Centers. Measurements were made using ImageJ (Schneider et al., 2012). Fifty spores were measured per available spore type per sample. Teliospore length was measured from the base of the first cell to the end of the second, disregarding the hilum and apical projections, and width was measured at the widest point. Measurements are given as the mean +/- one standard deviation with the minima and maxima in parentheses.

### Sample acquisition and survey methodology

Users of iNaturalist.org across the United States who reported rust on glossy buckthorn were contacted by direct message, provided with information about the rust fungus and the research project, and asked to provide a dried sample. From iNaturalist, nine samples were acquired from eight users across seven states. Additional samples were acquired through travel to the North Dakota State University Dale E. Herman Research Arboretum in Absaraka, ND, which maintains a small collection of *Rhamnus* and *Frangula* species, incidentally through travel to other states and within Minnesota, and by planned surveys in Minnesota.

Between May and December 2020, four extensive survey trips were conducted. Preliminary observations indicated that *Pcc* was present throughout central Minnesota and the Twin Cities Metro area, where glossy buckthorn is common, so survey routes were designed to visit reports of glossy buckthorn in the westernmost and northernmost limits of its range in the state (EDDMapS, 2022). Beyond these limits, reed canarygrass, abundant throughout the state, was inspected for uredinia and telia on the current and previous year's growth. Reed canarygrass rhizomes were collected from survey sites to assess their

susceptibility to *Pcc.* Plants were grown in greenhouses and inoculated with aeciospores when plants were 10-25 cm tall. After 14 days, plants were rated on a 0 – 4 scale adapted from Murphy (1935). Ratings of 3 or 4 indicated susceptibility.

### Development of isolates

Spores from live aecial and uredinial samples were vacuum collected into gelatin capsules, desiccated for five days, and stored in a -80 °C freezer. Live aeciospores or urediniospores, from field samples or storage, were suspended in Soltrol® 170 (Chempoint) and sprayed onto reed canarygrass plants grown in 3-inch diameter pots. Inoculated plants were kept overnight in a dew chamber at 100% relative humidity and then maintained in isolation in a greenhouse for 2-4 weeks in plexiglass cubicles fitted with plastic sheets to reduce contamination. For the development of single-pustule, or pure isolates, leaf segments with one uredinium were excised, washed of loose spores with distilled water, and incubated in a moist chamber to induce sporulation. The new spores were transferred by toothpick to the young leaf of another plant, which was incubated overnight in a dew chamber and maintained in a cubicle in the greenhouse. After 2-3 weeks, spores were collected. Isolates were maintained by repeated inoculation onto reed canarygrass. Dried leaf tissue from field samples and inoculations was stored in glycine bags or herbarium presses for later reference.

### DNA extraction, amplification, and sequencing

Four pure isolates and 18 field samples were selected for sequencing, primarily to maximize geographic range within Minnesota and the United States (Table 1.1). DNA from approximately 1 $cm^2$ of rust-infected plant tissue was extracted with an OmniPrep™ genomic DNA extraction kit (G-Biosciences).

Following Liu and Hambleton (2013), four loci were selected for sequencing: segments of *COI,* β-*tubulin*, and *RPB2*, and the ITS region. For the ITS region, primers ITS1rustF10d (Barnes and Szabo, 2007) or Rust2inv (Aime, 2006) were used with the reverse primer ITSRu1 (Hambleton et al., 2019, Rioux et al., 2015). For the β-*tubulin* segment, new primers were designed with Primer-BLAST (Ye et al., 2012) based on sequences from Liu and Hambleton (2013): BtubPcF (5′-CCCTACAACGCCACCTTGTC-3′) and BtubPcR (5′-GTGTACCAATGCAGGAAGGC-3′). For *COI* and *RPB2*, we follow Liu and Hambleton (2013). To each primer sequence, a tail was added to the 5′ end to enable barcoding amplicons in a later step. All primers are available as sequences in Supplementary Table 1.1. PCRs were conducted with One*Taq*® 2x Master Mix with Standard Buffer (NEB Inc.). A solution of 5x trehalose, bovine serum albumin, and Tween-20 solution was added to reactions in place of an equal quantity of water (Samarakoon et al., 2013). Otherwise, reactions were conducted according to manufacturer instructions. PCRs were run with the following conditions: initial denaturation at 94 °C for 120 s; 30 cycles of denaturation at 94° C for 30 s, annealing at 54 °C for 30 s, and extension at 68 °C; final extension at 68 °C for 180 s. Products were cleaned up with HighPrep™ PCR (Magbio Genomics) and quantified with a Qubit™ 4 fluorometer (ThermoFisher Scientific). Products originating from the same sample were pooled, with each amplicon equally represented by molarity. Samples (with multiple amplicons per sample) were barcoded and sequenced with an Oxford Nanopore MinION following manufacturer instructions (kits EXP-PBC001 and SQK-LSK109, R9.4.1 flow cells, and MinKnow v21.10.4).

Output files were processed with Guppy v. 5.0.16 (Oxford Nanopore) with the High Accuracy basecalling model, output as FASTQ files, and separated by barcode. With BBDuk v38.84 (Bushnell, 2014), raw reads (PRJNA802333) were filtered for quality (≥Q14) and length (≥200 bp, or ≥500 bp for ITS). With Bowtie2 v2.3.2 (Langmead and Salzberg, 2012) using default settings, reads were mapped to consensus sequences generated (65% agreement) for each of the four regions from sequences of *P.* series *Coronata* in Liu and Hambleton (2013) (*COI, RPB2*, β-*tubulin*) or Szabo (2006) (ITS). Mapped reads were aligned with MAFFT v7.450 (Katoh and Standley, 2013) with default settings. Consensus sequences were generated (65% agreement) and primers trimmed. Sequences were uploaded to NCBI Genbank (Table 1.1). Coverage is provided in Supplementary Table 1.2. Samples selected for sequencing, except one as noted, were deposited at the Arthur Fungarium at Purdue University.

#### Phylogenetic analysis and identification of samples

For each of the four single pustule isolates (Table 1.1), consensus sequences generated for the ITS, *COI*, *RPB2*, and β-*tubulin* loci were used for phylogenetic analysis. Original sequences and selected sequences from Liu and Hambleton (2013), Szabo (2006), and Kenaley et al. (2017) were aligned with MAFFT v7.450 and edited in Geneious. In MEGA v. 7.0.26 (Kumar et al., 2016), the best fit models for the maximum likelihood method were calculated, and with this model, trees were generated using the bootstrap method with 1,000 replications and default settings. Trees were reformatted in FigTree v1.4.4 (Rambaut, 2009) and Microsoft PowerPoint. Other samples were identified with a local BLASTn search (Zhang et al., 2000) using a library containing all

sequences from Liu and Hambleton (2013), Szabo (2006), Kenaley et al. (2017), and from the four isolates identified by phylogenetic analysis.

### Separation of ITS variants

For the single pustule isolates, alignments of the ITS region were visually inspected, and reads were separated into variants based on single nucleotide polymorphisms at four loci where there were ambiguous base calls in one or more of the consensus sequences. Reads with calls of 'N' at any of the four loci were discarded. Consensus sequences were generated for each variant, and only variants with at least 10x coverage are reported (Supplementary Table 1.3). Variants were not uploaded to GenBank but are available as Supplementary Material.

## Results

### Disease description and spore morphology

*Pcc* is a macrocyclic, heteroecious rust fungus with a life cycle similar to *Puccinia coronata* var. *avenae* and other cereal rust fungi. In spring, teliospores embedded in reed canarygrass straw germinate and produce basidiospores that are infectious on glossy buckthorn. From basidiospores, small green to yellow-orange pycnial clusters, or spermagonia, (Fig. 1.1A) are produced on glossy buckthorn leaves, young stem tissue, floral tissues, and fruits. Upon fertilization by pycniospores (spermatia), aecia form. On leaves, aecia occur predominantly on the abaxial surface (Fig. 1.1B). With multiple foliar infections or infections of leaf petioles, plants may drop leaves, sometimes resulting in significant defoliation that can persist through summer. On young shoots, aecia may cause

the formation of shepherd's crooks or swollen galls (Fig. 1.1C), sometimes causing the death of shoots and producing dark persistent scars (Fig. 1.1D). Floral tissue, pedicels, and fruits are often infected, and plants may have reduced flower and fruit production (Fig. 1.1B). Aecial infection is not persistent from year to year in plants maintained in the greenhouse. Uredinia form on the grass host following the production of aecia and aeciospores in late spring or early summer. In repeated greenhouse trials, aeciospores collected from glossy buckthorn were successfully used to inoculate reed canarygrass. Uredinia cause chlorosis and premature senescence of leaves with high levels of infection (Fig. 1.1E) and may persist through fall. In Minnesota, telia form beginning in mid-summer (Fig. 1.1F). Teliospores require an overwintering period for germination. Pycnial clusters small, 0.2 to 0.4 cm, sometimes growing larger with age, especially along leaf veins. Individual pycnia 60 to 80 μm (Fig. 1.2A). Pycniospores 3-5 (6) x 2-3 μm (Fig. 1.2B). Aecia highly variable in size, up to 5 cm or more on large galls. Aeciospores verrucose, (16)19-22(26) x (13)16-19(21) μm (Fig. 1.2C). Uredinia with few clavate paraphyses, amphigenous. Urediniospores echinulate, small, (14)17-21(26) x (13)15-18(21) μm (Fig. 1.2D). Germ pores highly obscure, unable to be observed following the Congo-Red staining procedure used by Liu and Hambleton (2013) and Urban (1963). Telia amphigenous but more common on the abaxial surface. Usually partly covered by the host epidermis. Linear, but sometimes fusing together. Teliospores two-celled, generally clavate but somewhat variable in shape, (27)33-45(56) x (8)12-15(18), modestly constricted at septum (Fig. 1.2E). Length of longest appendage 3 to 7 μm, rarely with short branches. Telial paraphyses present but not abundant. Three-celled teliospores not observed. Basidia four celled (Fig. 1.2F).

Specimens examined: Aecia: PUR N24020, PUR N24026, PUR N24029, PUR N24030, PUR N24033, and PUR N24037. Uredinia: PUR N24017, PUR N24021, PUR N24025, PUR N24023, and PUR N24019. Telia: PUR N24017, PUR N24018, PUR N24021, PUR N24022, and PUR N24040. Images and measurements of pycnia, pycniospores, and basidiospores are from sequenced field samples.

Naturally infected hosts in Minnesota are most commonly glossy buckthorn and reed canarygrass. Bluejoint grass (*Calamagrostis canadensis*) and sweetgrass (*Hierochloe odorata*) have each been observed weakly infected with crown rust near glossy buckthorn. Uredinia were successfully transferred to reed canarygrass, indicating likely infection by *Pcc*.

#### Identification of samples by phylogenetic analysis and BLAST

In maximum likelihood trees generated for the β-*tubulin*, ITS, and *RPB2* loci, consensus sequences from each of the four single pustule isolates consistently grouped with *P. coronata* var. *coronata* samples reported by Liu and Hambleton (2013) (Fig. 1.3). In each tree, the *Pcc* clade was monophyletic and was supported with moderate to high bootstrap support: 90, 84, and 64, respectively. This confirms the results of the first report (Kenaley et al., 2017). *COI* sequences grouped with *P. coronata s.s.* and some other *P.* series *Coronata* clades but did not provide enough definition to identify sequences to variety level, as noted by Liu and Hambleton (2013). Field samples were identified by local BLASTn queries: for each sequence, the closest match, % match, and number of base pairs matching are provided in Supplementary Table 1.4.

### Distribution of *Puccinia coronata* var. *coronata* in Minnesota and North America

Sequencing confirmed the presence of *Pcc* in across the range of glossy buckthorn in the United States from eastern North Dakota east to New England (Fig. 1.4). We report *Pcc s.s.* for the first time in thirteen states: Illinois, Indiana, Iowa, Massachusetts, Michigan, Minnesota, New Hampshire, New York, North Dakota, Ohio, Pennsylvania, Vermont, and Wisconsin. Reports on iNaturalist indicate that *Pcc* likely also occurs in eastern Canada in Ontario and the maritime provinces, as well as Quebec, but we have not confirmed reports by sequencing (Fig. 1.5).

In multiple surveys in the southwest of Minnesota and a survey in the northwest and north central regions, where glossy buckthorn is absent or very uncommon, reed canarygrass plants were usually observed without rust infection (Fig. 1.6). In a survey in the northeast of Minnesota along MN Highway 61 (Cook, Lake, and St. Louis counties), rust infection was absent in the northeasternmost sites where glossy buckthorn is absent but was present at sites nearer to glossy buckthorn populations around Duluth, MN. In greenhouse trials, however, reed canarygrass plants from all survey sites were susceptible to *Pcc*. These surveys suggest *Pcc* is uncommon outside the range of glossy buckthorn despite the presence of widespread, susceptible telial hosts. In contrast, in central and eastern Minnesota, where glossy buckthorn is common, rust on glossy buckthorn and reed canarygrass is ubiquitous.

### Variation in ITS region

In each of the four single-pustule isolates confirmed to be *Pcc* through phylogenetic analysis, we observed consistent variation at four loci in the rDNA region sequenced: at two adjacent positions in the ITS 1 and at two positions in

the ITS 2. In each of the four single pustule isolates, three to five variants were detected with >10x coverage. Each isolate differed in which variants were present, and between the four isolates, nine total variants were detected. Additionally, there are some differences around homopolymer regions between otherwise identical variants, but these are likely due to errors, common near homopolymer regions with Nanopore sequencing. For field samples, alignments were not separated into variants, although in each case, reads similarly vary at some of or all the same positions.

## Discussion

*Puccinia coronata* var. *coronata*, a crown rust pathogen recently observed in North America, is now widespread across the range of glossy buckthorn in the United States. The prairie of western Minnesota marks the western border of naturalized glossy buckthorn, but the range of reed canarygrass, in contrast, stretches across temperate regions of the continent. The range of *Pcc* may extend beyond the one we reported, and continued study will be needed to assess the distribution, especially on other potential aecial hosts such as native *Frangula* spp. To our surprise, in Minnesota, *Pcc* seems mostly restricted to the range of glossy buckthorn despite susceptible reed canarygrass across the state. We hypothesized that urediniospores of *Pcc*, like those of cereal rust fungi, would spread far beyond the vicinity of the aecial host. However, outside the range of glossy buckthorn, *Pcc* is absent or rare. The reason for the limitation of the spread is unknown but has been noted in Europe as well. According to Urban and Marková (1993), who summarize Norwegian sources (Jørstad 1940, 1964), crown rust of *Calamagrostis* spp., presumably *Pcc*, is reported throughout the range of

glossy buckthorn in Norway, but north of the upper limit of the buckthorn, rust is absent despite the presence of susceptible grass hosts.

Prior to the first published report of *Pcc* in North America (Kenaley et al., 2017) and the collection of the sample in Quebec in 2013 (DAOM 242990; GenBank ID OP477344), crown rust of glossy buckthorn and reed canarygrass was rare. While more than 350 herbarium specimens from Canada and the United States are listed as *Pcc* (MyCoPortal, Miller and Bates, 2017; accessed Sept. 16, 2022), apart from the Quebec collection, all samples date from 1995 or earlier and were determined using older and more expansive concepts of the taxon, which included crown rust fungi now placed in other taxa (Liu and Hambleton, 2013). Samples will need to be reexamined given recent revisions in taxonomy. In the USDA fungal host database (Farr et al., accessed Feb. 10, 2022), only three reports of *P. coronata* on *Phalaris* spp. (e.g. reed canarygrass) are listed in North America—far fewer reports than for grass hosts of other crown rust fungi. Similarly, only one report of *P. coronata* is listed for glossy buckthorn. Melhus et al. (1922) reported that glossy buckthorn in Ames, Iowa was never observed infected with aecia and that aecia were believed to be rare on the species throughout the "Middlewest." One report (Carleton, 1899; see Melhus et al. 1922) describes aeciospores from lanceleaf buckthorn (*R. lanceolata*) infecting *Phalaris caroliniana*. This is possibly consistent with *Pcc* but is more likely another crown rust form. Grass genera and species are often able to host more than one species of crown rust fungus (Eshed and Dinoor, 1980; Liu and Hambleton, 2013). Glossy buckthorn had been observed with rust infection in the Twin Cities by Dr. Yue Jin in years prior to the observation of *Pcc*, but rust infection was very uncommon locally. Two samples of rust on glossy buckthorn collected near St.

Paul, Minnesota in 2012 were each identified with sequencing as *P. coronati-agrostidis* (unpublished). In contrast, since 2018, when we began carefully observing *Pcc* infection, from mid-May to mid-June in the Twin Cities, we have not seen a single glossy buckthorn plant without rust infection.

Aecial infection in Quebec in 2013 implies the presence of the uredinial and telial stage in 2012 in North America. Similarly, in 2016, Kenaley et al. (2017) observed rust infection of glossy buckthorn in Connecticut, implying an introduction to the United States by 2015 at the latest. It is possible the rust was present earlier and escaped detection. *Pcc* infection is dramatic, however, and it is unlikely it would have been present for long without notice. There is another possibility that the rust was present earlier but that some other factor led to its recent proliferation. For example, the introduction of a new pathotype or the acquisition of virulence on an important resistance gene in reed canarygrass could have facilitated the spread of *Pcc*. The available evidence suggests a recent introduction, or at least a recent emergence.

Liu and Hambleton (2013) did not sequence any specimens of *Pcc* on reed canarygrass, and neither they nor Kenaley et al., (2017) list it as a host. Earlier taxonomic literature (Brown, 1937; Cummins, 1971; Urban and Marková, 1993) however did consider reed canarygrass as a host of *Pcc*, and Brown (1937) determined through host specificity assays that the crown rust fungi in England that infected *Calamagrostis* spp. and reed canarygrass were likely the same. In our study, teliospore morphological characteristics are consistent with *P. coronata* var. *coronata* Liu and Hambleton (2013), especially the type specimen, as shown in an image panel. In contrast to their report, three-celled teliospores with long first cells were not observed in any samples and, as a result, the given teliospore

length is lesser, on the low end of the given range. Other spore types were not reported. Differences in our morphological description may be attributable to the population in North America or due to the telial hosts examined. Cummins (1971) and Urban and Marková (1993) each noted small urediniospores as typical of *Pcc*, and likewise, we report urediniospores that are smaller than those of *Puccinia coronata* var. *avenae* (Cummins, 1971). Phylogenetic analysis clearly groups our samples with those identified by Liu and Hambleton (2013) as *Puccinia coronata* var. *coronata* and confirms reed canarygrass as a host of *Pcc.*

Variation in the ITS region of single pustule isolates could be explained by contamination of isolates or of PCR products and by variation between rather than within isolates. However, this seems unlikely to have occurred with all four isolates. A likely explanation is intragenomic variation. With Nanopore sequencing, each read generally corresponds to a single strand of DNA. Thus, when multiple copies of a gene or multiple alleles occur and are amplified by the same PCR, different amplicons will be generated and may later be differentiated. In fungi, multiple copies of the rDNA region, which includes the ITS, are present in the genome (Stadler et al. 2020; Maleszka and Clark-Walker, 1990). It is often taken for granted that rDNA copies are identical within an individual, but studies have repeatedly documented intragenomic variation in the rDNA regions in diverse taxa of fungi (Lindner and Banik, 2011; Paloi et al., 2021; Vydryakova et al., 2011;). In rust fungi, the occurrence of multiple ITS variants in single isolates or samples has been reported several times (Alaei et al., 2009; Freire, 2008; McTaggart and Aime, 2018; Virtudazo et al., 2001). Aecia, uredinia, and telia are all dikaryotic stages of the rust life cycle, and some variation may occur between nuclei within isolates as well. Before next generation sequencing

technologies, investigation of ITS variants required Sanger sequencing of multiple clones per sample, a labor intensive and costly, while valuable, process. Sanger sequencing of uncloned PCR products of the ITS region can produce inconclusive results due to variation. As Nanopore sequencing of rDNA regions becomes more common, researchers will likely uncover much more intragenomic variation as well as intraspecific variation. User-friendly tools to detect likely ITS variants with Nanopore reads will be needed. A more thorough investigation of rDNA variation within pure rust isolates would be valuable.

For this project, iNaturalist.org and its users were instrumental in assessing the distribution of *Pcc*. In recent years, community or citizen scientists have been critical to efforts in detecting and mapping invasive species (Larson et al., 2020). Collaborators sent samples from seven states and were sometimes able to provide detailed information about the sample location such as the composition of the local plant community. Many users are natural resources workers or highly knowledgeable non-professionals. As of June 2022, on iNaturalist.org there are 97 confirmed observations of rust on glossy buckthorn spanning from Minnesota to Prince Edward Island (iNaturalist contributors and iNaturalist, 2022). In this case, several factors likely contribute to the number of reports: the rust is abundant, bright orange, and conspicuous on a widespread and well-known invasive species in densely populated regions of North America. Nevertheless, this project is a testament to the power of community science and of the amateur mycological and naturalist communities to help assess new threats to plant health and monitor the spread of introduced and emerging pathogens.

While *Pcc* is an exotic pathogen, it appears to have a positive effect locally: it primarily infects two highly aggressive plant species with possible costs to host fitness and competitive ability. Given the likely recent introduction of *Pcc* to North America, its significant effect on two invasive species, and its relation to important pathogens of cereal crops, research into *Pcc* is ongoing. Later publications will address its pathogenicity on other buckthorn and grass species, including on cereal crops, and the effects of *Pcc* on glossy buckthorn and reed canarygrass growth in greenhouse trials.

**Table 1.1.** Sample collection information with GenBank and herbarium accession numbers of *Puccinia coronata* var. *coronata* samples.

| Sample ID | Host | Location | Date of collection | Collector[2] | ITS | *COI* | β-*tub* | *RPB2* | Voucher number |
|---|---|---|---|---|---|---|---|---|---|
| **18_NH_Pa01** | *Phalaris arundinacea* | 43.136, -70.940 | 28-Jul-18 | Yue Jin | OP795932 | OP821271 | OP821268 | OP821272 | PUR N24040 |
| **18_WI_Pa01-1[1]** | *P. arundinacea* | 44.39, -92.02 | 26-Aug-18 | N. Greatens and Julia Mitchell | OM471916 | OM489424 | OM515198 | OM514671 | PUR N24017 |
| **19_IA_Pa01** | *P. arundinacea* | 43.227, -92.188 | 22-Oct-19 | N. Greatens | OM471915 | OM489423 | OM515199 | OM514670 | PUR N24018 |
| **19_MN_Pa01** | *P. arundinacea* | 47.464, -91.03 | 24-Aug-19 | N. Greatens | OM471914 | OM489422 | - | - | PUR N24019 |
| **19_ND_Fa01** | *Frangula alnus* | 46.988, -97.353 | 17-Jun-19 | N. Greatens | OM471913 | OM489421 | - | - | PUR N24020 |
| **19_OH_Pa01-1[a]** | *P. arundinacea* | 41.542, -81.633 | 3-Aug-19 | N. Greatens | OM471912 | OM489420 | OM515200 | OM514669 | PUR N24021 |
| **19_OH_Pa02** | *P. arundinacea* | 41.307, -81.577 | 6-Aug-19 | N. Greatens and Yue Jin | OM471911 | OM489419 | OM515201 | OM514668 | PUR N24022 |
| **19_WI_Pa01** | *P. arundinacea* | 44.994, -87.684 | 1-Jun-19 | Gordon Cisar | OM471910 | OM489418 | - | - | PUR N24023 |
| **20_IL_Fa01** | *F. alnus* | 41.719, -87.583 | 28-May-20 | **Cassi Saari** | OM471909 | OM489417 | - | - | PUR N24024 |
| **20_MN_Fa01-1[a]** | *F. alnus* | 44.995, -93.26 | 1-May-20 | N. Greatens | OM471907 | OM489415 | OM515203 | OM514666 | PUR N24025 |

**Table 1.1 continued**

| Sample ID | Host | Location | Date of collection | Collector[2] | ITS | *COI* | β-*tub* | *RPB2* | Voucher number |
|---|---|---|---|---|---|---|---|---|---|
| **20_MN_Fa02-1[a]** | *F. alnus* | 43.948, -91.399 | 7-Jun-20 | N. Greatens | OM471908 | OM489416 | OM515202 | OM514667 | PUR N24026 |
| **20_MN_Pa02** | *P. arundinacea* | 44.031, -94.601 | 6-Dec-20 | N. Greatens | OM471906 | OM489414 | - | - | NA |
| **21_IN_Fa01** | *F. alnus* | 41.602, -87.221 | 7-Jun-21 | **iNaturalist collaborator** | OM471905 | OM489413 | - | - | PUR N24028 |
| **21_MA_Fa01** | *F. alnus* | 42.137, -72.435 | 5-Jun-21 | Nick Klejeski | OM471904 | OM489412 | - | - | PUR N24029 |
| **21_MA_Fa02** | *F. alnus* | 42.285, -71.181 | 10-Jun-21 | **iNaturalist collaborator** | OM471903 | OM489411 | - | - | PUR N24030 |
| **21_MI_Fa01** | *F. alnus* | 43.617, -84.182 | 18-May-21 | **Adam Kranz** | OM471902 | OM489410 | - | - | PUR N24031 |
| **21_MI_Fa02** | *F. alnus* | 42.134, -84.393 | 8-Jun-21 | **iNaturalist collaborator** | OM471901 | OM489409 | - | - | PUR N24032 |
| **21_OH_Fa01** | *F. alnus* | 41.361, -81.856 | 27-May-21 | **iNaturalist collaborator** | OM471900 | OM489408 | - | - | PUR N24033 |
| **21_PA_Fa01** | *F. alnus* | 40.971, -75.714 | 24-May-21 | **Claire Ciafré** | OM471898 | OM489406 | - | - | PUR N24035 |
| **21_PA_Fa02** | *F. alnus* | 40.895, -75.748 | 24-May-21 | **Claire Ciafré** | OM471897 | OM489405 | - | - | PUR N24036 |

## Table 1.1 continued

| Sample ID | Host | Location | Date of collection | Collector | ITS | *COI* | β-*tub* | *RPB2* | Voucher number |
|---|---|---|---|---|---|---|---|---|---|
| **21_VT_Fa01** | *F. alnus* | 44.517, -73.24 | 21-Jun-21 | **Michael Sundue** | OM471896 | OM489404 | - | -- | PUR N24037 |
| | | | | | | | | | |
| **22_NY_Pa01** | *P.* | 42.443, - | 14-Aug-22 | N. Greatens | OP795933 | OP821270 | OP821269 | OP821273 | PUR N24041 |

[1]Single pustule isolates are indicated by a "-1" following the isolate name.

[2] Collaborators from iNaturalist are emphasized in bold. Some elected to remain unnamed or could not be reached again prior to publication.

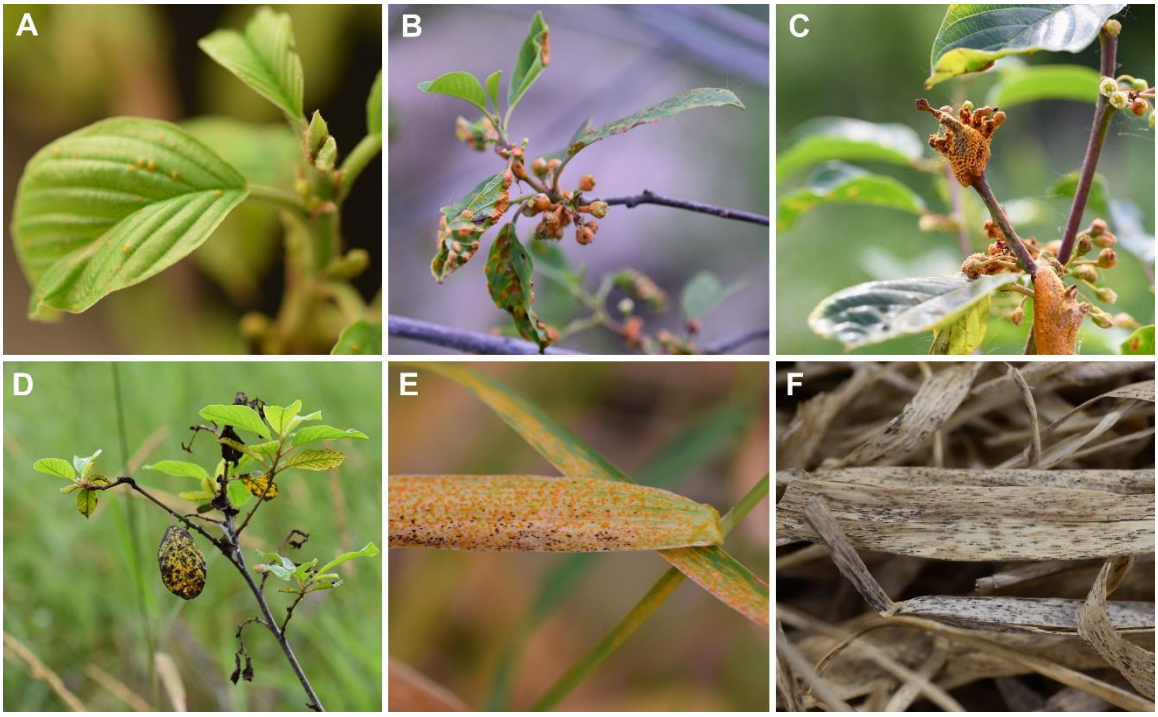


**Figure 1.1.** *Puccinia coronata* var. *coronata* (*Pcc*) and its effects on glossy buckthorn and reed canarygrass. **A)** *Pcc* pycnial clusters on glossy buckthorn. **B)** *Pcc* infection on leaves and fruits of glossy buckthorn. **C)** Glossy buckthorn with two rust galls on young stem tissue. **D)** Partially defoliated glossy buckthorn with blackened scar tissue and shoot dieback two months after rust infection. Dry reed canarygrass inflorescences are visible in the background. **E)** Uredinia and telia of *Pcc* on leaves of reed canarygrass cultivated in a greenhouse. **F)** *Pcc* telia in spring on the previous year's reed canarygrass straw.

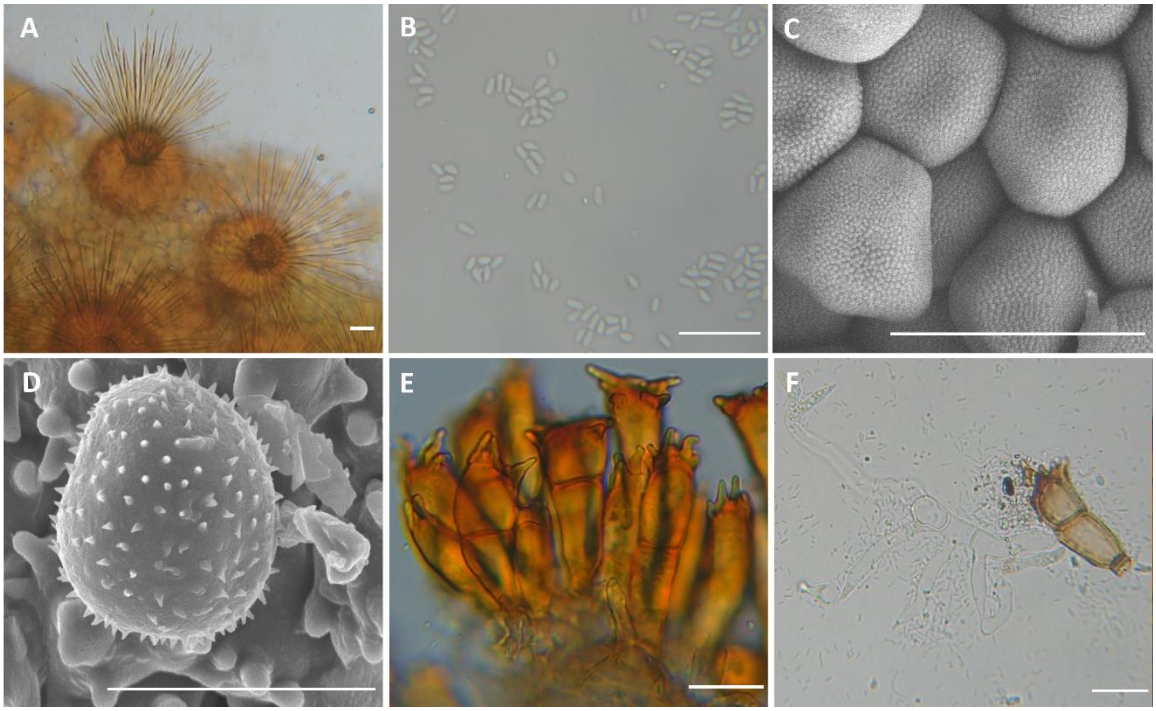


**Figure 1.2.** Microscopic images of *Pcc* spores and spore structures. **A)** Pycnia with receptive hyphae extended (field sample). 10x/.22 objective. Bar = 20 μm. **B)** Pycniospores (field sample). 40/.75 objective. Bar = 20 μm. **C)** Aeciospores (PUR N24035). 10kV. Sample dried, uncoated. Bar = 20 μm. **D)** Urediniospore resting on teliospores (PUR N24017). 10kV. Sample dried, uncoated. Bar = 20 μm. **E)** Teliospores (PUR N24017). 16x/.4 objective. Bar = 20 μm. **F)** A germinating teliospore with a four-celled basidium and a germinating basidiospore visible attached to the third cell (field sample). 16x/.4 objective. Bar = 20 μm. SEM images by the University of Minnesota University Imaging Centers.

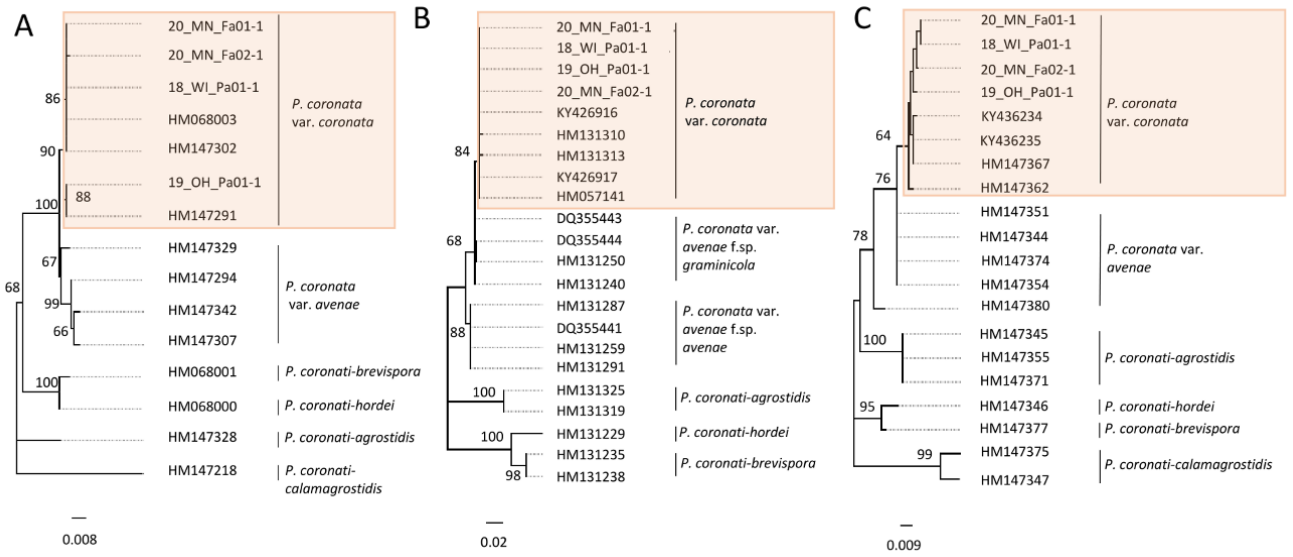


**Figure 1.3.** Phylogenetic analysis of β-*tubulin*, ITS, and *RPB2* consensus sequences from four single pustule isolates of *Puccinia coronata* var. *coronata*. **A)** ML tree for β-*tubulin*. Model: Kimura 2-parameter with Gamma distributed rates. **B)** ML tree for the ITS region. Model: Tamura 3-parameter with Gamma distributed rates. **C)** ML tree for the *RPB2* region. Model: Kimura 2-parameter with Gamma distributed rates. For each analysis, sequences representing all clades of *P. coronata s.s.* are included. Other *P. coronata sensu lato* species present in Minnesota are included as outgroups. Additional sequences are from Liu and Hambleton (2013), Szabo (2006) and Kenaley et al. (2017). Taxa are named following Liu and Hambleton (2013).

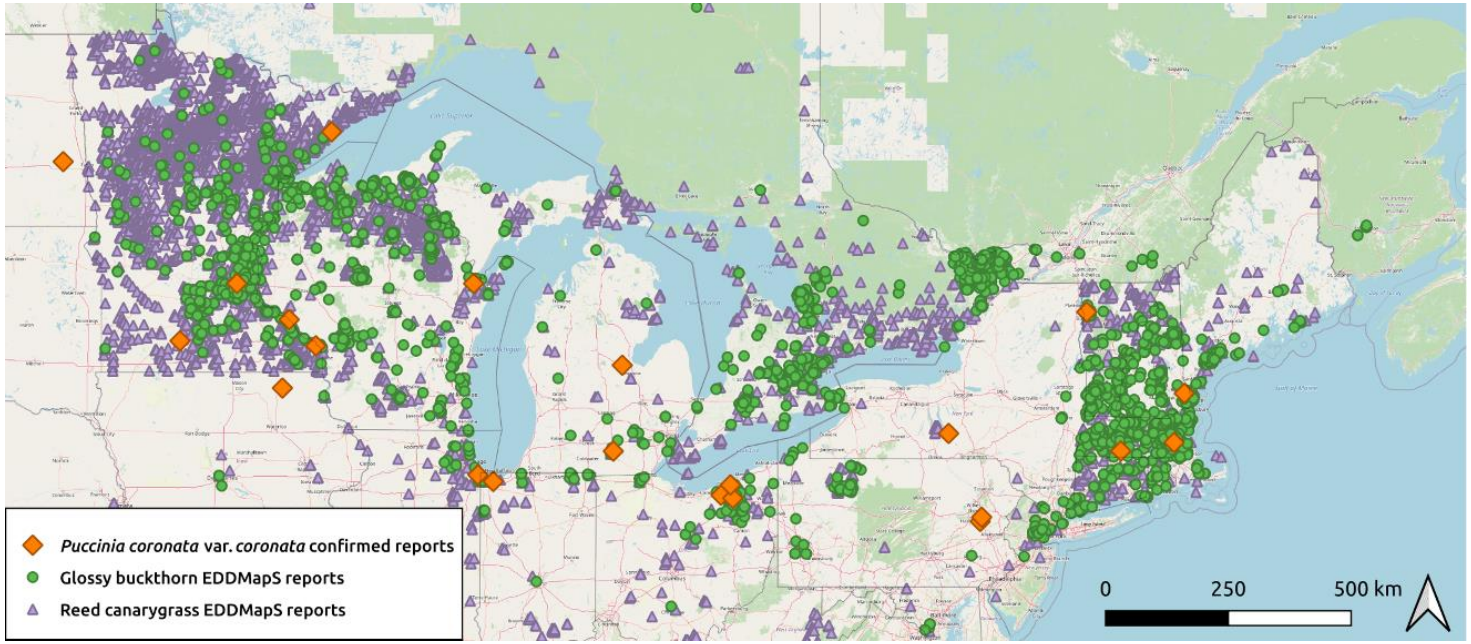


**Figure 1.4.** Confirmed reports of *Puccinia coronata* var. *coronata* in the United States from this study with reports of reed canarygrass and glossy buckthorn on EDDMapS. Map prepared in QGIS v3.10.4 with OpenStreetMap as the basemap. EDDMapS data downloaded Nov. 10, 2021. (EDDMapS is a mapping system in wide use by natural resources professionals in North America to document invasive species.) Only positive records with available coordinates appear in the map.

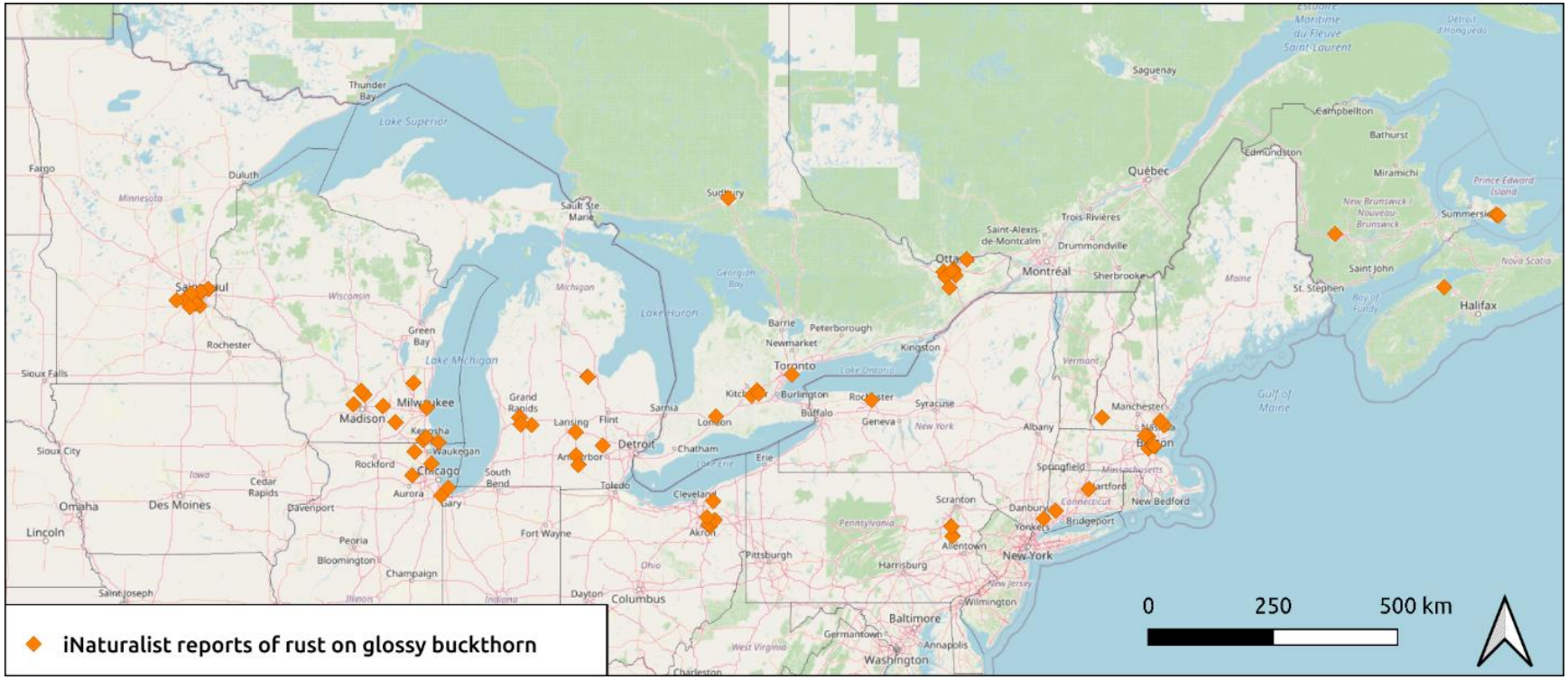


**Figure 1.5**. Reports of rust on glossy buckthorn on iNaturalist.org as of February 10, 2022. Map prepared in QGIS v3.10.4 with OpenStreetMap as the basemap.

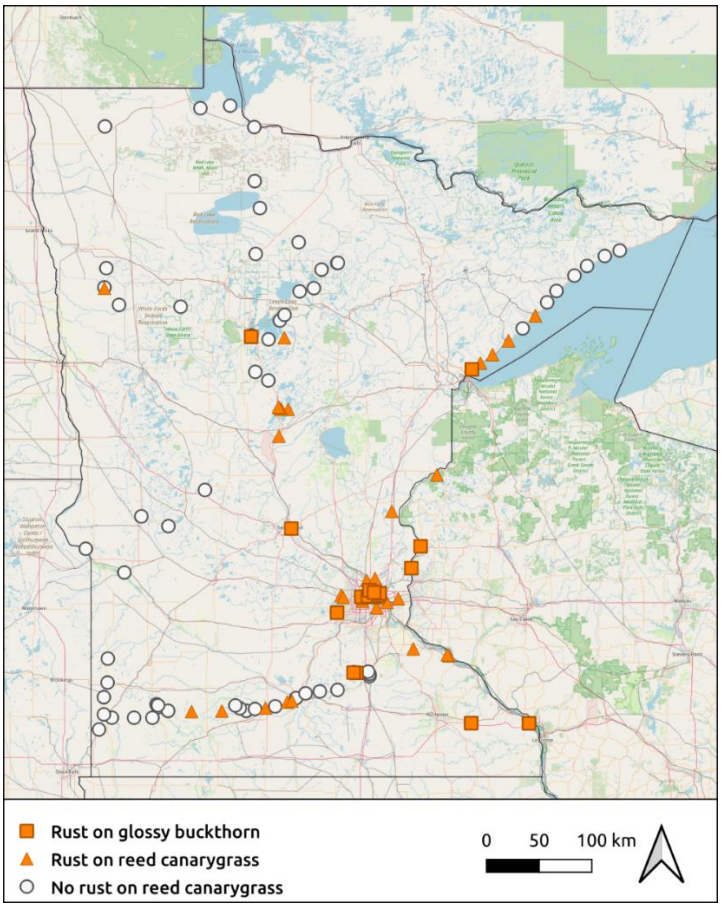


**Figure 1.6.** The results of surveys for *Puccinia coronata* var. *coronata* on glossy buckthorn and reed canarygrass in Minnesota. Four surveys were conducted in 2020: Southwest Minnesota from June 20-21; Northwest MN from July 6-8; Northeast MN on Aug 29; and Southwest MN on Dec. 9, 2020. Additional observations and sample collections were made during travel around the Twin Cities Metro Area from 2017-2022. Positive results are presumed to be predominantly *Pcc* but were not sequenced for confirmation except as noted elsewhere. Negative results indicate an absence of *Pcc* on reed canarygrass. Map prepared in QGIS v3.10.4 with OpenStreetMap as the basemap.

# Chapter Two

## Aecial and telial host specificity of *Puccinia coronata* var. *coronata,* a Eurasian crown rust fungus of two highly invasive wetland species in North America

This chapter in a similar form has been accepted for publication to Plant Disease as:

Greatens, N., Jin, Y., and Olivera Firpo, P.D. 2023. Aecial and telial host specificity of *Puccinia coronata* var. *coronata,* a Eurasian crown rust fungus of two highly invasive wetland species in North America. Plant Dis.

## Summary

The Eurasian crown rust fungus *Puccinia coronata* var. *coronata (Pcc)* was recently reported in North America and is widespread across the Midwest and Northeast United States. *Pcc* is a close relative of major pathogens of oats, barley, and turfgrasses. It infects two highly invasive wetland plants, glossy buckthorn (*Frangula alnus*) and reed canarygrass (*Phalaris arundinacea*) and could be useful as an augmentative biological control agent. We conducted large greenhouse trials to assess the host specificity of *Pcc* and determine any threat to cultivated cereals, turfgrasses, or native North American species. A total of 1,830 accessions of cereal crop species and 783 accessions of 110 other gramineous species were evaluated. Young plants were first inoculated with a composite uredinial inoculum derived from aecia. Accessions showing sporulation were further tested with pure rust isolates. Sixteen potential aecial hosts in the families Rhamnaceae and Elaeagnaceae were tested for susceptibility through inoculation with germinating teliospores. Thirteen grass species within five genera in the tribe Poeae, *Apera, Calamagrostis, Lamarckia, Phalaris,* and *Puccinellia,* and four species in Rhamnaceae, *Frangula alnus, F. californica*, *F. caroliniana*, and *Rhamnus lanceolata*, were found to be susceptible to *Pcc,* with some species native to North America. All assessed crop species and turfgrasses were resistant. Limited sporulation was observed on some other species within Poeae and four other tribes, Brachypodieae, Bromeae, Meliceae, and Triticeae. Among these species are oats, barley, and *Brachypodium distachyon*, suggesting the possible use of *Pcc* in studies of non-host resistance.

## Introduction

*Puccinia coronata* var. *coronata* (*Pcc*) is a crown rust fungus that alternates between reed canarygrass (*Phalaris arundinacea*) and glossy buckthorn (*Frangula alnus*) (Greatens et al. 2023), two highly invasive wetland plants in North America (Galatowitsch et al. 1999; Fiedler and Landis, 2012). *Pcc* was first observed on glossy buckthorn in the United States in 2016 (Kenaley et al. 2017) and has since been documented across fourteen states in the Midwest and Northeast (Greatens et al. 2023). Where glossy buckthorn and reed canarygrass grow together, *Pcc* proliferates, causing significant disease on both hosts with likely detrimental effects on host fitness and competitive ability. *Pcc* is a member of *Puccinia* series *Coronata,* or the crown rust fungi (Liu and Hambleton, 2013), a series that includes major pathogens of oats, barley, and turfgrasses (Nazareno et al. 2018; Jin and Steffenson, 1999; Bonos et al. 2006) as well as pathogens of several common weeds in North America including quackgrass (*Elymus repens)* and smooth brome (*Bromus inermis*) (Delgado et al. 2003; Jin and Steffenson, 1999). Early taxonomic treatments of *P. coronata sensu lato* relied on aecial and telial host specificity to delineate taxa, and a multitude of studies from Europe and North America dating back to the 19th century have reported the host specificities of various crown rust fungi (Brown, 1937; Melhus et al. 1922; Urban and Marková, 1993). The literature remains useful and guided this study, but previous results are complicated to interpret given changes in taxonomy. Older treatments of *Pcc* (Cummins, 1971; Urban and Marková, 1993) were polyphyletic (Liu and Hambleton, 2013), and the taxon is now more narrowly defined. The

host specificities of the various species, varieties, and *formae speciales* of *P.* ser. *Coronata* may need to be reevaluated.

Given the recent emergence of *Pcc* in North America, its relation to important agricultural pathogens, and its effects on two highly aggressive wetland plant species, we conducted a large trial to evaluate its host specificity on grasses and on potential aecial hosts in Rhamnaceae and Elaeagnaceae.

## Materials and Methods

### Plant Materials

In total, 2,614 accessions from 116 gramineous species were evaluated in this study. This collection includes: 1,830 accessions of six cereal crop species including oats (*Avena sativa*), barley (*Hordeum vulgare*), common wheat (*Triticum aestivum*), durum wheat (*T. turgidum* ssp. *durum*), rye (*Secale cereale*), and triticale (x*Triticosecale*) (Table 2.1); 719 accessions of 110 turfgrass and wild grass species in 45 genera (Table 2.2); and 65 accessions of the model species *Brachypodium distachyon* and related *Brachypodium* spp. Sixteen potential aecial hosts in the buckthorn (Rhamnaceae) and oleaster (Elaeagnaceae) families were also evaluated for susceptibility.

Initial selections of barley, bread wheat, and rye accessions from the USDA-National Small Grains Collection (NSGC) were made to maximize geographic diversity. In addition, one bread wheat line (PI 350005) susceptible to *P. coronati-hordei,* the causal agent of barley crown rust (Niu et al. 2014), the 20 wheat lines of the North American stem rust differential set (Jin et al. 2008), and four wheat genotypes carrying important stem rust resistance genes (*Sr7a, Sr13b, Sr22,* and *Sr35*) were included. Durum and triticale lines were selected from two

diverse collections recently screened for stem rust resistance (Olivera et al. 2013; 2021). A first series of 240 oat accessions was selected from NSGC to maximize geographic diversity, and 1,010 additional accessions were added based on their inclusion in trials investigating oat crown rust resistance: 603 accessions from GRIN collection "oat.crown.rust.batonrouge.16" and 410 random selections from collection "oat.crownrust.stpaul.99". (Suppl. Table 2.1)

Turfgrass and wild grass species were selected based on availability and on their inclusion in other studies of *P.* ser. *Coronata,* especially by Liu and Hambleton (2013) and Urban and Marková (1993). Seeds were sourced from the USDA National Plant Germplasm System (NPGS), part of the Germplasm Resources Information Network, GRIN. For *Phalaris* and *Calamagrostis* spp., all available accessions at NPGS were requested and screened. For other grass species, up to ten accessions per species were selected as available. Plants of nineteen varieties of creeping bentgrass (*Agrostis stolonifera*) were transplanted from the 2015 National Turfgrass Evaluation Program greens trials maintained by the University of Minnesota turfgrass science program, and live plants of four species were collected locally around Minnesota: *Ammophila breviligulata, Anthoxanthum nitens, Calamagrostis xarundinacea* 'Karl Forster', and *Phalaris arundinacea*. Seeds of *Brachypodium distachyon* were sourced from NPGS and by correspondence with Dr. Dave Garvin (USDA-ARS) and Dr. John Vogel (JGI). Initial selections were made based on availability. Other lines were later included based on their use in other investigations of *B. distachyon* and its response to various rust fungi (Ayliffe et al. *2013;* Figueroa et al. 2013; Omidvar et al. *2018*). (Suppl. Table 2.2)

Seeds or live plants in the families Rhamnaceae and Elaeagnaceae were acquired from NPGS, collected from parks in the Twin Cities, MN and the North Dakota State University Dale E. Herman Research Arboretum, and ordered from native plant nurseries in the United States (Suppl. Table 2.3).

### Rust evaluation of gramineous hosts

Seeds of cereal crop species and *Brachypodium* spp. were planted in square pots (6 cm x 6 cm x 6 cm) filled with vermiculite (Sun Gro Hort.). In each pot, four accessions were planted with five seeds per accession. After planting, pots were placed in a greenhouse maintained at 19 to 22°C with a photoperiod of 16 h. Seedling plants were inoculated at 8 to 9 days old when primary leaves were fully expanded, and *Brachypodium* accessions were inoculated three weeks after planting. Wild grasses were grown in Pro-Line C/B potting soil (Jolly Gardener) in 4 cm diameter x 16 cm length plastic cone-tainers for between 3-8 weeks, depending on plant growth rate. *Pcc* uredinial inoculum was generated as described in Greatens et al. (2023) and suspended in light mineral oil (Soltrol 170®, ChemPoint) for spray inoculations. After inoculation, plants were incubated overnight in a dew chamber and moved to a greenhouse. Cereals and *Brachypodium* spp. were scored 12-18 days after inoculation on a 0 to 4 scale adapted from Murphy (1935), where: 0 = immunity; ";" = chlorotic flecking; N = necrotic flecking; 1 = small uredinia with significant chlorosis and/or necrosis; 2 = small to midsized uredinia small to midsized with chlorosis; 3 = midsized to large uredinia with some chlorosis; 4 = large uredinia without chlorosis; "+" and "-" following numeric ratings indicate intermediate infection types. Wild grasses were rated 2 to 3 weeks after inoculation following the same scale. Accessions

exhibiting infection types (IT) ≥ 3 were considered susceptible. Susceptible reed canarygrass collections were used as positive controls.

Wild grasses and cereals were first inoculated with a bulk inoculum of urediniospores. The composite (bulk) uredinial inoculum was generated by inoculating aeciospores collected from glossy buckthorn onto reed canarygrass. This inoculum was presumed to have significant genetic diversity because it was a direct product of the sexual stage. Inoculation with bulk inoculum was done to assess broad-spectrum resistance. Accessions exhibiting sporulation were rescreened with one or both of the pure isolates of *Pcc* (20_MN_Fa02-1 and 18_WI_Pa01-1) that were derived from single pustules (Greatens et al. 2023). Some species or accessions that were added to trials later were screened only with the pure isolates.

#### Rust evaluation of aecial hosts

Species of Rhamnaceae and Elaeagnaceae tested for potential aecial hosts were acquired as live plants or as seeds (Suppl. Table 2.3). Seeds were stratified at 4 °C for three months in a mix of sand and peat moss and, in spring, transplanted into potting soil and grown in a greenhouse. All adult plants were kept in a vernalization chamber (3 to 4 °C) each winter (November through April) and returned to the greenhouses in spring. Young leaf tissue was present. In late April, naturally overwintered, telia-bearing reed canarygrass straw was collected from Acorn Park in Roseville, MN. Straw was soaked in water for 4 hours and suspended over plants in a dew chamber for 2 to 7 days (Jin and Steffenson, 1999). After inoculation, plants were incubated in a greenhouse and

rated for rust reaction 4-5 weeks later when aecia were fully developed on glossy buckthorn, the susceptible control.

## Results

All accessions of the cereal crop species oat, rye, barley, bread wheat, durum wheat, and triticale were resistant in our evaluations (Table 2.1). Although no symptoms (immune reaction) were observed in most of the accessions, some macroscopically visible resistant reactions, such as chlorosis and hypersensitive necrosis were observed in some accessions of all cereal species. These responses were most common in rye, barley, and oat, where individual lines varied in their degree of flecking. From the 1,250 oat accessions screened with the *Pcc* bulk inoculum, 147 lines exhibited macroscopic symptoms with infection types between fleck (;) and 2. These lines were re-evaluated with the two pure isolates. In this follow-up inoculation, uredinia were observed on thirty-two accessions (Suppl. Table 2.1, Fig. 2.1A). Responses to the two isolates were similar in most of the accessions. Variation in infection type was sometimes observed among replicates within lines when challenged with a pure isolate. A single oat accession, a landrace originally from Iran (PI 287308), was rated as IT = 2 to both isolates. Limited sporulation (ITs = ;1- and ;1) was observed on several rye and barley when inoculated with the bulk inoculum. On barley line CI7498, the IT = ;1- was also observed with the pure isolate 20_MN_Fa02-1 (Suppl. Table 2.1).

Grass species within five tribes of *Poaceae* were able to host *Pcc* to the point of sporulation: Brachypodieae, Bromeae, Meliceae, Poeae, and Triticeae (Table

2.2). Within the tribe Poeae, 13 species in five genera were found to be generally susceptible (IT ≥ 3) in at least one accession (Table 2.2). In all these species, except for *Lamarkia aurea* and *Puccinellia gigantea*, resistant reactions were also observed. Thirty-five other grass species were not considered susceptible but were able to host *Pcc* weakly with sporulation observed.

Three *Phalaris* spp. were considered susceptible to *Pcc* (Figs 2.1B and 2.1C). *Phalaris arundinacea* accessions varied significantly in their response to *Pcc*, ranging from complete immunity to high susceptibility (IT = 3+ or 4) when inoculated with bulk and single pustule isolates (Fig 2.1B). Susceptible responses were more common than resistant ones among the NPGS accessions. Among the highly susceptible *P. arundinacea* lines were 'Paloton' and 'Venture', two varieties grown as forages. The common ornamental variegated variety *P. arundinacea* var. *picta* 'Feesey' is highly resistant, although small uredinia formed in the areas of the leaves lacking chlorophyll (Fig. 2.1B2). While *P. angusta* and *P. paradoxa* were considered susceptible, only one accession of each was able to be acquired, and it is unknown how representative these accessions are of their species. *Phalaris coerulescens* was generally resistant, but one of the five accessions tested was susceptible. Other *Phalaris* spp. were highly to moderately resistant, with *Pcc* successfully sporulating in some species. Based on the location data of accessions deposited at NSGC, there was no clear association between the geographic origin (Eurasia or North America) and disease reaction.

Two of the five *Calamagrostis* spp. assessed were susceptible to *Pcc. Calamagrostis canadensis,* or bluejoint grass, a common North American species, was generally resistant, but five of the twenty-one accessions were rated as IT = 3 to at least one inoculum (Fig 2.1D1-3). Four of the five accessions of *C.*

*purpurascens* were highly susceptible, and *C. stricta* was consistently highly susceptible in two of the four accessions. One accession of both species was highly resistant (Fig 2.1D4-7). *Calamagrostis* x*arundinacea* 'Karl Forster', a popular ornamental grass, and *C. breviligulata* are highly resistant, although sporulation was observed.

Species within three other genera were considered susceptible: *Apera, Lamarckia,* and *Puccinellia*. Within *Apera,* accessions of both *A. spica-venti* and *A. intermedia* were susceptible (Fig 2.1E1-2). Others were resistant. *Lamarkia aurea* was moderately susceptible across inoculations (IT = 3; Fig 2.1E4), although only one accession was available through GRIN. All *Puccinellia* spp. assessed were able to host *Pcc* to varying degrees, with accessions of some species, *P. distans, P. gigantea, P. intermedia, P. nuttalliana,* and *P. stricta* rated as susceptible in at least one inoculation. *Puccinellia tenuiflora* was resistant. (Fig 2.1E5-10).

Of the 18 accessions of *Brachypodium distachyon* inoculated with the bulk inoculum, sporulation was observed on ten accessions at two weeks post inoculation (Suppl. Table 2.2). Infection types ranged from ';N' to '2'. Following these evaluations, 36 additional lines were acquired and inoculated with the two pure isolates. On two lines (PI 239713 and W6 39246), sporulation was observed when evaluated with both 20_MN_Fa02-1 and 18_WI_Pa01-1 isolates at 15 days post-inoculation. In three additional lines, sporulation was observed when inoculated with isolate 18_WI_Pa01-1. A strong hypersensitive response was common, and all plants had clear resistance responses (Fig 2.1F). All evaluated accessions of *B. stacei, B. sylvaticum,* and *B. hybridum (B. stacei* x *B. distachyon)* were immune.

Among the grasses considered resistant, but weakly infected by *Pcc* are *Vulpia octoflora,* sweetgrass (*Anthoxanthum nitens),* and orchardgrass (*Dactylis glomerata*) (Fig 2.1E3), an important forage. *Dactylis* is the sister genus to the susceptible *Lamarckia*. Four wild relatives of oats, *Avena barbosa, A. fatua, A. longiglumis,* and *A. strigosa* were each able to host *Pcc* weakly, although the degree of infection varied with inoculum and by accession. The turfgrasses creeping bentgrass (*Agrostis stolonifera),* perennial ryegrass (*Lolium perenne*), annual bluegrass (*Poa annua)*, Kentucky bluegrass (*Poa pratensis),* and *Festuca* spp. were highly resistant or immune.

Three species of buckthorns were found to be highly susceptible to *Pcc* with large, mature aecia forming: *Frangula alnus* (Fig 2.1G), *F. caroliniana* (Fig 2.1H), and *F. californica* (Fig 2.1I). On *Rhamnus lanceolata*, mature aecia were observed, although very small (Fig 2.1J). Other species tested and found to be resistant included: *Berchemia scandens, Ceanothus americanus, C. prostratus, C. pumilis, Frangula purshiana, Rhamnus alnifolia, R. cathartica, R. crenata,* and *R. japonica,* in Rhamnaceae, and *Elaeagnus angustifolia* and *Shepherdia argentea* in Elaeagnaceae.

## Discussion

This research is the first comprehensive assessment of the host range of *Pcc*, a likely recently introduced rust pathogen in North America. Understanding the host specificity of an introduced pathogen is critical to determine its potential impact on native species and cultivated crops. In addition, if *Pcc* were found to

be viable as an augmentative biological control agent of its invasive hosts, this assessment of host specificity would help predict and limit non-target effects.

Our results indicated that *Pcc* poses no threat to cereal crop species in North America as all accessions of tested cereal crops were considered immune or highly resistant. These results are consistent with previous reports from Europe, where *Pcc* originated (Urban and Marková 1993). Limited infection, however, was observed in some oat, rye, and barley accessions at the seedling stage. Limited rust infection was also observed in some lines of *Brachypodium distachyon,* a grass used as a model organism. As *Pcc* is closely related to *P. coronata* var. *avenae* f. sp. *avenae*, the causal agent of oat crown rust, it may be useful in studies of non-host resistance. Barley and *B. distachyon* have each been investigated for their susceptibility to numerous rust pathogens (Figueroa et al. 2013; Haghdoust et al. 2021; Omidvar et al. 2018). Haghdoust et al. (2021) report that in a screen of barley with the oat crown rust pathogen, sporulation was observed on one percent of the lines, as with our trials with *Pcc*. Assessments of the oat crown rust pathogen on *B. distachyon,* however, resulted in no sporulation, although infection types varied by lines and by rust isolate (Omidvar et al. 2018). This study is the first to show that *B. distachyon* can host *P. coronata sensu stricto* (*s.s.)* with successful sporulation. Given the many described taxa of crown rust fungi, totaling at least thirteen macrocyclic and four microcyclic species at the time of writing (Liu and Hambleton, 2013; Hambleton et al. 2019; Ji et al. 2022), crown rust fungi may be an underutilized resource in studies of non-host resistance. Our trials, however limited, suggest there is value in screening *B. distachyon* and barley with additional crown rust fungi.

In our evaluations, all grass species considered susceptible are within the tribe Poeae *sensu* Soreng et al. (2015), but do not fall within a single clade. Grass species within *Apera, Calamagrostis, Lamarckia, Phalaris,* and *Puccinellia* were susceptible to *Pcc.* Some susceptible species are native to North America, and others are Eurasian in origin, sometimes naturalized or weedy in North America. Grass species able to host *Pcc* weakly (IT ≤ 2) are within five tribes of Poaceae: Brachypodieae, Bromeae, Meliceae, Poeae, and Triticeae.

Reed canarygrass is widely considered an invasive species in North America, although recent studies have shown that riparian populations in Minnesota are likely native (Noyszewski et al. 2021). We found no correlation between origin and susceptibility to *Pcc,* and both susceptible and resistant plants are present among North American and Eurasian accessions. In the Twin Cities metro area, susceptible and resistant plants are readily observed in wild populations, although susceptible plants predominate. The forage varieties 'Paloton' and 'Venture' are highly susceptible, and *Pcc* could be a production issue for forage growers. However, in Minnesota, reed canarygrass is grown for forage in the far north of the state, where glossy buckthorn and *Pcc* are absent (Greatens, 2023). Several other *Phalaris* spp. may host *Pcc*. *Phalaris angusta*, a moderately susceptible species, is present in the southern U.S., although *Pcc* is not known there (Greatens, 2023). The susceptibility of other native North American *Phalaris* spp. is unknown since seed could not be acquired. *Phalaris canariensis*, from the Canary Islands and grown commercially for birdseed, and *P. aquatica* and *P. minor,* both weedy or invasive species of Mediterranean origin present in parts of North America (Barkworth, 2007; EDDMapS, accessed Nov 2022), were all highly resistant. *Phalaris paradoxa*, another weedy species of

Mediterranean and Eurasian origin naturalized in California (Barkworth, 2007), was highly susceptible, although only one accession was tested.

*Calamagrostis* is a large and diverse genus with many species in North America, some of which are of special concern (Marr et al. 2007). *Calamagrostis purpurascens*, highly susceptible, is listed as an endangered species in Minnesota and occurs rarely on cliff faces along the northeastern border with Ontario (MNDNR, 2008). *Calamagrostis stricta* (= *C. lacustris)*, also generally susceptible, is very rare in Minnesota and known only in the northeast part of the state (MNDNR, 2008). *Calamagrostis canadensis,* a common species in temperate North America, is often weakly infected by *Pcc* when growing near glossy buckthorn and reed canarygrass. Some accessions were rated as moderately susceptible in our trials. As shown in earlier host specificity studies (Brown, 1937) and taxonomic work (Liu and Hambleton, 2013), various *Calamagrostis* spp. were previously known to be susceptible to *Pcc*, including *C. arundinacea, C. epigejos,* and *C. lanceolata*. Urban and Marková (1993), working in Europe with an earlier and broader conception of *Pcc*, note three other *Calamagrostis* species able to host *Pcc: C. pseudophragmites, C. purpurea,* and *C. varia. Calamagrostis* spp. are infected by numerous other rust fungi, including *P. coronati-calamagrostidis,* an endemic North American crown rust fungus that has the native *Rhamnus alnifolia* as an aecial host (Liu and Hambleton, 2013).

*Puccinellia* spp., or alkali-grasses, are distributed across the Northern Hemisphere and 21 species are present in North America (Davis and Consaul, 2007). *Puccinellia distans,* susceptible, possibly native or a European introduction, is widely distributed in North America and is planted along roadsides for erosion control (Casler and Duncan, 2003). *Puccinellia nuttaliana,* moderately

susceptible, is native and is most common in the western U.S., but reaching Minnesota (Davis and Consaul, 2007). Other native *Puccinellia* species could not be acquired and were not assessed. *Puccinellia gigantea, P. intermedia,* and *P. stricta,* all susceptible, are not reported in North America. *Apera spica-venti* and *Lamarckia aurea*, both susceptible, are Eurasian and Mediterranean introductions in North America, sometimes becoming naturalized or weedy (Allred, 2007; Clark, 2007). *Apera intermedia,* susceptible, is not reported in North America. Several other genera were considered by Urban and Marková (1993) to include species susceptible to *Pcc sensu lato* but were not tested: *Catabrosa, Corynephorus, Milium, Molinia, Parapholis, Phragmites, Scolochloa, Sesleria,* and *Setaria* (see also Liu and Hambleton, 2013). *Phragmites australis*, a common species in Minnesota, has not been observed with crown rust infection locally.

Turfgrass species in *Poa, Agrostis, Schedonorus,* and *Lolium*, are all highly resistant to *Pcc*, although they each are susceptible to other crown rust fungi (Liu and Hambleton, 2013). Liu and Hambleton (2013) report one herbarium specimen of *Agrostis stolonifera* infected by *Pcc,* although the host identity could not be confirmed through sequencing. *Agrostis* and *Calamagrostis* spp. are easily confused, although the latter are generally larger (Marr et al. 2007). *Arrhenatherum elatius* and *Holcus lanatus*, hosts of *P. coronata* var. *avenae* f. sp. *graminicola* (Liu and Hambleton, 2013), are immune and highly resistant, respectively. *Anthoxanthum nitens* (=*Hierochloe odorata*), or sweetgrass, a plant of cultural importance to many Indigenous people in North America, was highly resistant in the two collections assessed, with a hypersensitive response clearly visible on leaves. The genus *Anthoxanthum* is sister to *Phalaris* (Soreng et al. 2015). *Vulpia octoflora* (=*Festuca octoflora*), six weeks fescue, is moderately resistant, with

some sporulation observed. Eshed and Dinoor (1981), working in Israel, report *V. octoflora* and *Lamarckia aurea*, among other grass hosts, to be susceptible to an array of crown rust isolates derived from various grass species, although the rust species identities and their phylogenetic diversity are difficult to determine.

The North American buckthorn species *F. californica, F. caroliniana,* and *R. lanceolata* are all susceptible to *Pcc* and could serve as the alternate hosts, although only very small aecia were observed on the latter. *Frangula carolinana* is distributed across the southeastern U.S., west to Texas; *F. californica*, or coffeeberry, is distributed throughout California, north to Oregon, and east to New Mexico; and *R. lanceolata* is distributed across parts of the central U.S., especially in Missouri, east to central Ohio (BONAP, accessed Dec. 2021. See Kartesz, 2014). The ranges of these potential aecial hosts overlap significantly with the ranges of susceptible grass species, and, thus, the range of *Pcc* may extend beyond the range of glossy buckthorn. Several other southwestern North American *Frangula* spp., *F. betulifolia, F. xblumeri,* and *F. obovata,* could not be acquired and were not tested. The highly invasive common buckthorn (*R. cathartica*) is not susceptible to *Pcc,* although it is host to other crown rust fungi (Liu and Hambleton, 2013).

*Puccinia coronata* var. *coronata*, despite likely being an exotic pathogen in North America, has some beneficial effects, causing disease on two highly invasive species. All tested cereal crop species and turfgrasses were resistant, but some native *Calamagrostis, Frangula, Phalaris*, and *Puccinellia* spp. were susceptible in greenhouse trials and may be vulnerable to disease under natural conditions. The net benefit of the pathogen in a given area is thus likely to vary significantly according to the local plant community composition, an important

consideration if *Pcc* were ever to be employed as an augmentative biological control agent.

**Table 2.1.** Summary of infection types observed in cereals in response to *Puccinia coronata* var. *coronata.*

| **Species** | **Total accessions screened**[1] | **Range of infection types**[2] | | |
|---|---|---|---|---|
| | | Inoculum | | |
| | | Bulk[3] | 20_MN_Fa02-1 | 18_WI_Pa01-1 |
| Oats (*Avena sativa*) | 1250 | 0 - 2 | 0 - 2 | 0 - 2 |
| Barley *(Hordeum vulgare)* | 128 | 0 - ;1 | 0 - ;1- | 0 - ; |
| Rye (*Secale cereale*) | 110 | 0 - ;1- | 0 - ; | 0 - ; |
| Triticale (*x Triticosecale*) | 114 | 0 - ; | - | - |
| Bread wheat (*Triticum aestivum*) | 106 | 0 - ; | - | - |
| Durum wheat (*Triticum turgidum* ssp. *durum*) | 125 | 0 - ; | - | - |

[1] Not all accessions were screened with all three inocula. See Supplementary Table 3.1 for full results for individual lines.

[2] Infection types are rated on a scale from 0 – 4. 0 = immunity. ; = chlorotic flecking. N = necrotic flecking. 1 = uredinia small with significant chlorosis. 2 = uredinia small to midsized with chlorosis. 3 = uredinia midsized to large with some chlorosis. 4 = uredinia large, no chlorosis. "+" and "-" following numeric ratings indicate intermediate infection types. Hosts with ratings ≥ 3 are considered susceptible.

[3]Uredinial inoculum derived from aecia and containing many diverse rust genotypes.

**Table 2.2.** Summary of infection types observed in other grasses in response to *Puccinia coronata* var. *coronata.*

| | **Tribe** | **Species** | **Total accessions screened**[1] | **Range of infection types**[2] | | |
|---|---|---|---|---|---|---|
| | | | | Inoculum | | |
| | | | | Bulk[3] | Isolate 20_MN_Fa 02-1 | Isolate 18_WI_Pa01-1 |
| Moderately to highly susceptible. (IT ≥ 3) | Poeae | *Apera intermedia* | 2 | 2+ - 3 | 2 – 3 | 2 – 4 |
| | | *A. spica-venti* | 3 | ;1 – 4 | ; - 3 | ;1 – 3 |
| | | *Calamagrostis purpurascens* | 5 | 0 – 4 | ;N – 4 | ;N – 4 |
| | | *Calamagrostis stricta* | 4 | 0 – 3 | 0 – 4 | 0 – 4 |
| | | *Lamarckia aurea* | 1 | 3 | 3 | 3 |
| | | *Phalaris angusta* | 2 | - | - | 2+ - 3+ |
| | | *P. arundinacea* | 93 | 0 – 4 | 0 – 4 | 0 – 3+ |
| | | *P. paradoxa* | 1 | - | 3 | 3+ |
| | | *Puccinellia distans* | 4 | 0 – 3 | 0 – 3 | - |
| | | *P. gigantea* | 1 | 3 | 3 | 2/3 |
| | | *P. intermedia* | 2 | 2 | 2 – 3 | 2 |
| | | *P. nuttalliana* | 1 | 3 | 2+ - 3 | 2 |
| | | *P. stricta* | 1 | 2 – 3 | 2 | - |
| Moderately to highly resistant. (IT ≤ 2 + with sporulation present) | Brachy-podieae | *Brachypodium distachyon* | 52 | 0 – 2 | 0 – 1+ | 0 - ;1 |
| | Bromeae | *Bromus diandrus* | 2 | ; - ;N | ; - 1 | ; - ;1 |
| | | *B. inermis* | 7 | 0 -1 | ; | 0 |
| | | *B. japonicus* | 5 | 0 – 1 | ;1 | ;1 |
| | Meliceae | *Melica nitens* | 6 | 0 - ;N | 0 - ;1 | 0 – 1 |

**Table 2.2. continued**

| | Tribe | Species | Total accessions screened[1] | Range of infection types[2] | | |
|---|---|---|---|---|---|---|
| | | | | Inoculum | | |
| | | | | Bulk[3] | Isolate 20_MN_Fa 02-1 | Isolate 18_WI_Pa01 -1 |
| Moderately to highly resistant. (IT ≤ 2 + with sporulation present) | Poeae | *Agrostis gigantea* | 10 | 0 - ;1 | ; | ; |
| | | *A. stolonifera* | 29 | 0 - ; | 0 – 1- | - |
| | | *Alopecurus geniculatus* | 4 | 0 – 1 | ;1 | ;1 |
| | | *A. pratensis* | 10 | 0 – 1 | ;1 | ; |
| | | *Anthoxanthum nitens* | 2 | 1 | ;1 – 1N | ;1 |
| | | *Avena barbata* | 10 | 0 | 0 – 1; | 0 - ;1 |
| | | *A. fatua* | 10 | 0 – 2 | 0 – 1; | 0 – 1; |
| | | *A. longiglumis* | 10 | 0 – 2 | 0 – 1+ | 0 – 1; |
| | | *A.* strigosa | 11 | 0 – 1 | 0 | 0 |
| | | *Beckmannia syzigachne* | 10 | 0 – 2 | 0 – 2+ | ; - 2 |
| | | *Briza media* | 9 | 0 - ;1 | 0 - ;1 | ; |
| | | *Calamagrostis xarundinacea* | 1 | - | 1 | 1 |
| | | *C. breviligulata* | 3 | - | 1 – 2 | 1 – 2 |
| | | *C. canadensis*[4] | 21 | 0 – 2 | ; - 3 | ; - 3 |
| | | *Dactylis glomerata* | 11 | 0 – 2 | 1+ - 2+ | 1+ - 2+ |
| | | *D. marina* | 9 | 0 – 1 | ; -1 | - |
| | | *Deschampsia cespitosa* | 9 | 0 – 1 | 0 - ; | 0 - ;1- |
| | | *Holcus lanatus* | 10 | - | 0 – 1+ | 0 - ;1- |
| | | *Lolium perenne* | 10 | 0 – 1 | 0 | 0 |
| | | *Phalaris aquatica* | 5 | 0/; | ; - ;1 | ; - ;1 |
| | | *P. brachystachys* | 7 | 0 - ; | ; - 1 | ; - ;1 |
| | | *P. coerulescens*[4] | 5 | 0 – 3 | ; - 3 | ;1 -2 |
| | | *P. minor* | 3 | 0 - ;1 | ; | ; |
| | | *P. platensis* | 1 | ;2 - 2+ | ;1 | 2+ |

**Table 2.2. continued**

| | Tribe | Species | Total accessions screened[1] | Range of infection types[2] | | |
|---|---|---|---|---|---|---|
| | | | | Inoculum | | |
| | | | | Bulk[3] | Isolate 20_MN_Fa 02-1 | Isolate 18_WI_Pa0 1-1 |
| Moderately to highly resistant. (IT ≤ 2 + with sporulation present) | Poeae | *Phleum phleoides* | 4 | ; - 1+ | ; - 1+ | ; |
| | | *Puccinellia tenuiflora* | 1 | 0 – 2 | ; - 2 | 0 – 2- |
| | | *Vulpia octoflora* | 14 | 0 -2 | ; - 2 | ;N – 2 |
| | Triti-ceae | *Elymus trachycaulus* | 6 | 0 – 1 | ; - ;1 | ; |
| | | *E. villosus* | 3 | 0 – 1 | ; - ;N | ; |
| Highly resistant to immune. (IT < 1. No sporulation observed.) | Various | *Aegilops cylindrica, Ae. tauschii, Ammophila breviligulata, Andropogon gerardi, Anthoxanthum odoratum, Aristida purpurea, Arrhenatherum elatius, Avena magna, A. murphyi, Avenula pubescens, Bouteloua curtipendula, B. gracilis, Brachypodium hybridum, B. stacei, B. sylvaticum, Bromus ciliatus, B. latiglumis, B. pubescens, B. secalinus, B. tectorum, Cinna arundinacea, Cynodon dactylon, Cynosurus cristatus, C. echinatus, C. elegans, Dasypyrum villosum, E. canadensis, E. elymoides, E. glaucus, E. hystrix, E. repens, E. virginicus, F. rubra, F. saximontana, F. subverticillata, F. trachyphylla, Glyceria canadensis, G. grandis, G. striata, Koeleria macrantha, Melica nutans, Panicum virgatum, Pascopyrum smithii, Phalaris canariensis, P. truncata, P. xdaviesii, Phleum pratense, Poa annua, P. compressa, P. palustris, P. pratensis, Polypogon monspeliensis, Schedonorus phoenix, Sporobolus cryptandrus, Stipa capillata, S. lagascae, S. orientalis, S. pennata, Thinopyrum intermedium, Trisetum flavescens, T. monococcum,* and *T. petropavloskyi* | | | | |

[1] Not all accessions were screened with all three inoculums. See Table S2 for complete results.

[2] Infection types are rated on a scale from 0 – 4. 0 = immunity. ; = chlorotic flecking. N = necrotic flecking. 1 = uredinia small with significant chlorosis. 2 = uredinia small to midsized with chlorosis. 3 = uredinia midsized to large with some chlorosis. 4 = uredinia large, no chlorosis. "+" and "-" following numeric ratings indicate intermediate infection types. Hosts with ratings ≥ 3 are considered susceptible.

[3] Uredinial inoculum derived from aecia and containing many diverse rust genotypes.

[4] Generally resistant, but with at least one accession showing a high infection type.

**Table 2.3.** Rust development on plants of Rhamnaceae and Elaeagnaceae inoculated with *Puccinia coronata* var. *coronata.*

| Family | Species | Growth stage | Number of plants | Rust development | Disease reaction |
|---|---|---|---|---|---|
| Elaeagnaceae | *Elaeagnus angustifolia* | Seedling | 1 | None | Immune |
| | *Shepherdia argentea* | Seedling | 6 | None | Immune |
| Rhamnaceae | *Berchemia scandens* | > 2 yrs. | 1 | None | Immune |
| | *Ceanothus americanus* | > 2 yrs. | 1 | None | Immune |
| | *C. prostratus* | Seedling | 2 | None | Immune |
| | *C. pumilis* | Seedling | 1 | None | Immune |
| | *Frangula alnus* | > 1 yrs. & seedling | > 500 | Numerous small to large aecia | Highly susceptible |
| | *F. californica* | Seedling | > 20 | Numerous small to large aecia | Highly susceptible |
| | *F. caroliniana* | > 2 yrs. & seedling | 1 | Numerous small to large aecia | Highly susceptible |
| | *F. purshiana* | > 2 yrs. & seedling | > 20 | None | Immune |
| | *F. rubra* | > 2 yrs. | 1 | None | Immune |
| | *Rhamnus alnifolia* | > 2 yrs. | 2 | None | Immune |
| | *R. cathartica* | > 1 yrs. seedling | > 200 | None | Immune |
| | *R. crenata* | Seedling | 5 | None | Immune |
| | *R. japonica* | Seedling | 3 | None | Immune |
| | *R. lanceolata* | > 2 yrs. | 1 | Few small aecia | Moderately susceptible |

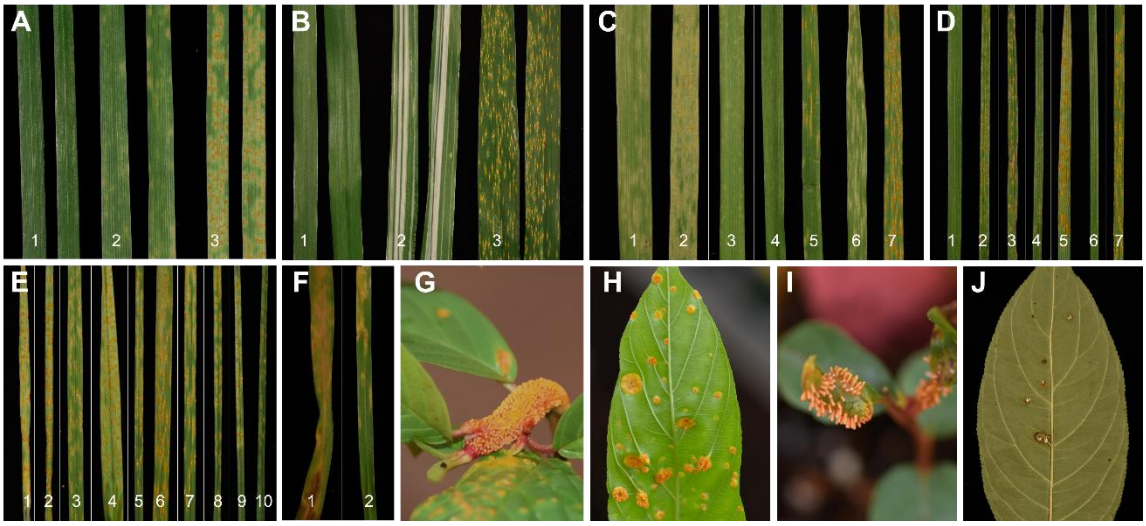


**Figure 2.1.** Grasses and buckthorns inoculated with *Puccinia coronata* var. *coronata* **A.** Range of infection types to isolate 20_MN_Fa02-1 in oats. 1) IT = 0 on Clav 1532. 2) IT = ; on PI 52820. 3) IT = 2 on Clav 637. **B.** Range of infection types in reed canarygrass in response to isolate 18_WI_Pa01-1. 1) IT = 0 on a wild-collected plant from Roseville, MN. 2) IT = 1 on *P. arundinacea* var. *picta* 'Feesey'. Sporulation occurs only in areas of the leaf where chlorophyll is absent. 3) IT = 3+ on a wild-collected plant from Falcon Heights, MN. **C.** Range of infection types in other *Phalaris* spp. in response to isolate 20_MN_Fa02-1. 1) IT = ; on *Phalaris aquatica* (PI 201943). 2) IT = ;1 on *P. brachystachys* (PI 207959). 3) IT = ; on *P. canariensis* (PI 165429). 4) IT = ; on *P. coerulescens* (PI 236527). 5) IT = 3 on *P. coerulescens* (PI 236526). 6) IT = ; on *P. minor* (PI 220033). 7) IT = 3+ on *P. paradoxa* (PI 185161). **D**. Range of infection types in *Calamagrostis* spp. in response to isolate 20_MN_Fa02-1. 1) IT = ; on *Calamagrostis canadensis*, (W6 49072). 2) IT = 1 on *C. canadensis* (W6 54495). 3) IT = 3 on *C. canadensis* (W6 56084). 4) IT = N on *C. purpurascens* (W6 54617). 5) IT = 4 on *C. purpurascens* (W6 56088). 6) IT = 0 on *C. stricta* (W6 49076). 7) IT = 3+ on *C. stricta* (W6 47071). **E.** Infection types observed in other selected grasses in response to isolate 20_MN_Fa02-1. 1) IT = 3 on *Apera*

*intermedia* (PI 203444). 2) IT = 3 on *A. spica-venti* (Ames 23688). 3) IT = 1+ on *Dactylis glomerata* (PI 231497). 4) IT = 3 on *Lamarckia aurea* (PI 378959). 5) IT = 3 on *Puccinellia distans* (PI 443386). 6) IT = 3 on *P. distans* ssp. *sevangensis,* (PI 229458). 7) IT = 3 on *P. gigantea* (PI 384943). 8) IT = 3 on *P. intermedia* (PI 628713). 9) IT = 2+ on *P. nuttalliana* (PI 675197). 10) IT = 2 on *P. tenuiflora* (PI 610870) . **F.** Infection types in *Brachypodium distachyon* inoculated with isolate 20_MN_Fa02-1. 1) IT = ;N on "Bd21"and 2) IT = ;1+ on W6 39246. **G.** Aecia on glossy buckthorn (*Frangula alnus*) in a greenhouse trial. **H.** Aecia on Carolina buckthorn (*F. caroliniana*). **I.** Aecia on coffeeberry (*F. californica*) **J.** Aecia on lanceleaf buckthorn (*Rhamnus lanceolata*).

# Chapter Three

## Effects of *Puccinia coronata* var. *coronata* on reed canarygrass and glossy buckthorn in greenhouse assays

## Summary

*Puccinia coronata* var. *coronata* (*Pcc*) is a crown rust fungus recently observed across the Midwest and Northeast United States that affects two highly invasive wetland species, glossy buckthorn (*Frangula alnus*) and reed canarygrass (*Phalaris arundinacea*). *Pcc* may be useful as an augmentative biological control for one or both of its hosts and likely offers some natural suppression. In greenhouse trials, glossy buckthorn seedlings were inoculated with germinating telia of *Pcc* in two separate experiments, at three- and eight-weeks following germination. Reed canarygrass plants collected from across Minnesota were propagated by rhizome and inoculated after three weeks with fresh inoculum collected from aecia on glossy buckthorn. Eight weeks after inoculation, in the two trials, respectively, treated glossy buckthorn plants had on average a height 86 and 94 % of the controls', total leaf area 57 and 85 %, and dry biomass 81 and 87 %. Plants treated at three weeks exhibited 6.8 % mortality. Fourteen weeks after inoculation, treated reed canarygrass plants had on average 71 % of the tillers of the controls' and had 75 % dry aboveground biomass. Plants were allowed to regenerate from belowground biomass, and five weeks later, treated reed canarygrass plants had 85 % of the height of the controls' and 48 % of the dry aboveground biomass. Results are consistent with observations in natural settings and suggest that infection by *Pcc* strongly affects both glossy buckthorn and reed canarygrass. Continued investigation as an augmentative biological control agent is warranted.

## Introduction

*Puccinia coronata* var. *coronata* (Pcc) is a macrocyclic crown rust fungus that causes disease of glossy buckthorn (*Frangula alnus*) and reed canarygrass (*Phalaris arundinacea*), two highly invasive plant species in North America. *Pcc* was confirmed for the first time in North America near Ottawa, Canada in 2014 and has since been observed across the Midwest and Northeastern United States (Greatens et al. 2023; Kenaley et al. 2017). In wetlands in the Twin Cities (MN, USA) where reed canarygrass and glossy buckthorn are common species, *Pcc* causes remarkable disease on glossy buckthorn, sometimes significantly defoliating plants and causing aborted flower and fruit production in late spring and early summer. Diseased reed canarygrass plants may show premature leaf senescence, with infection and production of uredinia occurring following maturation of aecia on glossy buckthorn and continuing through autumn (Greatens et al. 2023).

In North America, glossy buckthorn is an invasive species in the Midwest and Northeast US, southern Ontario and Quebec, and the maritime provinces (EDDMapS, 2023). It was originally introduced as an ornamental plant (Aiello-Lammens, 2014; DeKort et al. 2016) but has since been restricted as a noxious weed in many states. It most often invades fens, and bogs (Fiedler and Landis, 2012) and, in eastern North America, also invades forests managed for timber. Where gaps occur due to harvest or natural disturbance, glossy buckthorn proliferates, altering forest composition, and selecting for less profitable, shade tolerant species (Fagan and Peart, 2004). In Europe, within its native range, glossy buckthorn is often diseased by *Pcc* (Urban and Marková, 1991), but in

Minnesota, before the recent proliferation of *Pcc,* it was generally free of rust (Greatens et al. 2023).

Reed canarygrass is widespread across the same region, but is present west to the Pacific, and north to coastal Alaska (Sturtevant et al. 2021). Reed canarygrass is often assumed to have been introduced to North America, but examination of herbarium records and comparison of genetic markers from riparian populations in Minnesota and Eastern Europe have indicated that it is most likely a native species (Noyszewski et al. 2021). In wetlands with fluctuating water levels from periodic flooding, seasonal variation (Galatowitsch *et al.*, 1999), and stormwater runoff, reed canarygrass thrives. It is highly responsive to nitrogen (Kercher and Zedler, 2004) and, like many weedy plants, is advantaged by agricultural nitrate pollution (Green and Galatowitsch, 2002; Scherer-Lorenzen et al. 2007).

With the recent observation of *Pcc* in North America, a research project was designed to investigate its distribution on the continent (Greatens et al. 2023), assess its host specificity, and, with this study, to evaluate its effects on glossy buckthorn and reed canarygrass in greenhouse studies with the hypothesis that it may be useful as an augmentative biological control agent of one or both of its hosts.

## Materials and Methods

### Evaluation on glossy buckthorn

In September 2019, berries were collected from glossy buckthorn plants in Acorn Park, Roseville, MN. Seeds were removed and stored at 4 °C. Ninety days before planting, seeds were moist stratified in a 1:1:1 mix of gravel, peat moss, and pine bark. In 18 square 9 cm width x 8 cm height pots, approximately 100 seeds (estimated by weight) were planted in potting soil and maintained in a greenhouse at 19 to 22$^{o}$C with a photoperiod of 16 hrs. Four weeks after removal from stratification, when plants were approximately one to three weeks old, pots were randomly assigned to treated and control groups. The treated group was placed in a moist chamber, and naturally overwintered telia-bearing reed canarygrass straw soaked in water for four hours was suspended above plants for seven days. The control group was placed in a moist chamber with shredded cardboard suspended above plants to reduce light. After seven days, plants were returned to the greenhouse. Eight weeks after inoculation, plants were assessed for leaf area, biomass, height, and mortality. Seedlings which had died were not included in measurements of other variables.

In this trial, height was measured for all plants and plants in three randomly selected pots per treatment were selected for leaf area measurements. Each pot was assessed for total biomass. Height was measured from the first root to the apical meristem. Plant leaves were removed and placed on a white background with a 4 cm$^2$ red square for calibration, and the software "Easy Leaf Area" (Easlon and Bloom, 2014) and the associated mobile app "Easy Leaf Area Free" were used to calculate the ratio of green to red to estimate leaf area.

Software settings were customized so that diseased leaf tissue, often necrotic or chlorotic in treated plants due to rust infection, was not counted in leaf area estimates. Soil was removed from roots, and plants were weighed to assess dry biomass.

In a second experiment, 98 glossy buckthorn seedlings were produced by the same method. Plants were selected to be at approximately the same growth stage. Each plant was transplanted into 4 cm diameter x 16 cm length cone-tainers. A randomly selected treated group of 49 plants was inoculated at 2 months after removal from stratification. Height, leaf area, and biomass were calculated by the same method, but biomass was measured for each plant individually. Means of treatments were compared using an unpaired Student's t-test.

#### Evaluation on reed canarygrass

From 50 reed canarygrass plants collected from across Minnesota in surveys for *Puccinia coronata* var. *coronata* in 2020 (Greatens et al. 2023), 80 new plants were derived through 8 cm-long cuttings of rhizomes approximately equal in diameter and overall health. Rhizome segments were transplanted in Pro-Line C/B potting soil (Jolly Gardener) into round 9 cm diameter x 8 cm height plastic pots (Dillen Products) and maintained in a greenhouse at 19 to 22$^{o}$C with a photoperiod of 16 hrs. After three weeks, when plants had produced two to three leaves, they were transplanted into square 13 cm width x 16 .5 height pots ("Jumbo Junior," Belden Plastics), and fertilized with 4 g of 14-14-14 controlled release fertilizer (Osmocote). Plants were randomly assigned to

treated and control groups and placed into 61 x 30 x 12 cm tubs, with eight plants per tub. Plants were maintained with 2 – 5 cm of standing water in tubs.

Fresh aeciospores were vacuum-collected from mature aecia on glossy buckthorn plants harvested from Acorn Park, Roseville, MN, and two capsules of 25 mg of *P. coronata* var. *coronata* aeciospores suspended in 500 μL Soltrol 170 (Chempoint) were used to spray-inoculate each group of eight plants.

Control plants were mock inoculated with an equal quantity of Soltrol 170. All plants were incubated overnight in a moist chamber and returned to the greenhouse. Following production of uredinia on the leaves of treated plants, once per week, spores were gently spread from lower leaves to new leaves by gently running a hand from the base of the plant to the leaf tips, and treated plants were moved to a moist chamber overnight. This was repeated weekly for twelve weeks to simulate naturally occurring reinfection by urediniospores.

After 14 weeks, all plants were assessed for height, tiller production, and dry aboveground biomass. To assess height, leaves at the top of the plant were gathered and stretched upward, and the height was measured from the base to the farthest leaf tip. All aboveground stems that had fully emerged from the soil were considered tillers. All aboveground plant tissue for each plant was placed in a separate paper bag, dried for a week at 60 °C, and weighed. Plants were allowed to regrow for five weeks, after which they were again assessed for height and dry biomass. One plant in the treated group was entirely immune to *Pcc* and was omitted from the results. Means of treatments were compared using an unpaired Student's t-test.

## Results

Treatment of glossy buckthorn seedlings with *Pcc* significantly reduced height, leaf area, and biomass, and in young seedlings caused some mortality. In the trial with multiple plants per pot (3-weeks old plants), average height of treated plants was 86 % of the control ($p < .001$) (Fig 3.1A), and leaf area of treated plants was 57 % of the control ($p < .001$) (Fig 3.1B). Average biomass of treated plants was 81 % of the control, although means could not be compared with a t-test due to the experimental design. Seedlings exhibited a range of severity of infections, from nearly unaffected with very small aecia, to significant defoliation, partial stem loss, or death (Fig. 3.2). Mortality, which occurred in 6.8 % of plants (Table 3.1) usually occurred with the production of aecia on the main stem. In the trial where plants were grown individually in cone-tainers and inoculated at two months after germination, when compared with controls, treated plants had 94 % of the height ($p < .05$) (Fig 3.3A), 85 % of the total leaf area ($p < .05$) (Fig 3.3B), and 87% of the biomass ($p < .05$) (Fig 3.3C).

Similarly, treatment of reed canarygrass with *Pcc* significantly reduced tiller production, height, and dry biomass. When compared with the controls, treated plants produced 71 % of tillers (Fig 3.4A) and 75 % of aboveground biomass (Fig 3.4B). After plants were allowed to regrow for five weeks following the harvest of aboveground material, when compared with the control, treated plants had 85 % of the height (Fig 3.4C) and 48 % of the aboveground biomass (Fig 3.4D). Treated plants were notably diseased throughout the first growth period, with many leaves heavily diseased, often senescing with severe infection

(Fig 3.5A). Below-ground biomass production was not directly assessed but was notably reduced in treated plants (Fig 3.5B).

## Discussion

Greenhouse experiments show that *Pcc* significantly reduces the leaf area, height, and biomass production of glossy buckthorn. Between the two trials conducted for glossy buckthorn, reductions in height and biomass were comparable. Leaf area loss, however, was most significant in the first trial, likely because younger plants and younger leaves are more susceptible to rust infection. Likewise, mortality was only observed in the first trial, often due to rust infection on the young stem tissue. Under natural conditions, first-year seedlings of glossy buckthorn are rarely infected by *Puccinia coronata* var. *coronata* since seeds typically germinate after *Pcc* basidiospore production has ceased (N. Greatens, personal observation).

Similarly, *Pcc* reduces the height, tillering and biomass production of reed canarygrass. Critically, belowground biomass production is strongly affected. While belowground biomass could not be directly assessed due to the highly intertwined roots trapping soil particles, it was indirectly assessed through allowing regrowth of plants. Following an initial harvest, we observed substantially less growth in treated plants. In natural settings, reed canarygrass forms significant belowground biomass, more than two times the aboveground biomass (Adams and Galatowitsch, 2005), and in spring, reed canarygrass grows rapidly, producing abundant vegetation and limiting light availability for competitors. Reduced healthy leaf tissue, whether diseased or never produced

due to effects on belowground biomass production will likely advantage competitors and may promote species diversity.

While results are generally consistent with qualitative observations of wild populations, direct comparisons may not be appropriate since rust infection and its effects on plant health are likely to vary due to various factors in a field setting. For example, the viability and amount of inoculum may vary considerably between field and laboratory conditions—higher or lower. Other factors include plant age, presence of rust parasites or antagonists, systemic acquired resistance in host plants, and environmental conditions that may not favor rust infection. Notably one plant in the treated group was entirely resistant and was omitted from the results. Resistance is sometimes present in populations of reed canarygrass (Greatens, personal observation), raising the possibility that disease pressure may select for resistant plants. The durability of the resistance, however, is unknown as the pathogen may also acquire new virulences. Nevertheless, results are auspicious, and *Pcc,* whether eventually deployed as an augmentative biological control agent or left as a naturalized pathogen, is likely to provide some control of reed canarygrass and glossy buckthorn.

Rust fungi are usually highly host specific and are easily disseminated, making them valuable candidates for classical biological control agents. Twenty-four species have been authorized for release worldwide, including three in the mainland United States and one in Hawaii (Morin, 2020). Only two species, however, have ever been registered as augmentative biological control agents, or bioherbicides (Morin, 2020; Winston et al. 2014). *Puccinia canaliculata*, a macrocyclic rust, was successfully used for control of yellow nutsedge (*Cyperus esculentus*) in combination with herbicides (Beste et al. 1992; Phatak et al. 1983)

but was discontinued following production issues and the occurrence of the aecial stage on cultivated sunflower (*Helianthus annuus*) (Winston et al. 2014). *Puccinia thlaspeos,* a microcylic rust of dyer's woad (*Isatis tinctorum*), was registered and cultivated as teliospores embedded in the host, but was never commercialized (Thomson and Kropp, 2003; Winston et al. 2014).

*Puccinia coronata* var. *coronata* could conceivably be used as a bioherbicide of glossy buckthorn, but rust life cycle traits would complicate implementation. Teliospores are the overwintering spores of crown rust fungi and are firmly embedded in the grass host. Following a required natural overwintering period, teliospores germinate to produce basidiospores infectious on glossy buckthorn. Successful infection produces spermagonia and aecia. The stages on buckthorn are monocyclic and occur naturally once per year. Reinfection of glossy buckthorns would require introduction of viable teliospores through addition of infected reed canarygrass. Reed canarygrass is easily cultivated for hay production, and *Pcc*-susceptible varieties could be grown at scale as a source of rust inoculum. The environmental conditions to maintain spore viability, however, are poorly understood, and would require more research. Successful cultivation, storage, and application of rust teliospores at a scale useful for land managers would require significant investment of labor and resources.

The use of *Pcc* as a bioherbicide of reed canarygrass could be more straightforward. Urediniospores are produced in large quantities on susceptible reed canarygrass under laboratory and field conditions, and can be vacuum-collected, stored, transported, and spray-inoculated to produce new infection on the grass host. The uredinial stage is polycyclic, meaning urediniospores produced on reed canarygrass will reinfect the host. Infection naturally occurs on

reed canarygrass shortly after the maturation of aeciospores. In Minnesota, this occurs in mid-June, when reed canarygrass plants have already flowered and have begun producing seed. If inoculum were introduced earlier in the season, it is possible that plants would be more significantly infected. Use might be most beneficial in high value wetlands or ecological restorations, where reed canarygrass often establishes to the detriment of more desirable species (Adams and Galatowitsch, 2006). Effective use would require further research on inoculum development, storage, and implementation. It is possible that especially virulent isolates could be discovered and selected.

Greenhouse host specificity research on non-target effects suggests that some other species may be affected by *Pcc,* including some native North American *Calamagrostis, Frangula, Phalaris,* and *Puccinellia* spp. (Chapter Two). Use of *Pcc* may thus be limited to areas where these species are not present or are not of concern. Some evidence suggests that *Pcc* uredinial inoculum does not readily spread beyond the range of its buckthorn hosts and that, thus, local introductions would be to affect potential hosts far away (Greatens et al. 2023). *Pcc* in any case is already well-established, and in-field testing in these regions would pose minimal additional risk.

Successful implementation of a bioherbicide is a rare and complicated process, usually initially backed by public funding but commercialized in collaboration with a private entity (Morin, 2020). While ideally bioherbicides have some advantages over traditional herbicides, such as host specificity and reduced non-target effects, they generally require specific environmental conditions and have poor shelf life (Morin, 2020). Unfortunately, development of bioherbicides often ceases after proof-of-concept research, or, if products are

successfully commercialized, they are often discontinued after short periods (Morin, 2020). Successful and long-term use of *Pcc* as a bioherbicide would likely require sustained governmental funding or commercial interest.

**Table 3.1**. Effects of *Puccinia coronata* var. *coronata* infection on biomass of glossy buckthorn seedlings grown together in pots.

| | Total mass (g) | Number of plants | Avg. no. of plants per pot | Average mass per plant (g) | Mortality rate |
|---|---|---|---|---|---|
| Control | 55.16 | 427 | 53.4 | 0.129 | 0 |
| Treated | 37.15 | 355 | 44.4 | 0.105 | 6.8 % |

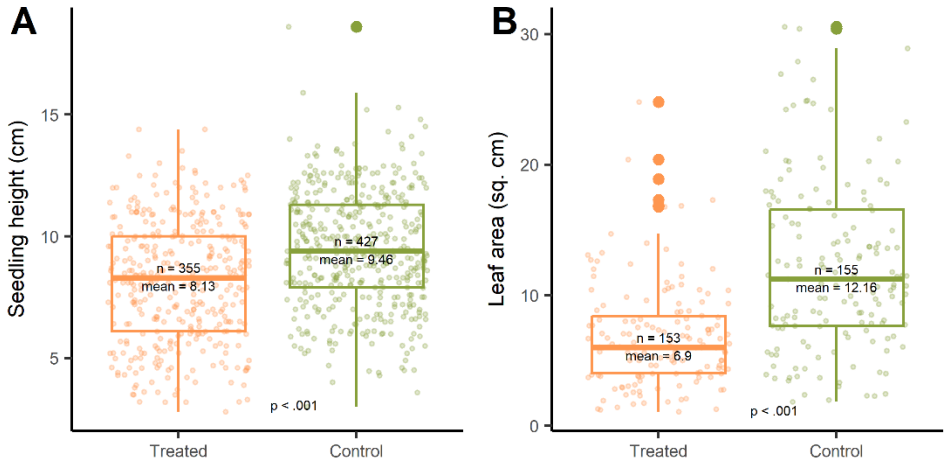


**Figure 3.1**. Effects of *Puccinia coronata* var. *coronata* infection on seedlings of glossy buckthorn grown in pots, with 100 seeds per pot. Overall, treated plants were reduced in **A)** aeedling height (86 % of the control, $p < .001$) and **B)** leaf area (57 %, $p < .001$). Leaf area was assessed in only a randomly selected subset of the plants.

**Figure 3.2.** Glossy buckthorn seedlings eight weeks after infection by *Puccinia coronata* var. *coronata* inoculated with germinating teliospores. In some plants, rust infection had caused severe shoot deformations, resulting in irregular growth, and partial or complete shoot death. Other plants, such as the one in the middle, appeared to minimally affected.

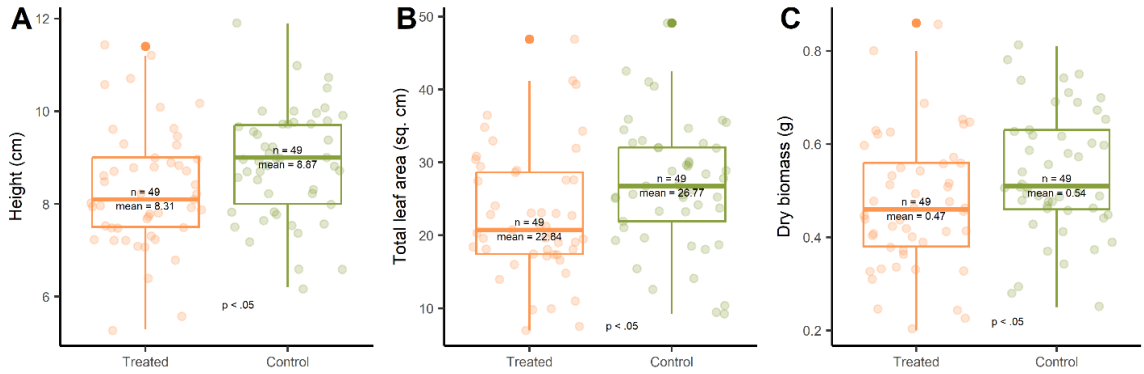


**Figure 3.3.** Effects of aecial infection by *Puccinia coronata* var. *coronata* on glossy buckthorn plants grown from seed in cone-tainers and assessed eight weeks after inoculation. Treated plants were reduced in **A)** height (94 % of control, $p < .05$), **B)** total leaf area (85 %, $p < .05$), and **C)** dry biomass (87, $p < .05$).

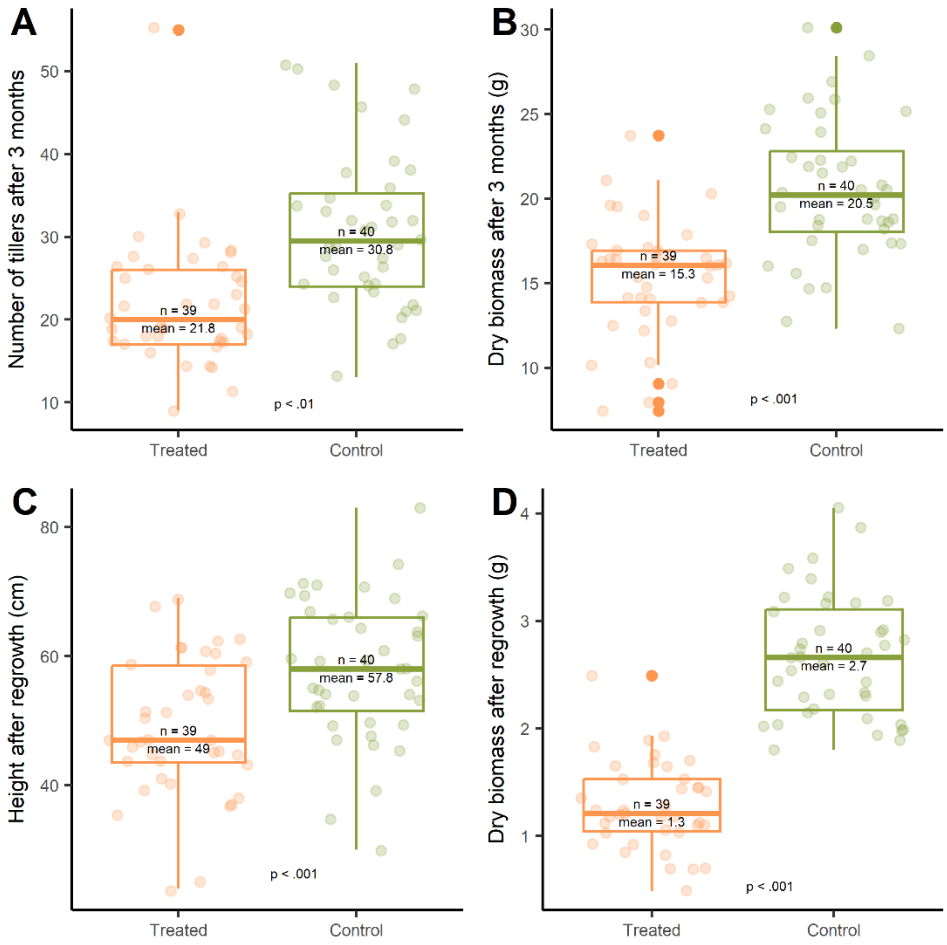


**Figure 3.4.** Effects of rust crown disease caused by *Puccinia coronata* var. *coronata* on reed canarygrass in a greenhouse trial. When plants were assessed after inoculation, treated plants had significant reductions in **A)** tiller production (71 % of the control, $p < .01$) and **B)** dry biomass production (75 % of control, $p < .001$). Plants were allowed to regenerate and were assessed after five weeks. After this period, treated plants had significant reductions in **C)** height after regrowth (85 %, $p < .001$) and **D)** dry biomass after regrowth (48 %, $p < .001$).

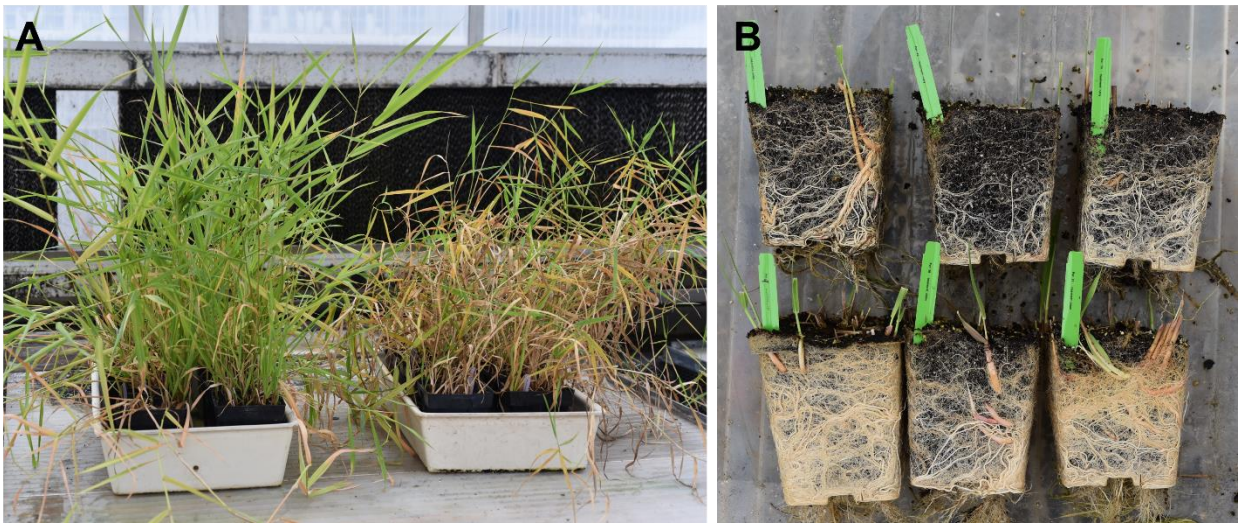


**Figure 3.5**. Photographs of the effects of *Puccinia coronata* var. *coronata* on reed canarygrass. **A)** Comparison of aboveground plant health in four randomly selected untreated (left) and treated (right) plants: treated plants had more chlorosis and leaf senescence. **B)** Comparison of belowground biomass production in three randomly selected untreated (bottom) and treated (top) plants: treated plants had much less root-mass production when compared with untreated plants.

# Chapter Four

## Host specificity of *Puccinia digitaticoronata*, a new crown rust fungus of Kentucky bluegrass in North America

This chapter has been submitted in a similar form to Plant Disease as:

Greatens, N., Miller, K., Watkins, E., Jin, Y., and Olivera Firpo, P.D. 2023. Host specificity of *Puccinia digitaticoronata*, a new crown rust fungus of Kentucky bluegrass in North America. Plant Dis. Awaiting decision.

## Summary

In North America, crown rust has emerged as a common disease of Kentucky bluegrass (*Poa pratensis*) and may cause significant damage. After observing crown rust on *Poa* spp. in the Twin Cities, Minnesota and on three East Asian buckthorn species (*Rhamnus crenata, R. davurica*, and *R. japonica*) in North Dakota, we sequenced one to four loci of nine samples for identification and to determine their placement within the *Puccinia* series *Coronata.* Sequence analysis identified the samples as *Puccinia digitaticoronata*, a crown rust fungus previously known only in northeast China. Earlier studies had shown that another pathogen in the *P. coronata* var. *avenae* f. sp. *avenae* clade was the cause of crown rust of Kentucky bluegrass in North America. Thus, two different crown rust fungi cause disease in Kentucky bluegrass in North America. To assess the pathogenicity on potential telial and aecial hosts, 789 total accessions of 98 gramineous species were evaluated with two inocula: one field collection and one pure isolate. Eleven species in Rhamnaceae and Elaeagnaceae were evaluated for potential aecial hosts by inoculation with germinating teliospores. Cereal crops and turfgrasses other than *Poa* were highly resistant. Twenty-two of the tested gramineous species were susceptible, including twelve *Poa* spp., and several other native and introduced species in North America. In addition to the three East Asian buckthorn species, the native *R. lanceolata* and the widespread invasive common buckthorn (*R. cathartica*) are susceptible and may serve as the aecial hosts of *P. digitaticoronata*.

## Introduction

Crown rust diseases are caused by fungi within *Puccinia* series *Coronata,* which includes at least 17 species (Hambleton et al. 2019; Ji et al. 2022; Liu and Hambleton, 2013). Macrocyclic crown rust fungi alternate between grasses as the telial hosts, and Rhamnaceae or Elaeagnaceae as aecial hosts. Crown rust has recently been recognized as a widespread disease of Kentucky bluegrass (*Poa pratensis*) in North America (Beirn et al. 2011). One pathogen causing crown rust of Kentucky bluegrass is within *P. coronata* var. *avenae* f. sp. *avenae*, a clade that contains the pathogens causing crown rust of oats (*Avena sativa*), perennial ryegrass (*Lolium perenne*) and tall fescue (*Schedonorus phoenix*) (Beirn et al. 2011; Liu and Hambleton, 2013). In 2022, Ji et al. working in northeastern China, described five new species of crown rust fungi, including *Puccinia digitaticoronata,* a pathogen of *Poa* spp. The ITS sequences of *P. digitaticoronata* were highly similar or identical to sequences reported by Karakkat et al. (2018) from samples collected on Kentucky bluegrass in Wisconsin and to previously unpublished sequences we generated from aecial samples collected in 2019 at a North Dakota arboretum on three East Asian species of buckthorn: *Rhamnus crenata, R. davurica,* and *R. japonica,* which in visits in 1992 and 1993 had been free of aecia.

*Poa* is a large genus of grasses that contains more than 500 species, and it is present on every continent, even Antarctica (Chwedorzewska et al. 2014; Soreng, 2007). Kentucky bluegrass and, to a lesser extent, several other *Poa* spp. and hybrids, are employed as turfgrasses, with Kentucky bluegrass widely cultivated in temperate regions worldwide (Huff, 2003). In North America, 61 *Poa* spp. and 5 hybrids are native (Soreng, 2007) and are common components of

prairie, forest, and wetland ecosystems (Judziewicz et al. 2014; Tisdale, 1982). Nine introduced species, including Kentucky bluegrass, have naturalized, sometimes becoming weedy or invasive (DeKeyser et al. 2015; Soreng, 2007). Similarly, buckthorns (*Rhamnus* and *Frangula* spp.), the aecial hosts of many crown rust fungi, are numerous, with some species native and others introduced in North America. Common buckthorn (*R. cathartica*) and glossy buckthorn (*F. alnus*) are major invasive species throughout much of the Midwest and Northeast United States and neighboring regions of Canada (EDDMapS, 2022). Aecial hosts of rust fungi enable the sexual cycle to occur and can facilitate the rapid evolution of virulence on resistance genes, significantly complicating breeding for disease resistance (Carson, 2008; Beirn et al. 2011).

Given the recent description of *P. digitaticoronata*, its presence in North America, and its occurrence on Kentucky bluegrass, we initiated this study to clarify the placement of the pathogen within *Puccinia* series *Coronata* and assess its host specificity on grasses and potential aecial hosts in the families Rhamnaceae and Elaeagnaceae

## Materials and Methods

### Sample collection and sequencing

Samples were collected incidentally around the Twin Cities, MN, and at the North Dakota State University Dale E. Herman Research arboretum, where a collection of buckthorn species is maintained (Table 4.1). Samples collected from *Rhamnus* spp. in 2019 were processed and sequenced as described in Szabo (2006) for the ITS region with partial 28S. For other samples collected later, DNA

extraction and PCRs were conducted for four loci, ITS, *COI*, *RPB2*, and β-*tubulin*, following protocols lightly modified from Liu and Hambleton (2013) as described in Greatens et al. (2023). Sequencing was conducted on an Oxford Nanopore MinION following manufacturer instructions (kits EXP-PBC001 and SQK-LSK109 with R9 flow cells). Consensus sequences were generated by the method described in Greatens et al. (2023). Raw reads are available at SRA PRJNA899065. Average coverage is given in Supplementary Table 4.1.

**Phylogenetic analysis and sample identification**

Sequences were subjected to two phylogenetic analyses. The first, using a maximum likelihood criterion, is based on the ITS2. A second analysis, more robust but with fewer taxa represented, uses Bayesian inference and is based on four loci and the datasets from Liu and Hambleton (2013) and Hambleton et al. (2019) to clarify the placement of *P. digitaticoronata* within *Puccinia* series *Coronata.* Trees were formatted for publication with the R package GGTree (Yu et al. 2017).

*ITS2 analysis*: From Liu and Hambleton (2013), Demers et al. (2016), Hambleton et al. (2019), and Ji et al. (2022), sequences were selected to represent named species and major clades within *Puccinia* series *Coronata* and to draw attention to certain hosts. For simplicity, with the exceptions of *P. coronata sensu stricto* (*s. s.*) and *P. digitaticoronata,* only one sequence per species is included. Sequences are from type specimens where possible (see Supplementary Table 4.2 for sample information). From Beirn *et al*. 2011, sequences of *P. coronata* on Kentucky bluegrass were selected based on collection location and uniqueness. Due to the high occurrence of 'N' basecalls in some sequences of crown rust

fungi on Kentucky bluegrass from Karakkat et al. (2018), sequences were first subjected to local BLAST searches (Zhang et al. 2000) against a library containing the ITS sequences from Liu and Hambleton (2013) and Ji et al. (2022). Sequences with < 99 % similarity, duplicate sequences, and sequences with an incomplete ITS2 were disregarded. Selected sequences were aligned with MAFFT v7.450 using the G-INS-I algorithm and default settings (Katoh and Standley, 2013) and edited and trimmed in Geneious. A maximum likelihood tree was generated in Mega7 (Kumar et al. 2016) with 1000 bootstrap replicates, and standard settings using the best-fit model of DNA evolution as estimated in Mega11 (Tamura et al. 2021): T92 + G.

*Four-loci analysis*: At least one sample was included for each named clade of *Puccinia* series *Coronata* for which *COI*, β-*tubulin*, or *RPB2* sequences were available (meaning they were included either in Liu and Hambleton [2013] or Hambleton et al. [2018], Supplementary Table 4.2). Outgroup taxa were selected from the same datasets and from Liu and Hambleton (2010) which examined stripe rust fungi. Not all samples or taxa had sequencing data available for all four loci (See Supplementary Table 4.2). For this analysis, obvious errors around homopolymer regions in the ITS1 in original sequences generated with the Oxford Nanopore were corrected by comparison with samples sequenced using Sanger sequencing. Sequences were aligned with MAFFT v7.450 (Katoh and Standley, 2013) using the G-INS-I for the ITS region and FFT-NS-i x 1000 algorithms for the other loci. Alignments were edited and trimmed in Geneious and concatenated. In MrBayes v3.2, the analysis was partitioned into four parts with the models of DNA evolution for each estimated in Mega11: T92 + I + G for the ITS, K2 + G for β-*tubulin* and *RPB2*, and JC for *COI.* Model parameters for

each partition were unlinked and an exponential shape parameter of 10 was specified. Two parallel MCMC searches were conducted for ten million generations with every thousandth tree saved.

As a second method of identification, for two samples, 19_MN_Pdc04 and 21_MN_Pdc01-1, the sources of inoculum for the host specificity assessments (described below), 50 urediniospores were measured following Greatens et al. (2023). Results were combined and reported as a range from the mean ± SD with the smallest and largest observations given in parentheses.

### Host specificity assays

In total, 370 accessions of six cereal crop species were assessed for reaction to *P. digitaticoronata* through seedling assays (Supplementary Table 4.3): oat (*Avena sativa*), barley (*Hordeum vulgare*), rye (*Secale cereale*), common wheat (*Triticum aestivum*), durum wheat (*T. turgidum* ssp. *durum*), and triticale (×*Triticale*). Accessions of oat, barley, rye, and common wheat were selected to maximize geographic diversity. Accessions of durum wheat and triticale are from collections recently screened for stem rust resistance (Olivera et al. 2017; Olivera et al. 2021). Seeds were planted in 6 cm x 6 cm x 6 cm pots filled with vermiculite (SunGro Hort.) with four accessions seeded per pot and five plants per accession.

In total, 418 accessions of non-cereal grasses within 92 species belonging to 35 genera were assessed for susceptibility to *P. digitaticoronata* (Supplementary Table 4.4). Grass species were selected for screening based on their relation to *Poa* (Soreng et al. 2015), previous reports of susceptibility to *Puccinia* series C*oronata* (Cummins, 1971; Ji et al. 2022; Liu and Hambleton, 2013; Urban and Marková,

1993), and availability from the USDA-NPGS (National Plant Germplasm System). Seeds of *Brachypodium* spp. were acquired from the USDA-NPGS and by correspondence with Dr. Dave Garvin (USDA-ARS, St. Paul) and Dr. John Vogel (Joint Genome Institute). Lines were selected based on their availability and inclusion in previous studies of rust resistance in *Brachypodium* spp. (Ayliffe et al. 2013; Figueroa et al. 2013; Omidvar et al. 2018). *Brachypodium* spp. were planted in vermiculite, as per the cereal crop species. Other non-cereal grasses were grown in potting soil in 4 cm diameter x 16 cm length cone-tainers. Plants were fertilized after emergence and after inoculation with 20-20-20 water-soluble fertilizer. All plants were maintained in a greenhouse at 19 to 22 °C with a 16-hour day length.

Grass accessions were screened with one field collection of *P. digitaticoronata*, 19_MN_Pdc04, and one pure isolate, 21_MN_Pdc01-1. The pure isolate was developed as described in Greatens et al. (2023) using collections derived from field samples of rust on *Poa* spp. Inoculum was suspended in light mineral oil (Soltrol 170®, ChemPoint) and sprayed onto plants. Cereal crop species and wild *Avena* spp. were inoculated at 8-9 days old after the primary leaf had fully expanded. Non-cereal species were inoculated at three to five weeks after planting, depending upon the plant sizes. After inoculation, plants were incubated overnight in a dew chamber at 100 % relative humidity, moved to a greenhouse for incubation, and rated for infection at 12 to 14 days. Field collections of susceptible Kentucky bluegrass were used as a positive control. Plants were rated on a scale from 0 - 4 adapted from Murphy (1935) where: 0 = immunity; ";" = chlorotic flecking; N = necrotic flecking; 1 = small uredinia with significant chlorosis and/or necrosis; 2 = small to midsized uredinia with

chlorosis; 3 = midsized to large uredinia with some chlorosis; 4 = large uredinia with no chlorosis; "+" and "-" following numeric ratings indicate intermediate infection types.

Plants of Rhamnaceae and Elaeagnaceae were obtained through seed or cuttings locally, at the North Dakota State University Dale E. Herman Research Arboretum, from the USDA NPGS, and through native plant nurseries. As a result, some species were screened as seedlings and others as older plants. Inoculations were conducted following Jin and Steffenson (1999) using naturally overwintered, telia-bearing Kentucky bluegrass straw collected on the University of Minnesota St. Paul campus in April 2021. The species identities of aecia were confirmed by sequencing the ITS region (data not shown).

## Results

### Phylogenetic analysis and sample identification

Collections of *Puccinia* series *Coronata* on *Poa* spp. in North America group into two distinct clades with high bootstrap support (Fig. 4.1A). *Puccinia coronata* var. *avenae* f. sp. *avenae sensu* Liu and Hambleton, within *P. coronata s. s.*, includes all samples from Beirn et al. (2011), two samples on *Poa* spp. from Liu and Hambleton (2013), and some of the samples from Karakkat et al. (2018). This clade also includes the oat, ryegrass, and tall fescue crown rust pathogens, which cannot be distinguished based on ITS2. *Puccinia digitaticoronata* includes all nine samples from this study collected in North Dakota and Minnesota, sequences of several samples collected in Wisconsin reported by Karakkat et al. (2018), and sequences of the type and paratype specimens of the species described by Ji et al.

(2022) in northeast China. Similarly, in the Bayesian consensus tree, original samples from this study form a well-supported clade sister to *P. coronata sensu stricto* (Fig 4.1B). Urediniospores measured (12)15-18(21) x (10)12-15(17), consistent with *P. digitaticoronata.*

### Host specificity

All accessions of cereal crop species (oat, barley, rye, common wheat, durum wheat, and triticale) were resistant to both inocula (Table 4.2). Sporulation was observed on 21 out of 103 accessions of oats with a maximum infection type (IT) of 1, a highly resistant response (Fig 4.2A). In rye, sporulation occurred on 10 out of 57 accessions with a maximum IT of ;1. (Fig 4.2B). Other cereal crop species were immune, with no visible symptoms. Some wild *Avena* spp., however, had more infection by *P. digitaticoronata* (Fig 4.2C). Uredinia were produced on five of the wild *Avena* spp. assessed. One accession of *A. barbata* (PI 166026) was rated IT = 2/3 to isolate 21_MN_Pdc01-1, meaning some individuals within the accession were rated IT = 2 and others IT = 3, a susceptible response. Similarly, some individuals within the *A. strigosa* accession (Clav 2524) were rated IT = 3-N to the field collection, 19_MN_Pdc04. All *A. strigosa* had notable necrosis (e.g. Fig. 4.2C14) regardless of the level of sporulation observed. All ten A. *murphyi* accessions were immune, with no visible symptoms.

Most of the thirty-one accessions of Kentucky bluegrass screened with *P. digitaticoronata* were highly susceptible (Table 4.3; Fig 4.2D). Two accessions (PI 591637 and PI 591639) were found to be resistant, and four accessions (PI 206740, PI 220616, PI 595593, and PI 601092) were heterogenous with resistant and susceptible plants present in the same accession (Supplementary Table 4.4). *Poa*

*pratensis* ssp. *angustifolia* was especially susceptible, with all eleven accessions rated as IT = 4. Sporulation was observed on all other *Poa* spp. assessed in our trials, although infection types varied both by species, and within species by accession (Table 4.3; Fig 4.2E). Of the *Poa* spp. assessed, only *Poa annua* was generally resistant, with eight of nine accessions rated as immune or highly resistant. One accession (PI 220109) was rated IT = 3. The remainder of the *Poa* spp. assessed were susceptible, including six native North American species (*P. arida, P. chaixii, P. interior, P. glauca*, *P. palustris*, and *P. secunda*); four introduced and sometimes weedy species (*P. bulbosa, P. compressa, P. nemoralis*, and *P. trivialis*); and *P. attenuata,* an East Asian species not known to be present in North America. Resistant responses were observed in some accessions of *P. palustris* and *P. trivialis. Poa compressa*, a species with limited use as a turfgrass in North America, is highly susceptible, with nine of ten accessions rated as IT = 4. We have observed natural infections on *P. glauca*, *P. palustris*, and *P. pratensis* by *P. digitaticoronata* (Table 4.1).

Various degrees of sporulation were observed on twenty-nine other grass species (Table 4.3; Fig 4.2F). Of these species, ten were rated as susceptible to at least one of the inocula, including *Apera intermedia* and *A. spica-venti*, *Lamarckia aurea, Phalaris minor*, five *Puccinellia* spp. (*P. distans, P. gigantea*, *P. intermedia*, *P. nuttaliana,* and *P. tenuiflora*), and *Vulpia octoflora.* In some of these species, resistance was observed among or within some accessions. Other turfgrass species assessed were immune to *P. digitaticoronata*, including creeping bentgrass (*Agrostis stolonifera*), tall fescue, five fine fescue species or subspecies (*F. brevipila, F. rubra* ssp. *commutata, F. rubra* ssp. *rubra, F. saximontana*, and *F. subverticillata*); and perennial ryegrass. Several other common grasses known to be hosts of

other crown rust fungi were immune to *P. digitaticoronata*, including *Arrhenatherum elatius, Bromus* spp., *Elymus* spp., *Holcus lanatus*, *Lolium multiflorum*, and *Phalaris arundinacea*. The model organism *Brachypodium distachyon* is highly resistant: while no sporulation occurred on any of the 51 accessions, variation in infection type was observed, with some accessions showing a hypersensitive response (Fig 4.2G).

Inoculation with germinating telia of *P. digitaticoronata* resulted in infection and production of aecia on *R. cathartica, R. lanceolata,* and *R. japonica* (Table 4.4). Three East Asian species, *R. crenata, R. dahurica,* and *R. japonica,* were found naturally infected in North Dakota. While adults were naturally infected, *R. crenata* seedlings from another source (NPGS: NA 56664) were resistant in greenhouse inoculations with necrotic flecking apparent. All five *Frangula* species tested were resistant: *Frangula alnus* and *F. purshiana* seedlings exhibited an immune reaction with no visible symptoms, whereas *F. californica, F. caroliniana* and *F. rubra* seedlings produced necrotic flecking. An immune reaction was also observed in silver buffaloberry (*Shepherdia argentea*) of Elaeagnaceae and Alabama supplejack (*Berchemia scandens*) of Rhamnaceae.

## Discussion

*Puccinia digitaticoronata*, a recently described crown rust pathogen of Kentucky bluegrass (Ji et al. 2022), is present in North America. In the Twin Cities Metropolitan Area in Minnesota, we have observed heavy crown rust infection by *P. digitaticoronata* on Kentucky bluegrass in unmowed or shaded areas surrounding field edges and at the bases of trees and buildings. Disease

development, however, was limited in research plots of Kentucky bluegrass maintained by the University of Minnesota turfgrass science program, where plants are mowed regularly and grow under full sun. Differences in plant age, environmental conditions, and abiotic and biotic stresses may explain differences between greenhouse assessments and field infection.

Our phylogenetic analyses clearly showed that our samples collected from buckthorns in North Dakota and *Poa* spp. in Minnesota, belong to *P. digitaticoronata,* a species morphologically and genetically distinct from *P. coronata s.s*. Based on published sequences, several crown rust samples from *P. pratensis* reported previously in Wisconsin (Karakkat et al. 2018) also belonged to *P. digitaticoronata*, although the species was undescribed at the time of publication and samples were determined as *P. coronata* (*s. l.*). Notably, a second crown rust pathogen of Kentucky bluegrass is within *P. coronata* (*s. s.*) var. *avenae* f. sp. *avenae* (Liu and Hambleton 2013). This pathogen is also present in North America (Beirn et al. 2011) and in Minnesota (Karakkat et al. 2018, Liu and Hambleton, 2013), although in our limited collections, we only found *P. digitaticoronata*. The Bayesian consensus tree based on four loci, following Liu and Hambleton (2013), shows with strong posterior probability that *P. digitaticoronata* is sister with *P. coronata* (*s. s.*). Several taxa, however, which were included in the analysis by Ji et al. (2022) and grouped more closely with *P. digitaticoronata* were excluded since sequencing data was limited to ITS and 28S. Future work may resolve the placement of these taxa within *Puccinia* series *Coronata*. The results of our phylogenetic analysis are similar to those of Liu and Hambleton (2013) and Hambleton et al. (2019). *Puccinia digitaticoronata* should not be confused with *P. digitata* or *P. pseudodigitata,* two recently described North

American microcyclic crown rust fungi hosted by *Endotropis crocea* ssp. *ilicifolia* (syn. *Rhamnus ilicifolia*) (Hambleton et al. 2019).

The uredinia of *P. digitaticoronata* are similar in appearance to those of other crown rust fungi or *P. pseudostriiformis*, but *Puccinia digitaticoronata* could be distinguished based on its small urediniospores (14.5 – 19.5 x 12.5 - 15.5 μm in the original description) and long telial appendages (7.2 - 14.5 μm; Ji et al. 2022). The urediniospores of *P. coronata* var. *avenae* f. sp. *avenae* and *P. pseudostriiformis,* as well as other common *Puccinia* spp. on bluegrass in North America (e.g., *P. graminis, P. poarum*, and *P. poae-nemoralis*), are all generally larger (Beirn et al. 2015; Cummins, 1971; Liu and Hambleton, 2010; Liu and Hambleton, 2013). Liu and Hambleton (2013) describe urediniospores of *P. coronata* var. *avenae* f. sp. *avenae* as ranging from 15–29 (32) x 13–25 μm with a mean length of 20.5 μm, and Beirn et al. (2015) report a range of 20-25 × 17-22 μm for a single *Poa* crown rust isolate collected in New Jersey, presumably within *P. coronata* var. *avenae* f. sp. *avenae* like their other samples (this study; Beirn et al. 2011), although identified as *P. coronata* (*s. l.*). Rust fungi of turfgrasses are often confused based on macroscopic appearance and spore morphology (Beirn et al. 2011), and reliable real-time PCR-based identification methods have been developed (Beirn et al. 2011; Karakkat et al. 2018). While Karakkat et al. (2018) reports success in identifying *P. digitaticoronata* samples as *P. coronata s. l.* using real-time PCR, new probes would be needed to distinguish between clades of *Puccinia* series *Coronata.*

In addition to *P. digitaticoronata,* Ji et al. (2022) also described a closely related species, *P. eleganticoronata*, another pathogen of *Poa* spp. presently known only in northeast China. It is thus likely that *P. digitaticoronata* originated in East

Asia. It is not clear when *P. digitaticoronata* or crown rust fungi more broadly began to cause widespread disease in Kentucky bluegrass in North America since they may have evaded detection due to confusion with other rusts (Beirn et al. 2011). In the U.S. National Fungus Collections (Farr and Rossman, accessed Dec. 2, 2022), there are thirteen samples of *Puccinia coronata* (*s. l.*) on *Poa* spp. from North America, including several on *P. pratensis*. Many of these samples will need to be reevaluated given recent species descriptions, taxonomic revisions, and frequent misidentifications of rusts and their grass hosts. *Puccinia digitaticoronata* was not collected in the survey by Beirn et al. (2011), which reported *P. coronata* on Kentucky bluegrass in 21 samples collected in six states, Maine, Massachusetts, New Jersey, North Carolina, Ohio, and Wyoming. *Puccinia digitaticoronata* has been detected in three states, in eastern North Dakota and in Minnesota with this study, and in Wisconsin, based on sequences reported by Karakkat et al. (2018). Given the notably small urediniospores, ornate telial appendages, and host association, it is unlikely that *P. digitaticoronata* would have gone unnoticed by earlier North American uredinologists or turfgrass pathologists. Together with the appearance of *P. digitaticoronata* on *R. crenata, R. davurica,* and *R. japonica* in North Dakota, this may indicate a recent introduction. A range expansion into other regions of North America where buckthorn hosts are present is likely.

All 370 accessions of the six cereal crop species assessed (barley, oat, rye, bread wheat, durum wheat, and triticale) were highly resistant, and *P. digitaticoronata* is of no apparent concern for food production. Some varieties of oat and rye, however, may serve as weak hosts, with limited sporulation occurring, and some accessions of wild *Avena* spp., relatives of cultivated oats,

were susceptible. Wild *Poa* spp., except for *P. annua,* are generally susceptible to *P. digitaticoronata.* Among these species are native grasses in North America as well as common weeds and invasive species. Kentucky bluegrass too has escaped cultivation and naturalized in much of temperate North America (Soreng, 2007). In the northern Great Plains, for example, it is a major invasive species associated with declining plant species richness in endangered prairie ecosystems (DeKeyser et al. 2015), although some evidence shows invasive populations are genetically distinct from commercially available cultivars (Dennhardt et al. 2017). In some contexts, it is possible *P. digitaticoronata* may benefit native plant communities, but where susceptible native *Poa* spp. are present, the pathogen may be of concern.

Some species able to host *P. digitaticoronata* to varying degrees were also found to host *P. coronata* var. *coronata* in a recent study (N. Greatens, unpublished) including *Alopecurus geniculatus*, *Apera* intermedia, *A. spica-venti*, *Beckmannia syzigachne*, *Lamarckia aurea*, all six *Puccinellia* spp. assessed here, and *Vulpia octoflora.* Similarly, Eshed and Dinoor (1981) find that *L. aurea* and four *Vulpia* spp., among other grass species, are susceptible to isolates of crown rust from eight grass hosts in Israel, including oats, although the precise identities of the rusts are ambiguous. These grasses that can host diverse crown rust fungi may be valuable as bridging hosts of interspecific hybrids or as sites of asexual hybridization or lateral gene transfer between sexually incompatible rust fungi (Eshed and Dinoor, 1981)

In North America, several crown rust fungi have recently emerged as pathogens: *P. coronati-hordei* on barley (Jin et al. 1992; Jin and Steffenson, 1999); *P. coronati-brevispora* on bromegrasses (*Bromus* spp.) (Delgado et al, 2003); *P.*

*coronata* var. *avenae* f. sp. *avenae* on Kentucky bluegrass (Beirn et al. 2011); and *P. coronata* var. *coronata* on glossy buckthorn and reed canarygrass (Greatens et al. 2023). In addition, *P. coronata* (*s. l.*) var. *gibberosa* was recently reported for the first time in North America on the ornamental blue oat grass (*Helictotrichon sempervirens*) (Demers et al. 2016). Recent disease emergences may be explained by the introduction of new host species, new pathogen strains, or by the evolution of new virulences. Improved taxonomy and phylogenetics also facilitate the detection and recognition of new pathogens. Abundant and widespread invasive buckthorn and grass hosts have likely contributed to the recent occurrence and establishment of novel crown rust species and strains.

Some cultivars of Kentucky bluegrass are resistant, but the durability of the resistance is unknown. Growers should be vigilant for changes in crown rust susceptibility in previously resistant varieties. In the field, it is difficult to distinguish *P. coronata* var. *avenae* f. sp. *avenae* from *P. digitaticoronata*, and field ratings that are not verified by laboratory examination will be of limited use to identify resistant cultivars. A coordinated effort to identify rust pathogens on Kentucky bluegrass cultivars, in both turfgrass and seed production trials, would help illuminate how prevalent this pathogen is in North America. Future investigations may build off research in cereal rust fungi to characterize pathogen populations, screen germplasm, and identify sources of resistance.

Rust diseases of turfgrasses can make sod unsalable (Beirn et al. 2011), diminish customer satisfaction, and reduce yield in seed production (Pfender, 2004). Turfgrasses are often perennial and long-living, and replacement of susceptible turf is generally not feasible for consumers (Karakkat et al. 2018). As with other rusts of turfgrasses, impact of disease can likely be mitigated by

maintaining adequate levels of nitrogen and keeping turf in otherwise good health, by regular mowing with removal of clippings, and if desired, by applications of sterol-inhibiting fungicides (Smiley et al. 2005). Diversification of species or cultivars within seed mixes may also help reduce disease severity (Roscher et al. 2007).

**Table 4.1.** *Puccinia digitaticoronata* sample collection information with GenBank and herbarium accession numbers.

| Voucher number | Sample ID | Year | Location | Host | ITS | *COI* | β-*tubulin* | *RPB2* |
|---|---|---|---|---|---|---|---|---|
| PUR N24042 | 19_ND_Pdc01 | 18-Jun-19 | Absaraka, ND (46.988, -97.353) | *Rhamnus japonica* | OP802575 | - | - | - |
| PUR N24043 | 19_ND_Pdc02 | 18-Jun-19 | Absaraka, ND (46.988, -97.353) | *R. davurica* | OP802576 | - | - | - |
| PUR N24044 | 19_ND_Pdc03 | 18-Jun-19 | Absaraka, ND (46.988, -97.353) | *R. crenata* | OP802577 | - | - | - |
| PUR N24045 | 19_MN_Pdc04 | 2-Oct-19 | Minneapolis, MN (44.997, -93.255) | *Poa pratensis* | OP796492 | OP821256 | OP821250 | OP821262 |
| PUR N24046 | 21_MN_Pdc01-1[1] | 12-Jul-21 | Roseville, MN (45.0167, -93.124) | *P. palustris* | OP796493 | OP821257 | OP821251 | OP821263 |
| PUR N24047 | 21_MN_Pdc02 | 21-Aug-21 | Chaska, MN (44.857, -93.616) | *P. glauca* | OP796494 | OP821258 | OP821252 | OP821264 |
| PUR N24048 | 21_MN_Pdc03 | 1-Oct-21 | St. Paul, MN (44.990, -93.181) | *P. pratensis* | OP796495 | OP821259 | OP821253 | OP821265 |
| PUR N24049 | 21_MN_Pdc04 | 18-Aug-21 | Roseville, MN (45.018, -93.124) | *P. palustris* | OP796496 | OP821260 | OP821254 | OP821266 |
| PUR N24050 | 21_MN_Pdc05 | 1-Oct-21 | St. Paul, MN (44.989, -93.181) | *P. pratensis* | OP796497 | OP821261 | OP821255 | OP821267 |

[1] The "-1" following the sample ID indicates a purified isolate

**Table 4.2.** Summary of infection types in cereal crops inoculated with *Puccinia digitaticoronata.*

| **Species** | **Number of accessions tested** | **Range of infection types**[1] | |
|---|---|---|---|
| | | Inoculum | |
| | | Field collection 19_MN_Pdc04 | Isolate 21_MN_Pdc01-1[2] |
| Oats (*Avena sativa*) | 103 | 0 - 1 | 0 - 1; |
| Barley (*Hordeum vulgare*) | 56 | 0 | 0 |
| Rye (*Secale cereale*) | 54 | 0 - 1; | 0 - 1;- |
| Common wheat (*Triticum aestivum*) | 55 | 0 | 0 |
| Durum wheat (*Triticum turgidum ssp. durum*) | 54 | 0 | 0 |
| Triticale (*x Triticosecale*) | 48 | 0 | 0 |

1. Infection types are rated on a scale from 0 – 4. 0 = immunity. ; = chlorotic flecking. N = necrotic flecking. 1 = uredinia small with significant chlorosis and/or necrosis. 2 = uredinia small to midsized with chlorosis. 3 = uredinia midsized to large with some chlorosis. 4 = uredinia large, no chlorosis. "+" and "-" following numeric ratings indicate intermediate infection types. Multiple ratings separated by a slash indicate multiple infection types observed within the accession.

2. The "-1" following the sample ID indicates a purified isolate.

**Table 4.3.** Summary of infection types in other grass species inoculated with *Puccinia digitaticoronata.*

| | Species | Number of accessions tested | Range of infection types[1] Inoculum | |
|---|---|---|---|---|
| | | | Isolate 19_MN_Pdc04 | Isolate 21_MN_Pdc01-1[2] |
| Moderately to highly susceptible. (Highest IT ≥ 3.) | *Apera intermedia* | 2 | 2 - 3 | 1+ - 3 |
| | *A. spica-venti* | 5 | ;1 - 3 | 2 - 3 |
| | *Lamarckia aurea* | 1 | 3 | 3 |
| | *Phalaris minor* | 1 | 2 | 3 |
| | *Poa arida* | 1 | 3 | 3 |
| | *P. attenuata* | 1 | 3+ | 4 |
| | *P. bulbosa* | 4 | 3+ - 4 | 3+ - 4 |
| | *P. chaixii* | 2 | 3 - 3+ | 3 - 3+ |
| | *P. compressa* | 10 | 3+ - 4 | 3+ - 4 |
| | *P. glauca* | 2 | 3+ | 3+ |
| | *P. interior* | 4 | 3+ - 4 | 3 - 3+ |
| | *P. nemoralis* | 4 | 3 - 3+ | 3 - 4 |
| | *P. palustris* | 7 | 2+ - 3 | 1 - 3+ |
| | *P. pratensis* | 31 | ; - 4 | ;1 - 4 |
| | *P. secunda* | 4 | 3+ | 4 |
| | *P. trivialis* | 8 | 1+ - 3+ | 1+ - 3+ |
| | *Puccinellia distans* | 1 | 3 | 4 |
| | *P. gigantea* | 1 | 3/3+ | 3 |
| | *P. intermedia* | 1 | 3+ | 3+ |
| | *P. nuttalliana* | 1 | 3+ | 4 |
| | *P. tenuiflora* | 1 | 3/4 | 3 |
| | *Vulpia octoflora* | 4 | 2 - 3 | 2 - 3 |

**Table 4.3. continued**

| | Species | Number of accessions tested | Range of infection types[1] Inoculum Isolate 19_MN_Pdc04 | Isolate 21_MN_Pdc01-1[2] |
|---|---|---|---|---|
| | *Alopecurus geniculatis* | 3 | 0 - 2+ | 0 - 2+ |
| | *Avena barbata*[3] | 10 | N - 2+ | ;N - 3 |
| | *A. fatua* | 8 | 0 - 1 | ;N - 1+ |
| | *A. longiglumis* | 8 | 0 - 2+ | 0 - 2+ |
| | *A. magna* | 6 | 0 - 1 | 0 - ;1 |
| Moderately | *A. strigosa* | 10 | 0N - 3-N | 0N - 1+ N |
| to highly | *Beckmannia syzigachne* | 5 | 0 - 1 | 0 - 1 |
| resistant. | *Calamagrostis purpurascens* | 5 | 0 | 0 - ;1 |
| (Highest IT | *C. stricta*[3] | 4 | 0 - ; | 0 - 3 |
| ≤ 2 + with | *Cinna arundinacea* | 3 | 0 -1 | 0 -1 |
| sporulation | *Dactylis glomerata* | 4 | ; - 1 | ; - 1 |
| present.) | *Phalaris brachystachys* | 3 | 0 - 1 | ;N |
| | *P. truncata* | 2 | 0 - ; | ;1 |
| | *Phleum phleoides* | 2 | 0 | 0 - 1 |
| | *Poa annua*[3] | 9 | 0 - 3 | 0 - 3 |
| | *Puccinellia stricta* | 1 | ; | ;1 |
| | *Trisetum spicatum* | 2 | 0 | 0 - 1 |

**Table 4.3. continued**

| | Species | Number of accessions tested | Range of infection types[1] Inoculum: Isolate 19_MN_Pdc04 | Inoculum: Isolate 21_MN_Pdc01-1[2] |
|---|---|---|---|---|
| Highly resistant to immune. (IT < 1. No sporulation observed.)[4] | *Agrostis gigantea, A. stolonifera, Alopecurus pratensis, Arrhenatherum elatius, Avena murphyi, Avenula pubescens, Brachypodium distachyon, B. stacei, B. sylvaticum, Briza media, Bromus ciliatus, B. inermis, B. japonicus, B. pubescens, B. secalinus, Calamagrostis breviligulata, C. canadensis, C. nutkaensis, Cynosurus cristatus, Dactylis marina, Deschampsia cespitosa, Digitaria ischaemum, Elymus canadensis, E. hystrix, E.repens, E. trachycaulus, E. villosus, E. virginicus, Festuca arundinacea, F. rubra, F. saximontana, F. subverticillata, F. trachyphylla, Glyceria canadensis, G. grandis, G. striata, Holcus lanatus, Koeleria macrantha, Lolium multiflorum, L. perenne, Melica nitens, M. nutans, Pascopyrum smithii, Phalaris arundinacea, P. canariensis, P. coerulescens, P. paradoxa, P.platensis, Phleum pratense, Polypogon monspeliensis, Thinopyrum intermedium, Trisetum flavescens,* | | | |

[1] Infection types are rated on a scale from 0 – 4. 0 = immunity. ; = chlorotic flecking. N = necrotic flecking. 1 = uredinia small with significant chlorosis and/or necrosis. 2 = uredinia small to midsized with chlorosis. 3 = uredinia midsized to large with some chlorosis. 4 = uredinia large, no chlorosis. "+" and "-" following numeric ratings indicate intermediate infection types. Multiple ratings separated by a slash indicate multiple infection types observed within the accession.

[2] The "-1" following the sample ID indicates a purified isolate.

[3] Accessions in this species are generally resistant, but one accession was rated as susceptible to at least one inoculum.

[4] Number of accessions evaluated and infection types are available in Supplementary Table S4. Here formatted as a list for brevity.

**Table 4.4.** Infection types in Rhamnaceae and Elaeagnaceae in response to *Puccinia digitaticoronata*

| Species | Growth stage | Number of plants | Origin of plant material | Infection type |
|---|---|---|---|---|
| *Berchemia scandens* | > 2 yrs. | 1 | Missouri Wildfowers Nursery, Missouri | Immune |
| *Frangula alnus* | Seedling | > 50 | Acorn Park, St. Paul MN | Immune |
| *F. californica* | Seedling | 5 | NPGS: W6 55073 | Hypersensitive response |
| *F. caroliniana* | > 2 yrs. | 1 | Mail Order Natives, Maryland | Hypersensitive response |
| *F. purshiana* | Seedling | 2 | NPGS: W6 53277 | Hypersensitive response |
| *F. rubra* | > 2 yrs. | 1 | Forestfarm Nursery, Oregon | Immune |
| *Rhamnus cathartica* | Seedling | > 50 | Theodore Wirth Park, Minneapolis, MN | Susceptible |
| *R.crenata* | Seedling | 1 | NPGS: NA 56664 | Hypersensitive response |
| *R. lanceolata* | > 2 yrs. | 1 | Missouri Wildfowers Nursery, Missouri | Susceptible |
| *R. japonica* | Seedling | 4 | Dale E Herman Research Arb., North Dakota | Susceptible |
| *Shepherdia argentea* | Seedling | 3 | NPGS: W6 47220 | Immune |

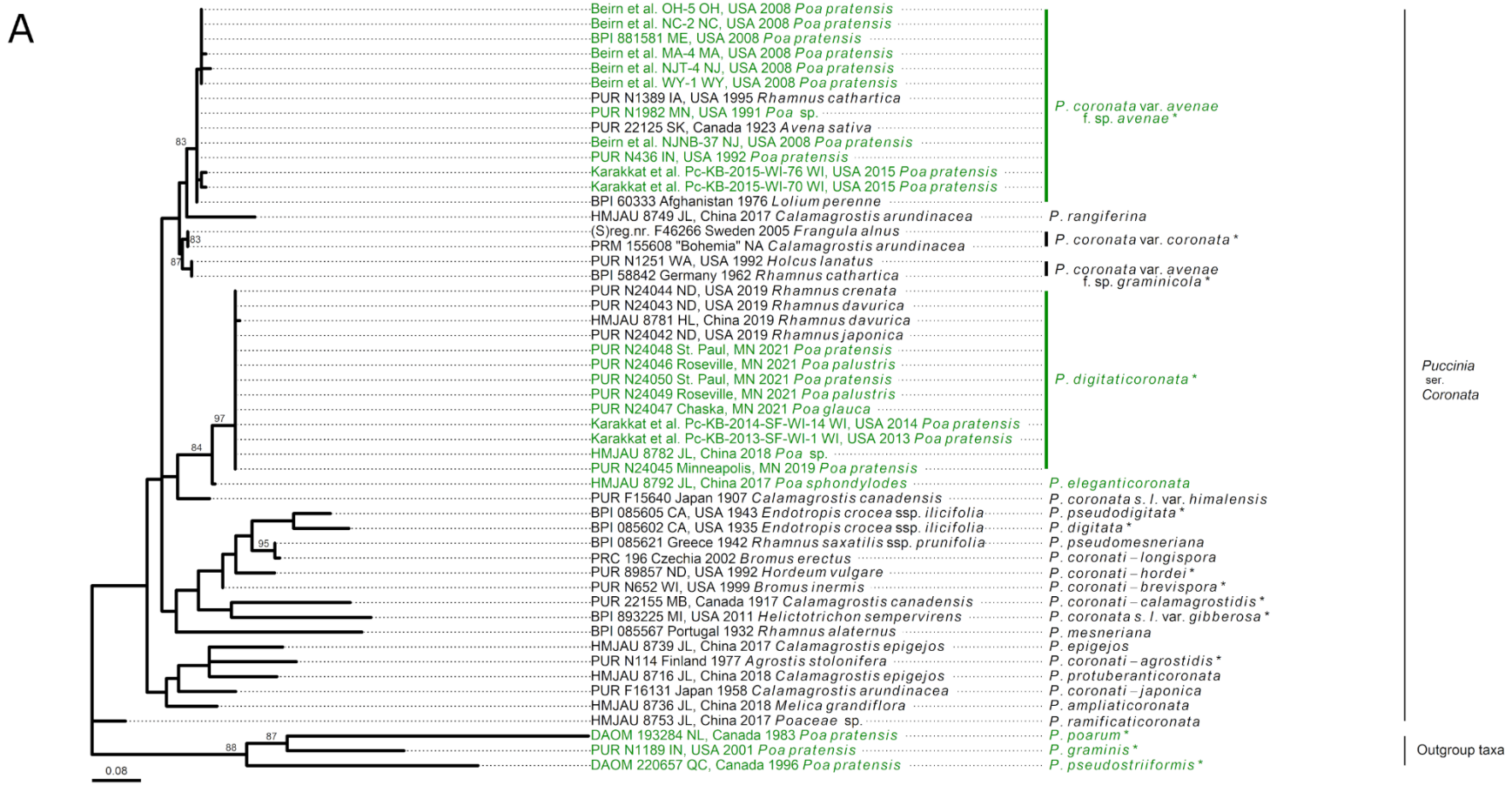


**Figure 4.1.** Results of phylogenetic analyses of *Puccinia digitaticoronata* samples and related crown rust fungi. **A)** Maximum likelihood phylogram inferred from ITS2 sequences. Bootstrap values > 70 are shown.

B


**Figure 4.1 continued** Results of phylogenetic analyses of *Puccinia digitaticoronata* samples and related crown rust fungi. **B)** Consensus tree from Bayesian analysis of four loci: the ITS region, and partial β-*tubulin, COI,* and *RPB*2 genes. Summarized from 15,002 trees with a burn-in of 25%. Posterior probabilities > 95 are shown. Taxa reported in North America are denoted with an asterisk. Additional sample collection data and NCBI GenBank accession numbers are provided in Suppl. Table 4.2.

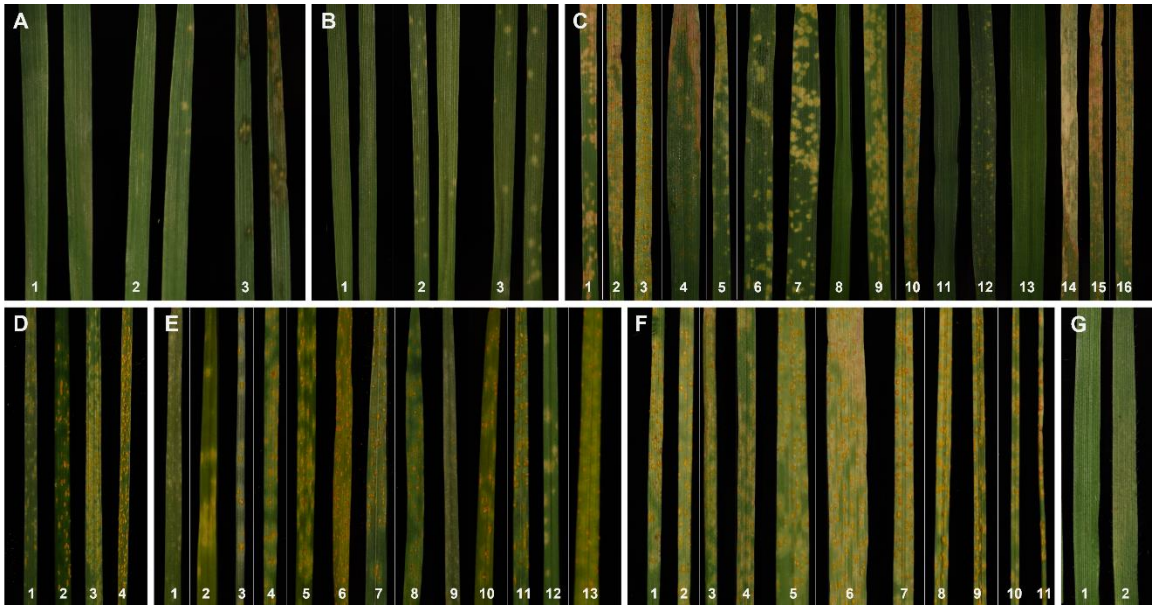


**Figure 4.2.** Range of infection types in gramineous species inoculated with *Puccinia digitaticoronata* isolate 21MN_Pdc01-1. **A)** Oat. 1) IT = 0 on CIav 3038. 2) IT = ; on CIav 3312. 3. IT = ;N1- on CIav 4639. **B)** Rye. 1) IT = 0 on Clse 20. 2) IT = ; on Clse 30. 3) IT = ;1 on Clse 122. **C)** Wild oats. 1) IT = ;1N on *Avena barbata* (Clav 8086). 2) IT = 2+N on *A. barbata* (Clav 9039). 3) IT = 3 on *A. barbata* (PI 166026). 4) IT = N on *A. fatua* (Clav 1778). 5) IT = ;1 on *A. fatua* (Clav 1779). 6) IT = 1 on *A. fatua* (Clav 2526). 7) IT = 1+ on *A. fatua* (Clav 2527). 8) IT = 0 on *A. longiglumis* (Clav 9089). 9) IT = 1+ on *A. longiglumis* (PI 282730). 10) IT = 2+ on *A. longiglumis* (PI 657388). 11) IT = 0 on *A. magna* (Clav 8330). 12) IT = ;1 on *A. magna* (PI 657516). 13) IT = 0 on *A murphyi* (PI 657363). 14) IT = N on *A. strigosa* (Clav 1782). 15) IT = 1N on *A. strigosa* (Clav 2524). 16) IT = 2 on *A. strigosa* (Clav 5057). **D)** Kentucky bluegrass. 1) IT = 1+ on *Poa pratensis* (PI 595593). 2) IT = 3+ on *P. pratensis* (PI 303053). 3) IT = 1+ on *P. pratensis* ssp. *angustifolia* (PI 221948). 4) IT = 4 on *P. pratensis* ssp. *angustifolia* (PI 220036). **E)** Wild *Poa* species. 1) IT = ; on *P. annua* (PI 217625). 2) IT = ;1 on *P. annua* (W6 28171). 3) IT = 3 on *P. arida* (PI 578806). 4) IT =

3+ on *P. bulbosa* (PI 211070). 5) IT = 3+ on *P. chaixii* (PI 249766). 6) IT = 4 on *P. compressa* (PI 206738). 7) IT = 3+ on *P. glauca* (PI 109350). 8) IT = 3 on *P. interior* (PI 236911). 9) IT = 1+ on *P. palustris* (PI 236913). 10) IT = 3+ on *P. palustris* (PI 369297). 11) IT = 4 on *P. secunda* (PI 284248). 12) IT = 1+ on *P. trivialis* (PI 601315). 13) IT = 3+ on *P. trivialis* (PI 594396). **F)** Other wild grasses. 1) IT = 3 on *Apera intermedia* (PI 203444). 2) IT = 3 on *A. spica-venti* (Ames 23686). 3) IT = 1+ on *Calamagrostis stricta* (W6 54550). 4) IT = 1 on *Dactylis glomerata* (PI 237601). 5) IT = 3 on *Lamarckia aurea* (PI 378959). 6) IT = 3 on *Phalaris minor* (PI 220033). 7) IT = 4 on *Puccinellia distans* (PI 230250). 7) IT = 3 on *P. gigantea* (PI 384943). 8) IT = 3+ on *P. intermedia* (PI 628715). 9) IT = 4 on *P. nuttalliana* (PI 675197). 10) IT = 3 on *P. tenuiflora* (PI 610863). 11) IT = 3 on *Vulpia octoflora* (W6 54351). **G)** *Brachypodium distachyon.* 1) IT = 0 on "Tek-4". 2) IT = ;N on W6 39359.

# Chapter Five

## First reports of *Puccinia glechomatis* infecting creeping charlie in Minnesota and of a rust fungus (*Puccinia* sp.) infecting lemongrass in Minnesota

The two sections of this chapter were published in similar form as first reports in the journal *Plant Disease*:

Greatens, N., Klejeski, N., Szabo, L.J., Jin, Y. and Olivera Firpo, P.D. 2023. First report of *Puccinia glechomatis*, a rust fungus of creeping charlie, in Minnesota. Plant Dis. In press. 10.1094/PDIS-10-22-2315-PDN

Greatens, N., Klejeski, N., Szabo, L.J., Jin, Y. and Olivera Firpo, P.D. 2023. First report of a rust fungus (*Puccinia* sp.) infecting lemongrass in Minnesota. Plant Dis. In press. 10.1094/PDIS-10-22-2314-PDN

## Summary

The following chapter consists of two first reports of rust fungi in Minnesota: *Puccinia glechomatis,* a rust fungus of the common weed creeping Charlie (*Glechoma hederacea*), and a *Puccinia* sp. not definitively identified to species level causing a rust of lemongrass (*Cymbopogon citriatus*), a plant used in some Asian cuisines and grown locally in small scale production. Rust fungi were identified based on a combination of morphological and life cycle traits and sequence similarity. Based on herbarium records, both species are new observations in Minnesota. *Puccinia glechomatis* was first recorded in North America in Pennsylvania in 1992. By 2001, it had spread to southern Wisconsin. Our report is evidence of its continued westward spread in the United States. *Puccinia glechomatis* is not known to affect any native species and may be welcomed as a pathogen of a notorious garden weed and invasive species. On lemongrass, rust disease had not previously been reported in Minnesota. Other reports of lemongrass rust in the United States, in California, Florida, and Hawaii identified the causal agent of *Puccinia nakanishikii,* but our sample clearly differed based on urediniospore morphology and the ITS2 region. A BLASTn search suggests a species identity of *Puccinia cesatii*, a rust not previously associated with lemongrass. More phylogenetic research will be necessary to resolve species relationships among *P. cesatii* and related rust fungi.

## Rust of creeping Charlie

In 2020 and 2021, at four locations around the Twin Cities, Minnesota (Sep. 2021: 44.980, -93.319; Aug 2021: 44.989, -93.186; Sep 2020: 45.000, -93.138; Sep. 2020: 44.870, -92.779), a rust fungus was observed infecting creeping Charlie (*Glechoma hederacea*), a weed of Eurasian origin (Hutchings and Price, 1999). Within stands, severity ranged from 1 to 30 % leaf loss. Telia were reddish-brown when young, starting small (0.1 to 0.5 mm), and growing into round but irregular sori measuring 1 to 5 mm, sometimes coalescing to form larger sori (Fig 5.1A). Sori are primarily abaxial, forming depressions on the adaxial surface, and sometimes occurring along stems and leaf petioles. Partial leaf necrosis occurs with high foliar infection and leaf dieback with high infection of petioles. Thin-walled, colorless leptosporic teliospores, or leptospores, (Fig 5.1B) were present in samples. Dark, thick-walled teliospores were not noted. Leaves bearing leptospores were soaked in water for four hours and suspended over young plants in a dew chamber at 20 °C for 16 hours. Plants were maintained in a greenhouse at 20-22 °C. After three weeks, nascent telia were observed on inoculated plants.

The ITS region and a segment of *EF1-α* were selected for sequencing for the samples from the first two listed locations. For the ITS, the primers ITS1rustF10d (Barnes and Szabo, 2007) and ITSRu1 (Rioux et al., 2015) were used, and for *EF1-α*, a reaction was conducted following van der Merwe (2007). Amplicons were barcoded and sequenced on an Oxford Nanopore MinIon following manufacturer instructions (kits EXP-PBC001 and SQK-LSK109 with R9 flow cells). Reads (PRJNA802185) were filtered for quality (> Q16), sorted and separated by length, and aligned. A consensus sequence was generated for each

amplicon with >50x coverage. BLAST searches of the EF1-$\alpha$ sequences OM489402 and OM489403 from the first two locations respectively showed 99.3 % (643/647) and 99.7 % (645/647) similarity with *Puccinia glechomatis* (EF560587). ITS sequences had not been reported for *P. glechomatis*, and there are no matches with >96 % homology in GenBank for the sequences OM470970 or OM470969, from the same samples. Morphological and life cycle traits are consistent with this identification.

Creeping Charlie is a common weed of turf, gardens, orchards, forests, and meadows. It is present in 46 of the lower 48 United States but is most common east of the Great Plains, in the Pacific Northwest, and in neighboring regions of southern Canada (Böllman and Scholler, 2004). *P. glechomatis* was recorded for the first time in 1992 in north-central Pennsylvania (Böllmann and Scholler, 2006). Examination of herbarium specimens and surveys established its distribution across the eastern U.S. and in a small area in the Pacific Northwest by the early 2000s, and in 2001 its presence was recorded in southern Wisconsin (Böllmann and Scholler, 2006). The basidiospores likely do not travel far, but the fungus may move long distances through plant matter and establish in new locations (Böllmann and Scholler, 2006). *P. glechomatis* is not known to affect native plants and may have a positive ecological effect, reducing the vigor of its undesirable host. To our knowledge, this is the first report of *P. glechomatis* in Minnesota. It is evidence of the continued westward spread of this rust in North America (Böllmann and Scholler, 2006). Sequenced samples were submitted to the Arthur Fungarium at Purdue University (PUR N24012 and PUR N24013, respectively).

## Rust of lemongrass

In July 2021 and July – Oct. 2022, in a community garden near Mankato, Minnesota, rust disease was observed on lemongrass (*Cymbopogon citriatus*). In 2022, all 20 plants in the garden plot were infected. Lemongrass is used in some Asian cuisines and for tea or medicine. It is not hardy in Minnesota but is grown in gardens and outdoors in small-scale production. Uredinia were cinnamon-brown on the abaxial surface of leaf blades. Pustules were small (0.2 - 0.5 x .01 - .05 mm) and numerous, causing large necrotic lesions and leaf dieback (Fig. 5.2A). Severity ranged from 5 – 50% leaf loss. Urediniospores were finely echinulate, slightly ovular (22 - 25 x 20 - 23 μm), thick-walled (2.5 - 4 μm), with 3 - 4 roughly equatorial, sometimes scattered germ pores (Fig 5.2B; 5.2C). Clavate paraphyses were abundant. Other spore types were not observed. The pycnidia of a mycoparasitic fungus were present within the uredinia. The specimen was submitted to the Arthur Fungarium at Purdue University (PUR N24011).

Primers ITS1rustF10d (Barnes and Szabo, 2007) and ITSRu1 (Rioux et al., 2015) were used to generate amplicons for the rust fungus, and ITS4 and ITSF+ (White, 1990) for the mycoparasite. Amplicons were sequenced on an Oxford Nanopore MinIon with R9 flow cells following manufacturer instructions. Reads (PRJNA802078) were filtered for quality (> Q13) and length (> 200 bp), mapped to reference sequences, aligned, and separated based on similarity. Consensus sequences were generated for the amplicons of the rust fungus and of two other fungi. BLASTn searches of the ITS sequences, OM442990 and OM442991, identified an *Alternaria sp.* (99.8% match (597/598) with MT548677) and *Sphaerellopsis filum* (syn. *Darluca filum*; 98.3% (529/538 bp) match with EF600974),

a common rust mycoparasite. A BLASTn search of the rust fungal ITS sequence (OM442989) yielded 98.9% (549/555) and 98.6% (633/642) match with MT955206 and MT955207, respectively, both *Puccinia cesatii* on *Bothriochloa ischaemum*. The third closest match is *P. cymbopogonis* on *C. citriatus* (97.1% (595/613) with KY764115). Urediniospore morphology is consistent with that of *P. cesatii* (Cummins, 1971). Available evidence suggests the fungus is *P. cesatii* or a closely related species.

*Puccinia cesatii* has been reported infecting *Cymbopogon* spp. (Stevenson, 1926), but lemongrass is not generally considered a host—possibly due to confusion of *P. cesatii* with *P. cymbopogonis*, a closely related rust pathogen of lemongrass that is morphologically very similar to *P. cesatii*. *P. cymbopogonis* has not been reported in the U.S. Rust diseases of lemongrass have been reported in three states: Hawaii (Gardner, 1985), California (Koike and Molinar, 1999), and Florida (Ploetz et al., 2014). In each case the rust was identified as *Puccinia nakanishikii.* Urediniospores of *P. nakanishikii* are larger (26 to 36 μm long) (Cummins, 1971) and the ITS2 has no significant sequence similarity. *P. cesatii* is widespread in Eurasia, the southwest U.S., and Mexico (Cummins, 1971). Cummins lists three genera closely related to *Cymbopogon* as telial hosts of *P. cesatii*: *Bothriochloa, Capillipedium,* and *Dicanthium.* He lists nine rust fungi that infect *Cymbopogon* but does not list *P. cesatii*. Of these nine species, only *P. cymbopogonis* is morphologically similar. Further research is needed to investigate the potential impact of rust fungi on lemongrass production and to elucidate phylogenetic relationships of rust fungi infecting lemongrass.

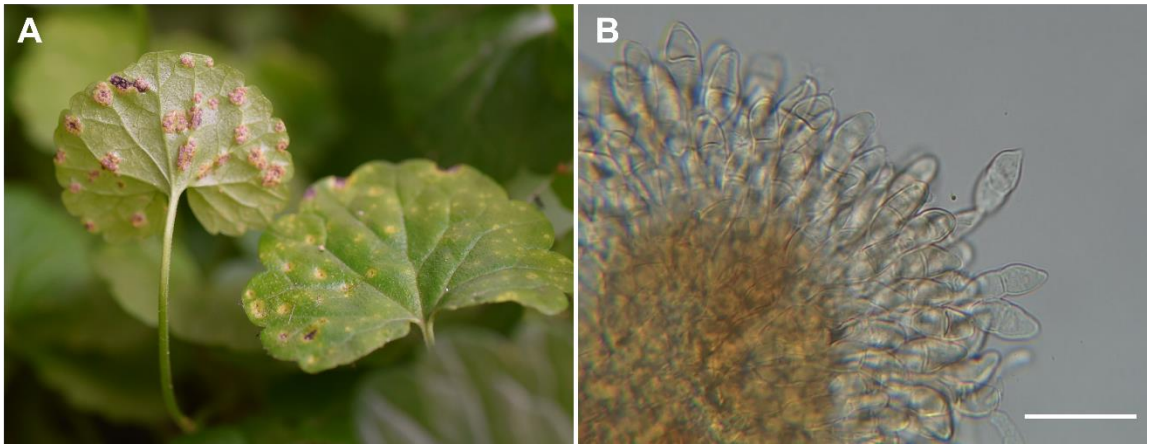


**Figure 5.1.** *Puccinia glechomatis* on creeping Charlie. A) Telia on live plants. B) Leptosporic teliospores. Scale bar = 50 μm. From the same location as sample PUR N24013.

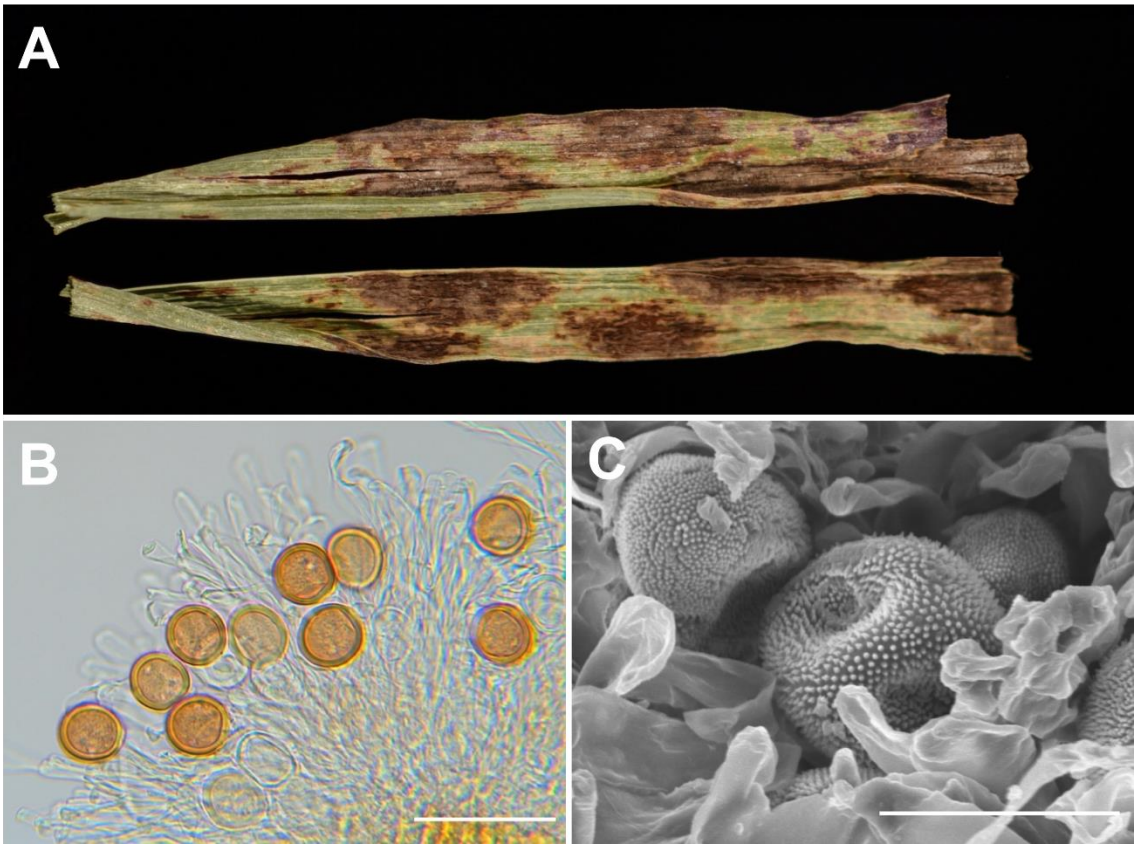


**Figure 5.2.** Images of lemongrass rust. A) Adaxial and abaxial surfaces of a rust infected leaf. B) Urediniospores with abundant paraphyses. Scale bar = 50 μ. Model: Zeiss Standard. Obj. 25x/.6. Sample mounted in lactophenol. C) SEM image of urediniospores. Scale bar = 20 μ. Model: Hitachi S3500N. 10 kV. Sample dried, uncoated. Image by the University of Minnesota Imaging Centers.

## Bibliography


Adams, C. R., and Galatowitsch, S. M. 2006. Increasing the Effectiveness of Reed canary grass (*Phalaris arundinacea* L.) Control in Wet Meadow Restorations. Restoration Ecology. 14:441–451.

Aiello-Lammens, M. E. 2014. Patterns and processes of the invasion of *Frangula alnus*: an integrated model framework (Doctoral dissertation, State University of New York at Stony Brook).

Aime, M. C. 2006. Toward resolving family-level relationships in rust fungi (Uredinales). Mycoscience 47:112–122.

Alaei, H., De Backer, M., Nuytinck, J., Maes, M., Höfte, M., and Heungens, K. 2009. Phylogenetic relationships of *Puccinia horiana* and other rust pathogens of *Chrysanthemum* x *morifolium* based on rDNA ITS sequence analysis. Mycol. Res. 113:668–683.

Allred, K.W. 2007 *Apera.* In: Flora of North America Editorial Committee (Eds. 1993+). Flora of North America: North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 24. Retrieved 26 August 2022 from floranorthamerica.org/Apera.

Anderson, N. O. 2019. Throwing Out the Bathwater but Keeping the Baby: Lessons Learned from Purple Loosestrife and Reed Canarygrass. HortTechnology. 29:539–548.

Anderson, N. O., Smith, A. G., Noyszewski, A. K., Ito, E., Dalbotten, D., and Pellerin, H. 2021. Management and control issues for native, invasive species (reed canarygrass): Evaluating philosophical, management, and legislative issues. HortTechnology 31:354–366.

Angeloni, N. L., Jankowski, K. J., Tuchman, N. C., and Kelly, J. J. 2006. Effects of an invasive cattail species (*Typha* × *glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. FEMS Microbiol Lett. 263:86–92.

Anikster, Y. 1984. The *formae speciales*. In: Bushnell, W. R., and Roelfs, A. P. (Eds.), The Cereal Rusts. Vol 1. Academic Press, Orlando, Florida.

Ayliffe, M., Singh, D., Park, R., Moscou, M., and Pryor, T. 2013. Infection of *Brachypodium distachyon* with selected grass rust pathogens. Mol. Plant-Microbe Interactions 26:946–957.

Barkworth, M.E. 2007. *Phalaris*. In: Flora of North America Editorial Committee (Eds. 1993+). Flora of North America: North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 24. Retrieved 14 February 2022 from floranorthamerica.org/Phalaris.

Barnes, C. W., and Szabo, L. J. 2007. Detection and identification of four common rust pathogens of cereals and grasses using real-time polymerase chain reaction. Phytopathology 97:717–727.

Barney, J. N., Tekiela, D. R., Dollete, E. S., and Tomasek, B. J. 2013. What is the "real" impact of invasive plant species? Frontiers Ecol. Environ. 11:322–329.

Barton, J. 2012. Predictability of pathogen host range in classical biological control of weeds: an update. BioControl. 57:289–305.

Beaury, E. M., Patrick, M., and Bradley, B. A. 2021. Invaders for sale: the ongoing spread of invasive species by the plant trade industry. Frontiers Ecol.Environ. 19:550–556.

Beenken, L., Lutz, M., and Scholler, M. 2017. DNA barcoding and phylogenetic analyses of the genus *Coleosporium* (Pucciniales) reveal that the North American goldenrod rust *C. solidaginis* is a neomycete on introduced and native *Solidago* species in Europe. Mycol Prog. 16:1073–1085.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., and Courchamp, F. 2012. Impacts of climate change on the future of biodiversity. Ecol. Lett. 15:365–377.

Beste, C. E., Frank, J. R., Bruckart, W. L., Johnson, D. R., and Potts, W. E. 1992. Yellow Nutsedge (*Cyperus esculentus*) Control in Tomato with *Puccinia canaliculata* and Pebulate. Weed Technology. 6:980–984.

Böllmann, J., and Scholler, M. 2006. Life cycle and life strategy features of *Puccinia glechomatis* (Uredinales) favorable for extending the natural range of distribution. Mycoscience. 47:152–158.

Bonos, S. A., Clarke, B. B., and Meyer, W. A. 2006. Breeding for disease resistance in the major cool-season turfgrasses. Annual Review of Phytopathology 44: 213–234.

Brown, M. R. 1937. A study of crown rust, *Puccinia coronata* Corda, in Great Britain: Physiologic specialization in the uredospore stage. Annals of Applied Biology, 24:504–527.

Burnham, K. M., and Lee, T. D. 2010. Canopy gaps facilitate establishment, growth, and reproduction of invasive *Frangula alnus* in a Tsuga canadensis dominated forest. Biol. Invasions 12:1509–1520.

Bushnell, B. 2014. BBTools. Retrieved from http://sourceforge. net/projects/bbmap

Carleton, M.A., 1899. Vol 16 in: "Cereal Rusts of the United States." USDA, Washington, D.C.

Carlson, I. T., Oram, R. N., and Surprenant, J. 1996. Reed Canarygrass and Other Phalaris Species. In: Cool-Season Forage Grasses. John Wiley & Sons, Ltd.

Carnegie, A. J., Kathuria, A., Pegg, G. S., Entwistle, P., Nagel, M., and Giblin, F. R. 2016. Impact of the invasive rust *Puccinia psidii* (myrtle rust) on native Myrtaceae in natural ecosystems in Australia. Biol Invasions. 18:127–144.

Casler, M. D., and Duncan, R. R. 2003. Origins of the Turfgrasses. In Casler, M. D., & Duncan, R. R., (Eds.), Turfgrass Biology, Genetics, and Breeding. John Wiley & Sons, Inc. New York, NY. pp. 5–23.

Catford, J. A., Jansson, R., and Nilsson, C. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers. Distrib. 15:22–40.

Catling, P. M. and Porebski, Z. S. 1994. The history of invasion and current status of glossy buckthorn, *Rhamnus frangula*, in southern Ontario. Can. Field-Nat.108:305-310.

Clark, L.G. 2007. *Lamarckia*. In: Flora of North America Editorial Committee (Eds. 1993+). Flora of North America: North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 24. Retrieved 26 August 2022 from floranorthamerica.org/Lamarckia.

Colautti, R. I., Ricciardi, A., Grigorovich, I. A., and MacIsaac, H. J. 2004. Is invasion success explained by the enemy release hypothesis? Ecol. Lett. 7:721–733.

Corda, A. C. I. 1837. Icones fungorum hucusque cognitorum. Apud. J. G. Calve, Prague, Czechia.

Cummins, G. B. 1971. The Rust Fungi of Cereals, Grasses, and Bamboos. Springer-Verlag, New York.

Davis, J. I. & Consaul, L. L. 2007. *Puccinellia*. In: Flora of North America Editorial Committee (Eds. 1993+). Flora of North America: North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 24. Retrieved 26 August 2022 from floranorthamerica.org/Puccinellia.

De Kort, H., Mergeay, J., Jacquemyn, H., and Honnay, O. 2016. Transatlantic invasion routes and adaptive potential in North American populations of the invasive glossy buckthorn, *Frangula alnus*. Annals of Botany. 118:1089–1099.

Delgado, N. J., Grau, C. R., and Casler, M. D. 2001. Host range and alternate host of a *Puccinia coronata* population from smooth brome grass. Plant Dis. 85:513–516.

Dietz, S. M. 1926. The alternate hosts of crown rust, *Puccinia coronata* Corda. J. Agric. Res. 33:935–970.

Dukes, J. S., and Mooney, H. A. 2004. Disruption of ecosystem processes in western North America by invasive species. Revista chilena de historia natural. 77:411–437.

EDDMapS. 2022. Early Detection & Distribution Mapping System. The University of Georgia - Center for Invasive Species and Ecosystem Health. Retrieved 8 April 2022 from http://www.eddmaps.org/

Erikkson, J. 1898. A general review of the principal results of Swedish research into grain rust. Bot. gaz. 25: 26-38

Eshed, N., and Dinoor, A. 1980. Genetics of pathogenicity in *Puccinia coronata*: Pathogenic Specialization at the Host Genus Level. Phytopathology. 70:1042–1046.

Eshed, N., and Dinoor, A. 1981. Genetics of pathogenicity in *Puccinia coronata*: The host range among grasses. Phytopathology, 71:156–163.

Fagan, M. E., and Peart, D. R. 2004. Impact of the invasive shrub glossy buckthorn (*Rhamnus frangula* L.) on juvenile recruitment by canopy trees. For. Ecol. Manag. 194:95–107.

Farr, D.F., and Rossman, A.Y. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved 10 February 2022 from https://nt.ars-grin.gov/fungaldatabases/

Fernandez Winzer, L., Berthon, K. A., Carnegie, A. J., Pegg, G. S., and Leishman, M. R. 2019. *Austropuccinia psidii* on the move: survey-based insights to its geographical distribution, host species, impacts and management in Australia. Biol. Invasions. 21:1215–1225.

Fiedler, A. K., and Landis, D. A. 2012. Biotic and Abiotic Conditions in Michigan Prairie Fen Invaded by Glossy Buckthorn (*Frangula alnus*). Nat. Areas J. 32:41–53.

Figueroa, M., Alderman, S., Garvin, D. F., and Pfender, W. F. 2013. Infection of *Brachypodium distachyon* by *formae speciales* of *Puccinia graminis*: Early infection events and host-pathogen incompatibility. PLoS ONE 8: p.e56857.

Frappier, B., Eckert, R. T., and Lee, T. D. 2003. Potential impacts of the invasive shrub *Rhamnus frangula* L. (glossy buckthorn) on forests of southern New Hampshire. Northeast. Nat. 10:277–296.

Fraser, W. P., and Ledingham, G. A. 1933. Studies of the crown rust, *Puccinia coronata* Corda. Sci. Agric 13:313–323.

Freire, M. C. M., Oliveira, L. O. de, Almeida, Á. M. R. de, Schuster, I., Moreira, M. A., Liebenberg, M. M., and Mienie, C. M. S. 2008. Evolutionary history of *Phakopsora pachyrhizi* (the Asian soybean rust) in Brazil based on nucleotide sequences of the internal transcribed spacer region of the nuclear ribosomal DNA. Gen. Mol. Biol. 31:920–931.

Galatowitsch, S. M., Anderson, N. O., and Ascher, P. D. 1999. Invasiveness in wetland plants in temperate North America. Wetlands 19:733–755.

Gardner, D. E. 1985. Lemongrass rust caused by *Puccinia nakanishikii* in Hawaii. Plant Dis. 69:1100.

Graper, A. L., Noyszewski, A. K., Anderson, N. O., and Smith, A. G. 2021. Variability in ITS1 and ITS2 sequences of historic herbaria and extant (fresh) *Phalaris* species (Poaceae). BMC Plant Biology. 21:515.

Greatens, N., Klejeski, N., Szabo, L., Jin, Y., and Olivera Firpo, P. D. 2022. *Puccinia coronata* var. *coronata*, a crown rust pathogen of two highly invasive species, is detected across the Midwest and Northeastern United States. Plant Dis. In press.

Green, E. K., and Galatowitsch, S. M. 2001. Differences in wetland plant community establishment with additions of nitrate-N and invasive species (*Phalaris arundinacea* and *Typha* ×*glauca*). Can. J. Bot. 79:170–178.

Haghdoust, R., Singh, D., Park, R. F., and Dracatos, P. M. 2021. Characterizing the genetic architecture of nonhost resistance in barley using pathogenically diverse *Puccinia* isolates. Phytopathology 111:684–694.

Hambleton S., Liu M., Eggertson Q., Wilson S., Carey J., Anikster Y. and Kolmer J.A. 2019 Crown rust fungi with short lifecycles – the *Puccinia mesnieriana* species complex. Sydowia 71:47–63.

Hamelin, C., Truax, B., and Gagnon, D. 2016. Invasive glossy buckthorn impedes growth of red oak and sugar maple under-planted in a mature hybrid poplar plantation. New Forests 47:897–911.

Holmes, T. P., Aukema, J. E., Von Holle, B., Liebhold, A., and Sills, E. 2009. Economic impacts of invasive species in forests. Ann. N. Y. Acad. Sci. 1162:18–38.

Huebner, C. D., Morin, R. S., Zurbriggen, A., White, R. L., Moore, A., and Twardus, D. 2009. Patterns of exotic plant invasions in Pennsylvania's Allegheny National Forest using intensive forest inventory and analysis plots. For. Ecol. Manag. 257:258–270.

Hunt, R. S. 2003. White pine blister rust. Recent Res. Dev. Mycol. 1:73-85.

Hutchings, M. J., and Price, E. A. C. 1999. *Glechoma hederacea* L. (*Nepeta glechoma* Benth., N. *hederacea* (L.) Trev.). J. Ecol. 87:347–364.

iNaturalist contributors and iNaturalist, 2022. iNaturalist Research Grade Observations. Retrieved 8 March 2022 from https://www.inaturalist.org/taxa/1057525

Jakubowski, A., Jackson, R., and D. Casler, M. 2012. Genetic evidence suggests a widespread distribution of native North American populations of reed canarygrass. Biological Invasions. 15.

Ji, J., Li, Z., Li, Y., and Kakishima, M. 2022. Phylogenetic approach for identification and life cycles of *Puccinia* (Pucciniaceae) species on Poaceae from northeastern China. Phytotaxa 533:1-48.

Jin, Y., and Steffenson, B. J. 1999. *Puccinia coronata* var. *hordei* var. nov.: Morphology and pathogenicity. Mycologia 91:877–884.

Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R., and Fetch, T. 2008. Detection of virulence to resistance gene Sr24 within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92:923–926.

Jones, D. R., and Baker, R. H. A. 2007. Introductions of non-native plant pathogens into Great Britain, 1970–2004. Plant Pathol. 56:891–910.

Jørstad, I. 1940. Uredinales of Northern Norway. Norske vidensk 1940:1-145.

Jørstad, I. 1964. The distribution within Norway of rust fungi (Uredinales) compared with the distribution of their hosts. Nytt. Mag. Bot. 11: 109-141.

Kartesz, J. T., and The Biota of North America Program (BONAP). 2015. North American Plant Atlas. Chapel Hill, N.C. Retrieved 28 February 2022 from http://bonap.net/napa.

Katoh, K., and Standley, D. M. (2013). MAFFT Multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30:772–780.

Keane, R. M., and Crawley, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecol. Evol. 17:164–170.

Kenaley, S. C., Ecker, G., and Bergstrom, G. C. 2017. First report of *Puccinia coronata* var. *coronata sensu stricto* infecting alder buckthorn in the United States. Plant Health Prog. 18:84–86.

Kercher, S. M., and Zedler, J. B. 2004. Multiple disturbances accelerate invasion of reed canary grass (*Phalaris arundinacea* L.) in a mesocosm study. Oecologia. 138:455–464.

Klebahn, H. 1894. Kulturversuche mit heteröcischen Uredineen. Zeitschrift Für Pflanzenkrankheiten 4:29–139.

Koike, S. T., and Molinar, R. H. 1999. Rust disease on lemongrass in California. Plant Dis. 83:304–304.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33:1870–1874.

Langmead, B., and Salzberg, S. L. 2012. Fast gapped-read alignment with bowtie 2. Nat. Methods 9:357–359.

Larson, E. R., Graham, B. M., Achury, R., Coon, J. J., Daniels, M. K., Gambrell, D. K., Jonasen, K. L., King, G. D., LaRacuente, N., Perrin-Stowe, T. I., Reed, E. M., Rice, C. J., Ruzi, S. A., Thairu, M. W., Wilson, J. C., and Suarez, A. V. 2020. From eDNA to citizen science: Emerging tools for the early detection of invasive species. Front. Ecol. Environ. 18:194–202.

Lavergne, S., and Molofsky, J. 2004. Reed canary grass (*Phalaris arundinacea*) as a biological model in the study of plant invasions. Crit. Rev. Plant Sci. 23:415–429.

Lavergne, S., and Molofsky, J. 2006. Control strategies for the invasive reed canarygrass (*Phalaris arundinacea* L.) in North American wetlands: the need for an integrated management plan. Nat. Areas. J. 26:208–214.

Lavoie, C., Dufresne, C., and Delisle, F. 2005. The spread of reed canarygrass (*Phalaris arundinacea*) in Québec: A spatio-temporal perspective. Écoscience. 12:366–375.

Lindner, D. L., and Banik, M. T. 2011. Intragenomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in genus *Laetiporus*. Mycologia 103:731–740.

Liu, M., and Hambleton, S. 2010. Taxonomic study of stripe rust, *Puccinia striiformis sensu lato*, based on molecular and morphological evidence. Fungal Biology. 114:881–899.

Liu, M., and Hambleton, S. 2013. Laying the foundation for a taxonomic review of *Puccinia coronata s.l.* in a phylogenetic context. Mycol. Prog. 12:63–89.

Liu, M., Szabo, L. J., Hambleton, S., Anikster, Y., and Kolmer, J. A. 2013. Molecular phylogenetic relationships of the brown leaf rust fungi on wheat, rye, and other grasses. Plant Dis. 97:1408–1417.

Maharjan, S., Devkota, A., Shrestha, B. B., Baniya, C. B., Rangaswamy, M., and Jha, P. K. 2020. Prevalence of *Puccinia abrupta* var. *partheniicola* and its impact on *Parthenium hysterophorus* in Kathmandu Valley, Nepal. J. Ecol.Environ. 44:25.

Maleszka, R., and Clark-Walker, G. D. 1990. Magnification of the rDNA cluster in *Kluyveromyces lactis*. Mol. Gen. Genet. 223:342–344.

Mann, C. C. 2011. 1493: Uncovering the new world Columbus created. Albert Knopf. New York, NY.

Marr, K.L., Hebda, R.J., Greenef, C. W. 2007. *Calamagrostis*. In: Flora of North America Editorial Committee (Eds. 1993+). Flora of North America: North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 24. Retrieved 26 August 2022 from floranorthamerica.org/Calamagrostis.

Marvier, M., Kareiva, P., and Neubert, M. G. 2004. Habitat destruction, fragmentation, and disturbance promote invasion by habitat generalists in a multispecies metapopulation. Risk Anal. 24:869–878.

McTaggart, A. R., Shivas, R. G., Nest, M. A. van der, Roux, J., Wingfield, B. D., and Wingfield, M. J. 2016. Host jumps shaped the diversity of extant rust fungi (Pucciniales). New Phytologist 209:1149–1158.

Melhus, I. E., Dietz, S. M., and Willey, F. 1922. The alternate hosts of crown rust, *Puccinia coronata* Corda. Pages 209-237 Vol 72 in: Iowa Agricultural Experiment Station Research Bulletin, Ames, IA.

Miller, A. N., and Bates, S. T. 2017. The Mycology Collections Portal (MyCoPortal). IMA Fungus 8:A65–A66.

Minnesota Department of Agriculture. 2023. Minnesota noxious weed list. Online. MN Dept. of Agriculture, St. Paul. MN. Retrieved 14 May 2023 from https://www.mda.state.mn.us/plants/pestmanagement/weedcontrol/noxiouslist/glossybuckthorn

Minnesota Department of Natural Resources (MNDNR) Division of Ecological Resources. 2008. Rare species guide: an online encyclopedia of Minnesota's rare native plants and animals. Minnesota Department of Natural Resources, St. Paul, Minnesota. Retrieved 17 November 2022 from www.dnr.state.mn.us/rsg/index.html.

Moore, J. W. 2017. The Capitalocene, Part I: on the nature and origins of our ecological crisis. J. Peasant Stud. 44:594–630.

Morin, L. 2020. Progress in biological control of weeds with plant pathogens. Annual Rev. Phytopathology. 58:201–223.

Mueller, K. E., Lodge, A. G., Roth, A. M., Whitfeld, T. J. S., Hobbie, S. E., and Reich, P. B. 2018. A tale of two studies: Detection and attribution of the impacts of invasive plants in observational surveys. J. App. Ecol. 55:1780–1789.

Mulhouse, J. M., and Galatowitsch, S. M. 2003. Revegetation of prairie pothole wetlands in the mid-continental US: twelve years post-reflooding. Plant Ecology. 169:143–159.

Murphy, H.C. 1935. Physiologic specialization in *Puccinia coronata avenae*. US Dep. of Agri. Tech. Bull. 433:1-48

Nazareno, E. S., Li, F., Smith, M., Park, R. F., Kianian, S. F., and Figueroa, M. 2018. *Puccinia coronata* f. sp. *avenae*: A threat to global oat production. Mol. Plant Path. 19:1047–1060.

Newcombe, G., and Dugan, F. M. 2010. Fungal pathogens of plants in the homogocene. In Molecular Identification of Fungi, eds. Youssuf Gherbawy and Kerstin Voigt. Berlin, Heidelberg: Springer Berlin Heidelberg, p. 3–34.

Newcombe, G., R. Gaylord, J. P. Yenish, J. Mastrogiuseppe, and F. M. Dugan. 2009. New records for pathogenic fungi on weedy or non-indigenous plants. North Am. Fungi 4: 1-12.

Niu, Z., Puri, K.D., Chao, S., Jin, Y., Sun, Y., Steffenson, B.J., Maan, S.S., Xu, S.S., and Zhong, S. 2014. Genetic analysis and molecular mapping of crown rust resistance in common wheat. Theor. Appl Genet. 127:609–619.

Noyszewski, A. K., Anderson, N. O., Smith, A. G., Kilian, A., Dalbotten, D., Ito, E., et al. 2021. Riparian populations of minnesota reed canarygrass (*Phalaris arundinacea*) are most likely native, based on SNPs (DArTseqLD). Wetlands Ecol Manage. 29:467–494.

Noyszewski, A. K., Anderson, N. O., Smith, A. G., Kilian, A., Dalbotten, D., Ito, E., Timm, A., Pellerin, H., Kubátová, B., Kávová, T., Januš, V., Čurn, V., Edwards, K. R., Bastlová, D., and Květ, J. 2021. Riparian populations of Minnesota reed canarygrass (*Phalaris arundinacea*) are most likely native, based on SNPs (DArTseqLD). Wetlands Ecol. Manage. 29: 467–494.

Olivera, P. D., Bulbula, W. D., Badebo, A., Bockleman, H. E., Edae, E. A., and Jin, Y. 2021. Field resistance to wheat stem rust in durum wheat (*Triticum turgidum* ssp. *durum*) accessions deposited at the USDA National Small Grains Collection. Crop Sci. 61: 2565-2578.

Olivera, P. D., Pretorius, Z. A., Badebo, A., and Jin, Y. 2013. Identification of resistance to races of *Puccinia graminis* f. sp. *tritici* with broad virulence in triticale (x *Triticosecale*). Plant Dis. 97:479-484.

Omidvar, V., Dugyala, S., Li, F., Rottschaefer, S.M., Miller, M.E., Ayliffe, M., Moscou, M.J., Kianian, S.F., and Figueroa, M. 2018. Detection of race-specific resistance against *Puccinia coronata* f. sp. *avenae* in *Brachypodium* species. Phytopathology 108:1443–1454.

Paloi, S., Mhuantong, W., Luangsa-ard, J. J., & Kobmoo, N. 2021. Using high-throughput amplicon sequencing to evaluate intragenomic variation and accuracy in species identification of *Cordyceps* species. J. Fungi 7:767.

Parker, A., Holden, A. N. G., and Tomley, A. J. 1994. Host specificity testing and assessment of the pathogenicity of the rust, *Puccinia abrupta* var. *partheniicola*, as a biological control agent of Parthenium weed (*Parthenium hysterophorus*). Plant Pathol. 43:1–16.

Pegg, G., Taylor, T., Entwistle, P., Guymer, G., Giblin, F., and Carnegie, A. 2017. Impact of *Austropuccinia psidii* (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland. PLOS ONE. 12:e0188058.

Perry, L. G., Galatowitsch, S. M., and Rosen, C. J. 2004. Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. Journal of Applied Ecology. 41:151–162.

Peturson B. 1954. The relative prevalence of specialized forms of *Puccinia coronata* that occur on *Rhamnus cathartica* in Canada. Can J Bot 32:40–47

Phatak, S. C., Sumner, D. R., Wells, H. D., Bell, D. K., and Glaze, N. C. 1983. Biological control of yellow nutsedge with the indigenous rust fungus *Puccinia canaliculata*. Science. 219:1446–1447.

Ploetz, R., Palmateer, A., Lopez, P., and Aime, M. 2014. First report of rust caused by *Puccinia nakanishikii* on lemongrass, *Cymbopogon citratus*, in Florida. Plant Dis. 98:156–156.

Rambaut, A. 2009. FigTree, version 1.4.34. http://tree.bio.ed.ac.uk/software/figtree/

Rioux, S., Mimee, B., Gagnon, A.-È., and Hambleton, S. 2015. First report of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat in Quebec, Canada. Phytoprotection 95:7–9.

Rull, V. 2017. The "Anthropocene": neglects, misconceptions, and possible futures. EMBO Rep. 18:1056–1060.

Samarakoon, T., Wang, S. Y., and Alford, M. H. 2013. Enhancing PCR amplification of DNA from recalcitrant plant specimens using a trehalose-based additive. Appl. Plant Sci. 1:1200236

Scherer-Lorenzen, M., Venterink, H. O., and Buschmann, H. 2007. Nitrogen enrichment and plant invasions: the importance of nitrogen-fixing plants and anthropogenic eutrophication. In: Wolfgang Nentwig (ed.). *Biological Invasions*. *Ecological Studies.* Berlin, Heidelberg: Springer, p. 163–180.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Scholler, M., and Böllmann, J. 2004. *Glechoma hederacea* (Lamiaceae) in North America: invasion history and current distribution. Feddes Repertorium 115:178-188.

Simberloff, D., and Von Holle, B. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biol. Invasions. 1:21–32.

Soreng, R.J., Peterson, P.M., Romaschenko, K., Davidse, G., Zuloaga, F.O., Judziewicz, E.J., Filgueiras, T.S., Davis, J.I., and Morrone, O. 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). J. of Systematics and Evol. 53:117–137.

Stadler, M., Lambert, C., Wibberg, D., Kalinowski, J., Cox, R. J., Kolařík, M., and Kuhnert, E. 2020. Intragenomic polymorphisms in the ITS region of high-quality genomes of the Hypoxylaceae (Xylariales, Ascomycota). Mycological Prog. 19:235–245.

Stevenson, J. A. 1926. Stevenson, J. A. 1926. Foreign plant diseases: A manual of economic plant diseases which are new to or not widely distributed in the United States. U.S. Government Printing Office, Washington, D.C.

Stokdyk, J. P., and Herrman, K. S. 2016. Effects of *Frangula alnus* on soil microbial communities and biogeochemical processes in Wisconsin forests. Plant Soil. 409:65–75.

Straib W. 1941 Weitere Beitrage zur Kenntnis der Specialisierung der Getreideroste und des Leinrostes. Arb. Biol. Reichsanst 23:233-263

Sturtevant, R., K. Dettloff, W. Conard, and Morningstar, C. 2023. *Phalaris arundinacea* L.: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL. Retrieved 27 April 2023 from https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2938

Szabo, L. J. 2006. Deciphering species complexes: *Puccinia andropogonis* and *Puccinia coronata*, examples of differing modes of speciation. Mycoscience 47:130–136.

Thomson, S. V., & Kropp, B. R. 2004. Production of *Puccinia thlaspeos* 'woad' strain inoculum using traditional farming equipment (abstract). Phytopathology, 94:6.

Thuiller, W., Richardson, D. M., and Midgley, G. F. 2007. Will climate change promote alien plant invasions? In: Wolfgang Nentwig (ed.). *Biological Invasions. Ecological Studies*. Vol. 193. Springer, Berlin, Heidelberg. p. 197–211.

Tilman, D. 1994. Competition and Biodiversity in Spatially Structured Habitats. Ecology. 75:2–16.

Uchida, J., Zhong, S., and Killgore, E. 2006. First report of a rust disease on Ohia caused by *Puccinia psidii* in Hawaii. Plant Dis. 90:524–524.

Urban, Z. 1963. A new method for observing urediospore germ-pores and its use in the taxonomy of graminicolous rust species. Ceska Mykol 17: 193–194.

Urban, Z. 1967. The taxonomy of some European graminicolous rusts. Ceska mykologie, 21:12-16.

Urban, Z., and Marková, J. 1993. The rust fungi of grasses in Europe. I. *Puccinia coronata* Corda. Acta Univ. Carol. Biol. 37:93–147.

USDA FEIS, 2023. *Phalaris arundinacea*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory (Producer). Retrieved 16 May 2023 from www.fs.usda.gov/database/feis/plants/fern/polmun/all.html

Valéry, L., Fritz, H., Lefeuvre, J.-C., and Simberloff, D. 2008. In search of a real definition of the biological invasion phenomenon itself. Biol Invasions. 10:1345–1351.

van der Merwe, M., Ericson, L., Walker, J., Thrall, P. H., and Burdon, J. J. 2007. Evolutionary relationships among species of *Puccinia* and *Uromyces* (Pucciniaceae, Uredinales) inferred from partial protein coding gene phylogenies. Mycol. Res. 111:163–175.

Virtudazo, E. V., Nakamura, H., and Kakishima, M. 2001. Phylogenetic Analysis of Sugarcane Rusts Based on Sequences of ITS, 5.8 S rDNA and D1/D2 Regions of LSU rDNA. J. Gen. Plant Path. 67:28–36.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., et al. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7:737–750.

Vydryakova, G. A., Van, D. T., Shoukouhi, P., Psurtseva, N. V., and Bissett, J. 2012. Intergenomic and intragenomic ITS sequence heterogeneity in *Neonothopanus nambi* (Agaricales) from Vietnam. Mycology 3:89–99.

White T.J., Bruns T., Lee S.J., and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protoc. 18:315-22.

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., and Losos, E. 1998. Quantifying threats to imperiled species in the United States. BioScience. 48:607–615.

Winston, R. L., Schwarzländer, M., Hinz, H. L., Day, M. D., Cock, M. J. W., and Julien, M. H. eds. 2014. Biological control of weeds: a world catalogue of agents and their target weeds. 5th ed. USDA For. Serv. For. Health. Enterp. Team. Morgantown, WV.

Wisconsin Reed Canary Grass Management Working Group. 2009. Reed Canary Grass (*Phalaris arundinacea*) Management Guide: Recommendations for Landowners and Restoration Professionals. University of Wisconsin Extension Services. Madison, WI.

Wrobel, C., Coulman, B. E., and Smith, D. L. 2009. The potential use of reed canarygrass (*Phalaris arundinacea* L.) as a biofuel crop. Acta Agriculturae Scandinavica 59:1–18.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. 2012. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13:1-11.

Zecchin, B., Caudullo, G., de Rigo, D., 2016. *Frangula alnus* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg. pp. e019ee2+.

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. 2000. A greedy algorithm for aligning DNA sequences. J. Comp. Biol. 7:203–214.

# Appendix:

# Supplementary tables

**Supplementary Table 1.1.** Primers used in chapter one.

| Primer | Locus | Sequence (5'-3') | Reference: |
|---|---|---|---|
| ITS1rustF10d | ITS | TGAACCTGCAGAAGGATCATTA | Barnes and Szabo (2007) |
| Rust2inv (F) | ITS | GATGAAGAACACAGTGAAA | Aime (2006) |
| ITSRu1 (R) | ITS | GCCTTAGATGGAATTTACCACCC | Hambleton et al (2019), Rioux et al. (2015) |
| P360f | *COI* | GCTAAGGATATTGCCATTCTATAT | Liu and Hambleton (2013) |
| P360r | *COI* | TCCATCCYGTYCCTGCYCC | Liu and Hambleton (2013) |
| Btub_Pc_F | β-*tubulin* | CCCTACAACGCCACCTTGTC | This study (Chapter 1) |
| Btub_Pc_R | β-*tubulin* | GTGTACCAATGCAGGAAGGC | This study (Chapter 1) |
| RPB2-187f | *RPB2* | CGATCCTGTGYTAYTCGGGMTAYAACCA | Liu and Hambleton (2013) |
| RPB2-492f | *RPB2* | CGGATGAAGACRCAYACKAARCG | Liu and Hambleton (2013) |
| RPB2-853r2 | *RPB2* | GCATCRCCYTCRTTVCCKGWG | Liu and Hambleton (2013) |

**Supplementary Table 1.2**. Coverage of consensus sequences of *Puccinia coronata* var. *coronata* samples evaluated in this study

| | Average coverage | | | |
|---|---|---|---|---|
| | **ITS** | **COI** | **RPB2** | **B-TUB** |
| 18_NH_Pa01 | 152 | 79 | 160 | 161 |
| 18_WI_Pa01-1 | 589 | 42 | 69 | 142 |
| 19_IA_Pa01 | 315 | 134 | 182 | 66 |
| 19_MN_Pa01 | 2234 | 119 | - | - |
| 19_ND_Fa01 | 2347 | 144 | - | - |
| 19_OH_Pa01-1 | 474 | 127 | 104 | 181 |
| 19_OH_Pa02 | 572 | 97 | 63 | 126 |
| 19_WI_Pa01 | 1378 | 552 | - | - |
| 20_IL_Fa01 | 1642 | 776 | - | - |
| 20_MN_Fa01-1 | 1115 | 13 | 12 | 51 |
| 20_MN_Fa02-1 | 354 | 40 | 72 | 32 |
| 20_MN_Pa01 | 1424 | 245 | - | - |
| 21_IN_Fa01 | 9554 | 9811 | - | - |
| 21_MA_Fa01 | 1378 | 1959 | - | - |
| 21_Ma_Fa02 | 698 | 698 | - | - |
| 21_MI_Fa01 | 1373 | 517 | - | - |
| 21_MI_Fa02 | 1758 | 370 | - | - |
| 21_OH_Fa01 | 993 | 1272 | - | - |
| 21_PA_Fa01 | 2530 | 2324 | - | - |
| 21_PA_Fa02 | 2157 | 2146 | - | - |
| 21_VT_Fa01 | 1768 | 1502 | - | - |
| 22_NY_Pa01 | 72 | 20 | 111 | 50 |

**Supplementary Table 1.3.** ITS variants and coverage of four *Puccinia coronata* var. *coronata* isolates.

| Isolate | ITS variant (named for SNPs) | % of reads (reads that span all four SNPs) | Coverage | Positions of SNPs |
|---|---|---|---|---|
| 18_WI_Pa01-1 | TGCC | 28.1 | 79 | 32, 33, 504, and 580 |
| | TGTA | 41.1 | 116 | 32, 33, 505, and 581 |
| | TGTC | 4 | 11 | 32, 33, 504, and 580 |
| 19_OH_Pa01-1 | CGCC | 13.5 | 29 | 32, 33, 504, and 580 |
| | CGTA | 21 | 46 | 32, 33, 504, and 580 |
| | TGCC | 34.1 | 73 | 32, 33, 503, and 579 |
| | TGTA | 8.7 | 19 | 32, 33, 503, and 578 |
| 20_MN_Fa01-1 | TACC | 26.8 | 142 | 32, 33, 503, and 579 |
| | TGCA | 2.5 | 13 | 32, 33, 503, and 579 |
| | TGTA | 40.4 | 213 | 32, 33, 503, and 579 |
| | TGTC | 5.7 | 30 | 32, 33, 504, and 580 |
| | CACC | 2.6 | 14 | 32, 33, 503, and 579 |
| 20_MN_Fa02-1 | CACC | 14.7 | 26 | 32, 33, 504, and 580 |
| | CATA | 26.8 | 48 | 32, 33, 504, and 580 |
| | TGCC | 8.9 | 16 | 32, 33, 504, and 580 |
| | TGTA | 11.6 | 22 | 32, 33, 504, and 580 |

**Supplementary Table 1.4.** Results of local BLAST searches for *Puccinia coronata* var. *coronata* sequences generated in this study.

| | ITS | | | *COI* | | |
|---|---|---|---|---|---|---|
| **Sample** | **Closest match(es)** | **% match** | **(XX/XX)** | **Closest match(es)** | **% match** | **(XX/XX)** |
| 18_NH_Pa01 | 18_WI_Pa01-1 | 99.8 | 881/883 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 19_IA_Pa01 | 20_MN_Fa01-1, 19_OH_Pa01-1 | 99.4 | 879/884 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 19_MN_Pa01 | KY426917; KY426916 | 99.8 | 614/615 | 20_MN_Fa02-1 | 100 | 309/309 |
| 19_ND_Fa01 | KY426917; KY426916 | 99.7 | 613/615 | 20_MN_Fa02-1 | 100 | 309/309 |
| 19_OH_Pa02 | 18_WI_Pa01-1 | 99.3 | 878/884 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 19_WI_Pa01 | 20_MN_Fa01-1 | 99.5 | 879/883 | 20_MN_Fa02-1 | 100 | 309/309 |
| 20_IL_Fa01 | 20_MN_Fa01-1 | 99.8 | 881/883 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 20_MN_Pa01 | 20_MN_Fa01-1 | 99.5 | 879/883 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 21_IN_Fa01 | KY426917; KY426916 | 100 | 615/615 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 21_MA_Fa01 | 20_MN_Fa01-1 | 100 | 615/615 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 21_MA_Fa02 | 20_MN_Fa01-1 | 99.6 | 880/883 | 20_MN_Fa02-1 | 100 | 309/309 |
| 21_MI_Fa01 | 20_MN_Fa01-1; 19_OH_Pa01-1 | 99.6 | 613/614 | 20_MN_Fa02-1 | 100 | 309/309 |
| 21_MI_Fa02 | 20_MN_Fa01-1; 19_OH_Pa01-1 | 99.6 | 881/884 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |

## Supplementary Table 1.4. continued

| Sample | ITS | | | *COI* | | |
|---|---|---|---|---|---|---|
| | **Closest match(es)** | **% match** | **(XX/XX)** | **Closest match(es)** | **% match** | **(XX/XX)** |
| 21_OH_Fa01 | KY426917; KY426916 | 100 | 615/615 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 21_PA_Fa01 | KY426917; KY426916 | 100 | 615/615 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 21_PA_Fa02 | 20_MN_Fa01-1 | 99.6 | 613/615 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 21_VT_Fa01 | KY426917; KY426916 | 99.8 | 614/615 | 18_WI_Pa01-1, HM147427, others | 100 | 309/309 |
| 22_NY_Pa01 | 20_MN_Fa01-1 | 99.5 | 881/883 | 18_WI_Pa01-1, HM147427, others | 99.7 | 308/309 |

## Supplementary Table 1.4. continued

| Sample | β-*tubulin* | | | *RPB2* | | |
|---|---|---|---|---|---|---|
| | **Closest match(es)** | **% match** | **(XX/XX)** | **Closest match(es)** | **% match** | **(XX/XX)** |
| 18_NH_Pa01 | - | - | - | - | - | - |
| 19_IA_Pa01 | 20_MN_Fa02-1 | 99.7 | 696/698 | 20_MN_Fa01-1, KY436234 others | 100 | 306/306 |
| 19_MN_Pa01 | 20_MN_Fa02-1 | 99.7 | 696/698 | 20_MN_Fa01-1, KY436234 others | 100 | 306/306 |
| 19_ND_Fa01 | - | - | - | - | - | - |
| 19_OH_Pa02 | - | - | - | - | - | - |
| 19_WI_Pa01 | 18_WI_Pa01-1 | 99.4 | 694/698 | 20_MN_Fa01-1, KY436234 others | 100 | 306/306 |
| 20_IL_Fa01 | - | - | - | - | - | - |
| 20_MN_Pa01 | - | - | - | - | - | - |
| 21_IN_Fa01 | - | - | - | - | - | - |
| 21_MA_Fa01 | - | - | - | - | - | - |
| 21_MA_Fa02 | - | - | - | - | - | - |
| 21_MI_Fa01 | - | - | - | - | - | - |
| 21_MI_Fa02 | - | - | - | - | - | - |

## Supplementary Table 1.4. continued

| Sample | β-*tubulin* | | | *RPB2* | | |
|---|---|---|---|---|---|---|
| | **Closest match(es)** | **% match** | **(XX/XX)** | **Closest match(es)** | **% match** | **(XX/XX)** |
| 21_OH_Fa01 | - | - | - | - | - | - |
| 21_PA_Fa01 | - | - | - | - | - | - |
| 21_PA_Fa02 | - | - | - | - | - | - |
| 21_VT_Fa01 | - | - | - | - | - | - |
| 22_NY_Pa01 | - | - | - | - | - | - |

**Note:** Supplementary tables 2.1 and 2.2 were omitted from the main text due to the size of the tables. They can be found online at the University of Minnesota Digital Conservancy as supplementary material.

**Supplementary Table 2.3.** Origins of Rhamnaceae and Elaeagnaceae and rust development after inoculation with *Puccinia coronata* var. *coronata.*

| Family | Species | Growth stage | Origin of plant material | Rust development |
|---|---|---|---|---|
| Elaeagnaceae | *Elaeagnus angustifolia* | Seedling | NPGS: NA 69101 | None |
| | *E. angustifolia* | Seedling | NPGS: PI 478005 | None |
| | *E. angustifolia* | Seedling | NPGS: PI 641697 | None |
| | *Shepherdia argentea* | Seedling | NPGS: W6 47538 | None |
| Rhamnaceae | *Berchemia scandens* | > 2 yrs. | Missouri Wildflowers Nursery, Missouri | None |
| | *Ceanothus americanus* | > 2 yrs. | Missouri Wildflowers Nursery, Missouri | None |
| | *C. prostratus* | Seedling | NPGS: PI 285234 | None |
| | *C. pumilis* | Seedling | NPGS: PI 376390 | None |
| | *Frangula alnus* | > 1 yrs. and seedling | Local collections around the Twin Cities, Minnesota | Numerous small to large aecia |
| | *F. californica* | Seedling | NPGS: W6 52191 | Numerous small to large aecia |
| | *F. californica* | Seedling | Klamath-Siskiyou seeds | Numerous small to large aecia |
| | *F. caroliniana* | > 2 yrs. | Mail Order Natives, Maryland | Numerous small to large aecia |
| | *F. purshiana* | seedling | NPGS: W6 53277 | None |
| | *F. purshiana* | > 2 yrs. | Forestfarm Nursery, Oregon | None |
| | *F. rubra* | > 2 yrs. | Forestfarm Nursery, Oregon | None |

**Supplementary Table 2.3. continued**

| Family | Species | Growth stage | Origin of plant material | Rust development |
|---|---|---|---|---|
| Rhamnaceae | *Rhamnus alnifolia* | > 2 yrs. | Johnson's nursery, Wisconsin | None |
| | *R. cathartica* | > 1 yrs. and seedling | Local collections around the Twin Cities, Minnesota | None |
| | *R. crenata* | Seedling | NDSU Dale E Herman Research Arb., Absaraka, North Dakota | None |
| | *R. japonica* | Seedling | NDSU Dale E Herman Research Arb., Absaraka, North Dakota | None |
| | *R. lanceolata* | > 2 yrs. | Missouri Wildfowers Nursery, Missouri | Few small aecia |

**Supplementary Table 4.1.** Coverage of consensus sequences of *Puccinia digitaticoronata* samples sequenced with Oxford Nanopore.

| Voucher number | Average coverage | | | |
|---|---|---|---|---|
| | **ITS** | ***COI*** | **β-*tubulin*** | **RPB2** |
| PUR N24045 | 233 | 106 | 81 | 71 |
| PUR N24046 | 173 | 102 | 173 | 174 |
| PUR N24047 | 233 | 118 | 408 | 141 |
| PUR N24048 | 56 | 46 | 107 | 68 |
| PUR N24049 | 164 | 264 | 80 | 200 |
| PUR N24050 | 206 | 124 | 92 | 132 |

**Note:** Supplementary tables 4.2, 4.3, and 4.4 were omitted from the main text due to the size of the tables. They can be found online at the University of Minnesota Digital Conservancy as supplementary material.