Obligate Rust Fungi as a Biological Control for Invasive Cheatgrass in North America.

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Authorization to Submit Thesis

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Abstract

In the Intermountain West of the United States, cheatgrass (*Bromus tectorum* L.) is a widespread invader. Its relative abundance is highest among all plants in many areas of the region. As a winter annual, it dries after seed set in late spring to become a fine, easily ignited fuel that sparks destructive fires. Many possible control measures for cheatgrass have been researched, but classical biological control with rust fungi has never been attempted; it would entail the deliberate introduction from the native range of obligate, and narrowly host-specific parasites in the genus *Puccinia*. For it to be worth attempting, cheatgrass would have to be in a state of release from rust pathogens in its invaded range in western North America, and rust in the native, Eurasian range would have to be virulent and aggressive on western North American genotypes of cheatgrass. The first chapter details the history of cheatgrass invasion in North America and the consequences to local ecosystems. A records review also shows, at least superficially, that USDA records of fungi on plants do not show release: records of rust fungi are as common on cheatgrass in western North America as they are in its Eurasian native range. However, in surveys in northern Idaho from 1999 to 2018 inclusive, rust was found on cheatgrass only in 2017, and then only minimally in terms of incidence and severity. Surveys in New Mexico in three years (2015, 2018, 2019) revealed no rust. Our surveys contradicted the records-based view and portray cheatgrass populations to be in a state of release.

Secondly, Idaho and Montana genotypes of cheatgrass developed severe rust in Israel. A study to determine the host specificity of *P. madritensis* to the *Genea* section of the genus *Bromus* is discussed. These studies will aim to indicate if native-range rust fungi should be considered for deliberate introduction into the invaded range of cheatgrass.

Acknowledgements

Dedication

I would like to dedicate this work to my family. My mother, Michelle, who has never been anything but loving, encouraging, and supportive, who showed me how to aspire for more by her own actions. My father, Curtis, who is the definition of unconditional love. My sister, Jessica, who has shown me how to live life for myself. My husband, Lionel, who worked every day to make sure that I had the opportunity to follow my dreams. And finally my children, Emmie and Asher. You are my greatest inspiration. Nothing of value comes easy and the hard path often leads to the most reward. Regardless of where you find yourself in life always remember not to be afraid of failure, it is the best way for all of us to learn. Remember that Mama will always love you and believe in you. Jen – thank you for the encouraging memes in my days of doubt, it saved me more than once. I love you all more than words can say and I thank you from my heart.

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Abstract

Cheatgrass (*Bromus tectorum* L.), is a damaging invader in western North America and especially prolific in the Intermountain West. Understanding the range of *B. tectorum* invasion and its effects on local ecosystems drives the need to find a method of control for this exotic species. This chapter is a review of the status of *B. tectorum* in North America and the current methods of control. The range and species of rust fungi present in North America are also examined. Currently, *B. tectorum* is present in every state of the continental United States, along with Canada and Mexico. In areas where it has become dominant, major shifts in local ecology have begun to degrade habitat for both plants and animals alike. An obligate rust fungus could be a possible method for control of this invasive grass, as it could reduce growth, fecundity, and overall fitness. However, according to surveys and compiled records, North American cheatgrass populations are not in a state of release from fungi, a fact that has perhaps discouraged any who might have gone to the native range of cheatgrass to find its most damaging rust.

Introduction

Cheatgrass (*Bromus tectorum* L.), also known as downy brome or downy chess, is a grass native to southern Europe, northern Africa, and central Asia (Huttanus 2011; CABI 2019). It is an ever-expanding invasive species within North America, and especially prolific in arid and semi-arid regions of the western United States. *Bromus tectorum* is an annual winter grass with a fine, shallow root system well adapted to xeric environments. Culm height can vary substantially by individual plant and growth region, ranging anywhere

between 4 and 30 inches (Klemmedson, 1964) as seen in *Figure 1.1*. Stems are typically

glabrous or marginally hairy with a marked pubescence of the leaves. *Bromus tectorum* is

characterized by its large, downy inflorescences produced as drooping panicles of slender

branches. Spikelets have between five and eight florets, each with a long, stiff awn that often

turns a deep purple at maturity. The overall "downy" look of this inflorescence is what gave

rise to one of the grass's common names, downy brome, and is often a useful tool when

spotting the plant along roadsides and in the field.

Figure 1.1. Drawing of cheatgrass (B. tectorum). USDA-NRCS PLANTS Database / Hitchcock, A.S. (rev. A. Chase). 1950. Manual of the grasses of the United States. USDA Misc. Publ. No. 200. Washington, DC.

Rust is a common term used when referring to a group of parasitic obligate fungi all

in the order Uredinales that colonize numerous cereals and grasses. The majority of rust

species have intricate, heteroecious life cycles, often with five distinct spore stages developed

on two separate and unrelated hosts (Kolmer et al. 2009; Cummins & Hiratsuka, 2003), as

seen in *Figure 1.2*. For many rust fungi, the secondary host represents the aecial host and is

the plant in which the sexual stage of the rust's life cycle takes place. The telial host is referred to as the primary host, wherein the asexual stage of the life cycle occurs and uredinia are formed. The uredinia phase is the only stage capable of repeated infections on the telial host, able to spread rapidly and cause potential epidemics (Leonard & Szabo 2005). Near the end of the growing season, uredinia are converted to telia and produce teliospores. These teliospores are hardy, resistant to cold or desiccation, and serve as the over-wintering phase for the fungus during the dormant state of the primary host. Once the growing season commences, haploid basidiospores produced from telia go on to infect the aecial host and begin the production of spermagonia. During this phase, fertilization occurs between spermatia and receptive hyphae, resulting in the formation of a functionally diploid aecium that produce aeciospores. Aeciospores go on to infect telial hosts, completing the rust fungus life cycle.
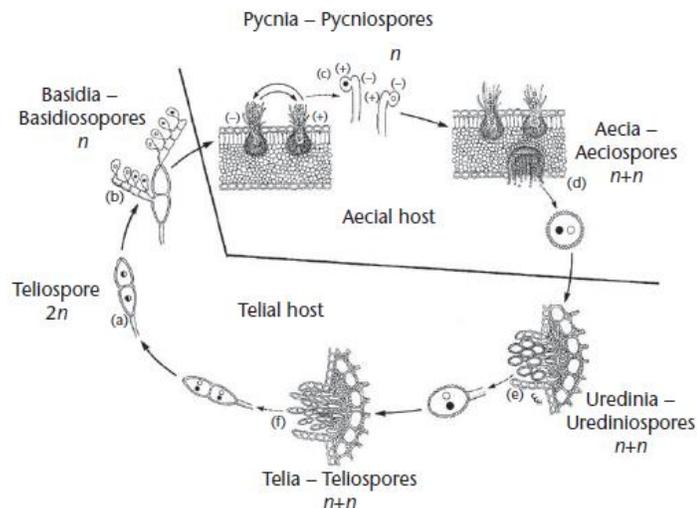
Figure 1.2. Life cycle of a macrocyclic-heteroecious rust. (a) mature, diploid teliospore, (b) basidia with basidiospores, (c) pycnial (sprermogonial) stage, (d) aecial stage, (e) uredinial stage and (f) telial stage. From *Introductory Mycology*, 4th Edition, 1996. Authors: Constantine J. Alexopoulos, Charles W. Mims, Meredith M. Blackwell, drawn by Carol Gubbins Hahn. (With permission from Wiley Books.)

With 7,000 species of documented rust fungi, approximately 4,000 are found within the genus *Puccinia* (Kolmer et al. 2009; Kirk et al. 2008). In the search for mechanisms of control for *B. tectorum*, these rust fungi may prove valuable. Among the *Puccinia* genus, taxonomy is an evolving framework with many species occurring as varieties and subspecies. *Puccinia madritensis* Maire is a putative species within the *Puccinia bromina* species complex, which has historically been confounded with *Puccinia recondita* Rob. ex Desm. (Cummins 1971; Urban & Markova 1999). As with many rust fungi, *Puccinia spp.* displays high host-specificity, a useful attribute when evaluating potential use as an agent of biological control (Anikster, 1984; Kolmer et al. 2009; Dracatos 2018). *Puccinia madritensis* is an endemic rust occurring on native populations of *B. tectorum* in Israel and surrounding regions (Savchenko et al. 2014; Urban & Markova 1999), where infected plants show reduced productivity (Cummins & Hiratsuka 2003).
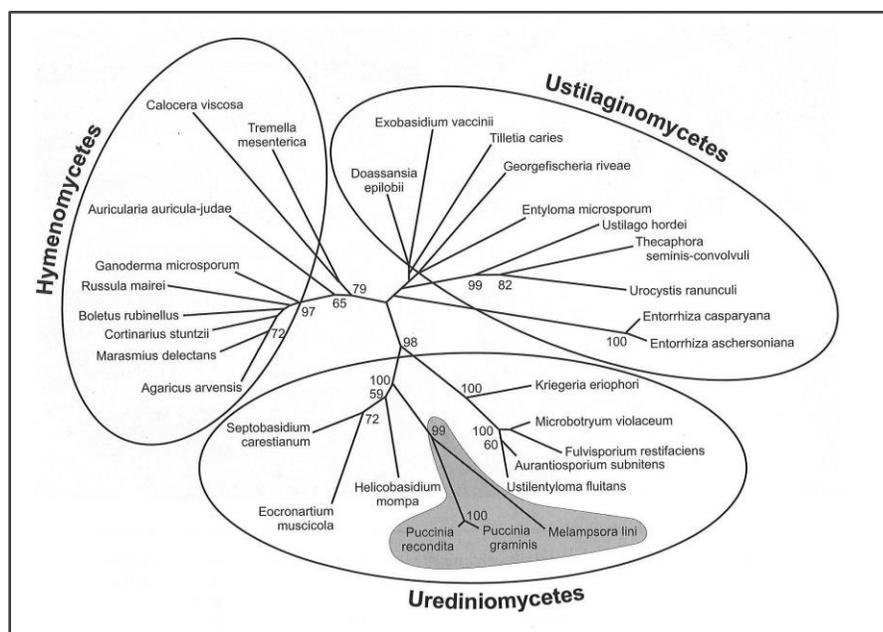
Figure 1.3. *Topology of division Basidiomycota. Obtained by neighbor-joining analysis of LSU rDNA sequences of 30 basidiomycetes. Shaded area indicates Uredinales. (With permission from Canadian Journal of Botany, Betegrow et al. 1997)*

Within its native Eurasian range, *B. tectorum* acts as the telial host for *P. madritensis* and Wisely Cream (*Clematis cirrhosa* L.)*, a moderately sized vine in the buttercup family, acts as the aecial host (Bachecioglu & Kabaktepe 2012; Gobelez, 1962). If native populations of *B. tectorum* are susceptible to *P. madritensis* infections, the question becomes whether this rust can be introduced to the invasive populations of North America as a form of population control. To propose this method of management, the *B. tectorum* populations of North America must be in a state of release from damaging rust infections and the invasive genotypes must be susceptible to *P. madritensis*.

Rust fungi can cause a number of symptoms in the telial host. Major symptoms displayed by these hosts include hypertrophy/hyperplasia, fasciation, malformation, and pseudoflower formation (Cummins & Hiratsuka 2003). Effects of grass and cereal rusts on their telial hosts typically involve damage due to repeated uredinial infections. This is due to the ability of urediniospores that can infect the hosts on which they are produced. This spore stage of the life cycle is known as the repeating stage and is responsible for the rapid development of disease outbreaks in telial hosts (grasses and cereals). *Figure 1.3* displays an example of rust infection on a wheat leaf (*Triticum* spp. L.).

Figure 1.4. Puccinia recondita uredinia releasing urediniospores on wheat leaf. With permission from Louisiana State University Ag Center, Louisiana State University AgCenter, Bugwood.org.

History of Cheatgrass Invasion in North America

The remote nature and isolation of the Intermountain West in the 19th century from the more populous eastern United States meant that development in the area was slow. As the population of the region increased, the sagebrush steppes became utilized as open range for cattle and homesteading. *Bromus tectorum* was introduced into North America sometime around 1850, most likely via contaminated packing materials and crop seed from Europe (Murray 1978; Whitson et al. 1991). Growing cultivation and grazing of the land marked the beginning of chronic, large-scale disturbances in the ecosystem, making it increasingly vulnerable to *B. tectorum* invasion. By 1890, *B. tectorum* was sweeping through the interior of the Pacific Northwest, as recorded by the Canadian naturalist, John Macoun (Mack 1981). In the late 19th century and early 20th century, *B. tectorum* was more than likely deliberately introduced as a means of replenishing the overgrazed ranges of the region and as a form of erosion control (Mack 1981). By the time the first quarter of the 20th century ended, *B.*

tectorum had reached the sagebrush-steppes of Utah and Nevada (Shantz, 1925) where it continues to persist today.

As a winter annual grass that is able to exploit moisture and nutrients prior to the germination of native plants, *B. tectorum* has become a serious and prolific invasive species (Thill 1984; Young 1969). The invasive power of *B. tectorum* has resulted in areas within the Great Basin region where the grass is the most abundant of all plant species (Mack 1981). The sagebrush-steppes have the lowest resiliency of Great Basin habitats (Zouhar 2003; Bunting et al. 1987) and recent studies have indicated *B. tectorum* cover is conservatively $\geq$ 15% in almost one-third of the sagebrush-steppes region ($\approx$ 210,000 km^2), dominating many areas within this scope (Bradley et al. 2018; Brummer et al. 2016; Downs et al. 2016). In some areas of the Great Basin, including southeastern Oregon, northeastern California, northwestern Utah, and north-central Nevada, mean percent cover has been reported at greater than 30% (Boyte et al. 2016). The Snake River Plain has the highest spatially consistent cover where reported values at and above 30% (*Figure 1.4*) are quite common (Boyte et al. 2016). Evidence suggests that low elevation areas have greater *B. tectorum* density when compared to higher elevations, but that higher elevations have greater spatial variability (Boyte et al. 2016). In addition, while the greatest documented effects have been observed in the sagebrush-steppes of the Great Basin, *B. tectorum* is present in all states of the United States to varying degrees (USDA Plants Database 2018). The invasive grass has also been reported in Canada, Greenland, and northern Mexico, further demonstrating how expansive and prolific *B. tectorum* has become in North America (USDA Plants Database 2018).
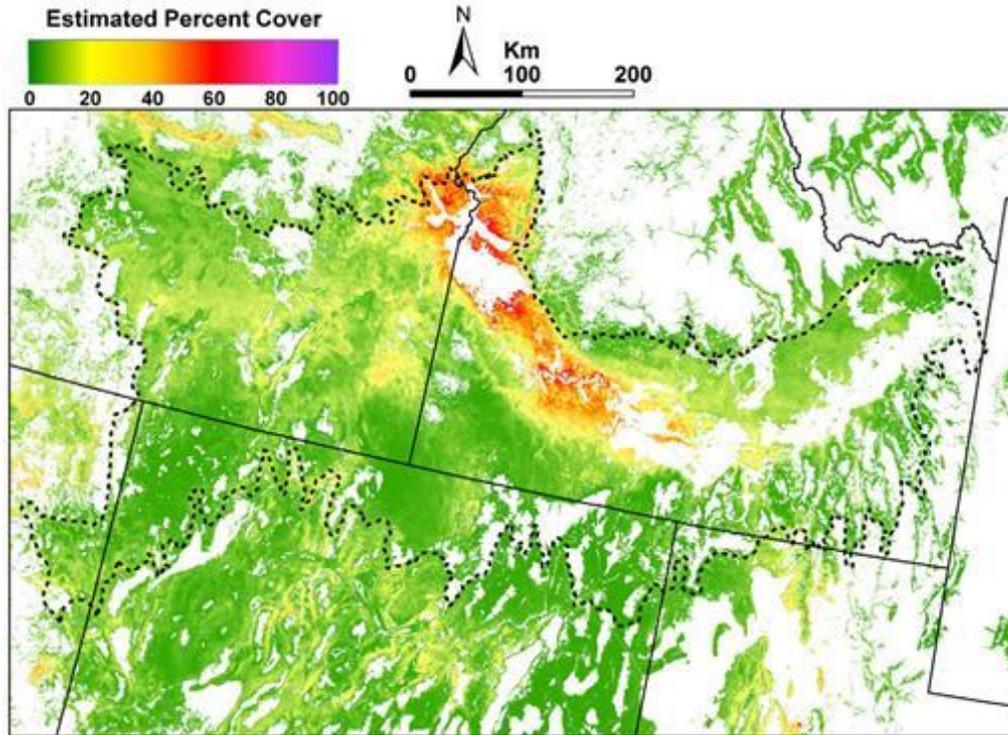
Figure 1.5. *Recent cheatgrass mean percent cover from 0−87. The black dashed line delineates the Northern Great Basin, which includes the Snake River Plain in Washington and Idaho. Analysis was conducted for all mapped areas that were unmasked. The mask (white) hides 2001 National Land Cover Database classifications other than shrub or grassland/herbaceous and elevations higher than 2,000 meters.*
Adapted from Rangeland Ecology and Management 69(4), Stephen P. Boyte, Bruce K. Wylie, and Donald J. Major, "Cheatgrass Percent Cover Change: Comparing Recent Estimates to Climate Change — Driven Predictions in the Northern Great Basin," Pages 265-279, Copyright [2016] with permission from Elsevier.

The mechanisms of *B. tectorum* expansion are numerous and often synergistic. In addition to *B. tectorum* acting as an early establisher in disturbed habitat, studies show many other methods by which the grass has become successful at occupying non-native ranges. The potential for fungal symbiosis is among these measures. There is growing evidence that certain cheatgrass populations may have formed symbiotic relationships with various thermo-tolerant fungi. Of these fungi, *Morchella* isolates found in New Mexico and other areas of the United States were shown to have positive effects on both the fecundity and growth of infected *B. tectorum* plants (Baynes et al. 2012). Infected seeds also showed increased viability when exposed to temperatures between 60°C and 65°C as compared to non-

inoculated, control seeds (Baynes et al. 2012). This relationship demonstrates one aspect of the positive feedback loop between fire and *B. tectorum* invasion.

Studies have shown harvester ants exhibit a strong preference for native grass seeds over *B. tectorum* seeds (Robertson, 2018; Schmasow & Robertson, 2016), contributing to increasing cheatgrass cover. Rodents may also play a part in the establishment of cheatgrass across America. Researchers have seen that native rodent populations within the Great Basin prefer to forage native seeds to *B. tectorum* seeds (Ostoja et al. 2013; Lucero et al. 2015). The effect of this foraging preference has been shown to limit the establishment of certain native grasses by at least 80% with no effect on the colonization of *B. tectorum* (Lucero and Callaway 2018).

Figure 1.6. *Bromus tectorum growing along sidewalk in White Rock, New Mexico. Taken May 2019 by author.*

The relationship between fire and *B. tectorum* is another important contributor to the invasive power of the grass. *Bromus tectorum* has altered local fire regimes by producing a positive feedback loop at the expense of native ecosystems (Zouhar 2003; Balch et al. 2012; Fenesi et al. 2016). Graminoids in general can encourage fire as the plants support large masses of standing, dead material that is highly combustible. The large surface-area-to-volume ratio also allows grass to desiccate rather quickly. Dry material combined with this large surface-area-to-volume ratio enhances the flammability of grass (D'Antonio and Vitousek 1992). Studies have consistently shown that the fire return interval for areas of the Great Basin invaded by *B. tectorum* have decreased from historical reference (Balch et al. 2013; D'Antonio and Vitousek 1992), with some data indicating return intervals are as frequent as every 3-5 years in cheatgrass-dominated habitat (Whisenat 1990). Along with the altered fire return intervals of many western landscapes, *B. tectorum* also provides a continuous source of fine fuels across these regions. This has resulted in not only decreased fire return intervals, but also larger, more expansive fires than those occurring prior to the arrival of *B. tectorum* (D'Antonio and Vitousek 1992).

While these fires have long-lasting and detrimental effects to local ecosystems, the financial burden placed on both citizens and government agencies has steadily increased over recent decades. In 1994, total fiscal appropriations to the United States Forest Service and Department of the Interior reserved for wildfire management was 1.7 billion dollars (Gorte 2011). In 2015, total fiscal appropriations to these agencies was approximately 3.5 billion dollars (Hoover & Bracmort 2015). These statistics do not include damages claimed by local residents or insurance agencies that suffer from loss or damage of property to wildfires.

The dominance of *B. tectorum* in large spans of the Great Basin affects not only the native vegetation, but also local wildlife (Freeman et al. 2014; Smith et al. 2017). As an example, the Greater Sage-Grouse is often used as an indicator species within the sagebrush-steppes. The loss of shrub-dominated territory to *B. tectorum* invasion has led to a marked decrease in Sage-Grouse populations (Lockyer et al. 2015; Coates et al. 2016). This is troubling news for areas overrun by *B. tectorum,* as the shift from shrubland to grassland can also affect small mammal diversity across ecosystems (Ostoja and Shupp 2009). The dense cover and increased litter created by *B. tectorum* invasion can impede the movement of small mammals, making them more vulnerable to predation (Reed et al. 2005; Ceradini and Chalfoun 2017). Additionally, the role of small digging mammals is especially important in numerous ecosystems as they can increase water infiltration, microbial activity, and trap litter and other organic material within the soil (Flemming et al. 2014). Maintaining a healthy wildlife community within *B. tectorum* invaded ranges is essential for overall ecosystem fitness.

Current methods of management for *B. tectorum* consist of mechanical and chemical (herbicidal) controls along with grazing practices and public education. Unfortunately, many of the methods proven to be effective must be implemented within a narrow range of application or the risk of *B. tectorum* dominance may actually increase (USDA 2014). As of now, no USDA approved form of classical biological control has been implemented. Agents such as head smut fungus and mold are being researched (USDA 2014) along with certain soil microbe populations (Kennedy, 2018), however these microbes are already present in the invaded range. The discovery of an effective biological control agent in the native Eurasian

range to inhibit the growth of *B. tectorum* may prove to be essential for the conservation of many ecosystems across North America, especially within the Great Basin.

Rust in Cheatgrass Populations of North America

A review was performed to gather reported incidence and severity of rust on North American populations of *B. tectorum*. This was done using searches of the literature along with resources available from the United States Department of Agriculture Plant-Fungal Database (USDA SMML) and the United States Department of Agriculture Plants Database (USDA Plants Database). According to the United States Department of Agriculture, rust fungi are well documented among North American *B. tectorum* populations, but incidence and severity of infection are unclear in a literature focused mostly on identification and taxonomy. The USDA SMML lists 71 separate species of fungi as infectious to *B. tectorum*, with 52 species reported on *B. tectorum* populations within North America (Farr & Rossman 2018) as shown in *Table 1.1*. Of the 52 reported fungi infecting *B. tectorum* populations in North America, eight are of the rust genus *Puccinia*. Records also show that of the 52 known *B. tectorum* fungal taxa present within the United States, 15 are documented in Idaho. These data show that of all the officially recognized rust species known to infect *B. tectorum* populations worldwide, 73% are present within the United States. These data also specify that 29% of all reported obligate fungi in the United States are confirmed in Idaho. *Puccinia madritensis* is not among the rust species described on *B. tectorum*-invaded ranges of North America as described by the USDA SMML Database. It is important to note that these USDA SMML records were compiled and that they do not represent an organized attempt to survey for rust on invasive *B. tectorum* in North America. We thus set out to perform simple

surveys to determine incidence and severity of rust fungi on invasive *B. tectorum* in two parts

of its invaded range: New Mexico and the Inland Pacific Northwest.

TABLE 1.1. Pathogenic fungi reported by the USDA Fungal Database to infect *Bromus tectorum*

Species	States/Countries Infected	Species	States/Countries Infected
*Ascochyta graminicola**	WI	*Puccinia graminis subsp. Graminis*	JAP
*Ascochyta sorghi**	MN, VI	*Puccinia madritensis - (Puccinia recondita)*	MOR
*Bipolaris sorokiniana**	VI	***Puccinia recondita****	CAN, AFG, BUL, CAN ISL, CHI, GER, GRE, IRA, KOR, NZ, TUR
Blumeria graminis	BUL, FRA, SPA, GER, ISR, MON, POL, ROM, SWE, SWI, TUR, USSR, UK	***Puccinia rubigo-vera - (Puccinia recondita)****	Uknown[a]
*Cercosporella herpotrichoides - (Oculimacula yallundae)**	ID, OR, WA	*Puccinia tsinlingensis*	CHI
*Chalastospora gossypii**	Uknown[a]	*Pyrenophora bromi*	UZB
Cladosporium graminum - (Cladosporium herbarum)	UZB	*Pyrenophora semeniperda - (Pyrenophora seminiperda)**	Uknown[a], CO, ID, UT, WA
Cladosporium herbarum	UZB	*Pyrenophora seminiperda*	GRE, TUR
*Claviceps purpurea**	KS	*Pyrenophora tritici-repentis*	RUS
Coniothyrium graminum	UZB	*Pythium debaryanum - (Globisporangium debaryanum)**	ID, NE, WY
*Erysiphe graminis - (Blumeria graminis)**	NE, WA	*Pythium graminicola**	NE, SD, WA
*Fusarium acuminatum**	NE, OR, SD, WY	*Pythium irregulare - (Globisporangium irregulare)**	ND, NE
*Fusarium culmorum**	ID, OR, WA	*Pythium perniciosum**	WA
*Fusarium equiseti**	ND, SD	*Pythium proliferum - (Globisporangium middletonii)**	NE
*Fusarium graminearum**	SD	*Rhizoctonia oryzae - (Waitea circinata)**	ID
*Fusarium nivale - (Monographella nivalis)**	ID, OR, WA	*Rhizoctonia solani**	MN, SD
*Fusarium oxysporum**	SD	*Rhizoctonia sp.**	ID, WA
*Fusarium scirpi var. acuminatum - (Fusarium acuminatum)**	ND, SD	*Septoria graminum*	UZB
*Gloeosporium bolleyi - (Microdochium bolleyi)**	NE, OR, SD, WY	*Septoria jacucella**	OR, WA
*Helminthosporium bromi - (Pyrenophora bromi)**	WA, WI	*Stagonospora montagnei**	VI
*Helminthosporium sativum - (Bipolaris sorokiniana)**	ND, SD, NE	*Stemphylium ilicis - (Ulocladium consortiale)*	UZB
*Hendersonia crastophila - (Septoriella hirta)**	ID, OR, WA	*Tilletia bromi**	CA, ID, OR, UT, WA, WY, SPA, Unknown[a]
Magnaporthe oryzae - (Pyricularia oryzae)	PAR	*Tilletia bromi-tectorum - (Tilletia bromi)**	WY
*Ophiobolus graminis - (Gaeumannomyces graminis)**	ID	*Tilletia caries**	CA, CO, ID, MT, NV, UT, WA
*Pellicularia filamentosa - (Rhizoctonia solani)**	ID, NE, OR, SD, WY	*Tilletia fusca**	CA, CO, ID, MT, OR, WA, WY, CAN, GER, SPA
Pleospora infectoria - (Alternaria infectoria)	UZB	*Tilletia fusca var. bromi-tectorum**	ID, WA
*Pleospora sp.**	OK	*Tilletia guyotiana - (Tilletia bromi)*	SPA
*Polymyxa graminis**	CANADA	*Typhula idahoensis - (Typhula ishikariensis var. idahoensis)**	WA
Puccinia bromina - (Puccinia recondita)	DEN, LIT, NOR, POL, ROM, SPA, SWE, UKR, UZB	*Typhula itoana - (Typhula incarnata)**	WA
Puccinia coronata	BUL, LIT	*Typhula sp.**	WA
Puccinia coronata var. hordei - (Puccinia coronati-hordei)*	MN, ND, SD	*Ustilago bromi - (Tilletia bromi)*	GER
Puccinia graminis - (Puccinia graminis subsp. graminis)*	CA, CO, WA, CHI, MAD, TUR	*Ustilago bromivora**	CA, CO, ID, MD, MT, NE, NA, OR, SD, CAN, CHI, GER, IRAN, IRA, MOR, NZ, POR, TUN, USSR, UZB
Puccinia graminis f. sp. Avenae*	CA, CO, WA	*Ustilago bullata**	AZ, CA, CO, ID, MT, ND, NE, NV, OR, SD, CAN, IRAN, ITA, NZ, POR, SPA, CAN ISL, Unknown[a]
Puccinia graminis f. sp. phlei-pratensis - (Puccinia graminis subsp. graminicola)*	CA, CO, WA	*Ustilago hypodytes - (Tranzscheliella hypodytes)*	
Puccinia graminis f. sp. Secalis*	CA, CO, WA	*Wojnowicia graminis - (Septoriella hirta)**	OR
Puccinia graminis f. sp. tritici - (Puccinia graminis subsp. graminis)*	CA, CO, WA		

* indicates reported infection in North American *B. tectorum* populations

[a] Listed as United States in database

These *B. tectorum* surveys were performed between 1999 and 2018 in northern Idaho and in 2015, 2018, and 2019 in northern New Mexico. Approximately 250 culms were examined in each field location to provide information on both the expansion of *B. tectorum* in New Mexico and incidence and severity of rust disease in North American *B. tectorum* populations. The method of examination consisted of a physical inspection of various *B. tectorum* stands within the named areas. The five New Mexico sites included the Salinas Pueblo Missions, Bandelier National Monument, Capulin Volcano National Monument, Sugarite Canyon near Raton, and in the Rio Grande Gorge between Espanola and Taos. All locations in northern Idaho were within 10 miles of Moscow. Surveys were also taken among *B. tectorum* populations in 26 sites from west to east in the Rhodope Mountains in southern Bulgaria in 2019. Thousands of plants were examined for fungal infection to gain insight into occurrences in the central part of the native range of *B. tectorum*.

TABLE 1.2 Surveys taken to identify rust infection in *Bromus tectorum* populations in Idaho, New Mexico, and Bulgaria

2015		2018	
Location	Pustules per 250 culms	Location	Pustules per 250 culms
Sugarite Canyon, NM	0	Sugarite Canyon, NM	0
Rio Grande Gorge, NM	0	Rio Grande Gorge, NM	0
Bandelier Ntl. Monument, NM	0	Bandelier Ntl. Monument, NM	0
Capulin Ntl. Monument, NM	0	Capulin Ntl. Monument, NM	0
Salinas Pueblo Missions, NM	0	Salinas Pueblo Missions, NM	0
Moscow, ID	0	Moscow, ID	0
Rhodope Mts, Bulgaria	*NA**	Rhodope Mts, Bulgaria	*NA**
2017		**2019**	
Location	Pustules per 250 culms	Location	Pustules per 250 culms
Sugarite Canyon, NM	*NA**	Sugarite Canyon, NM	*NA**
Rio Grande Gorge, NM	*NA**	Rio Grande Gorge, NM	*NA**
Bandelier Ntl. Monument, NM	*NA**	Bandelier Ntl. Monument, NM	0
Capulin Ntl. Monument, NM	*NA**	Capulin Ntl. Monument, NM	*NA**
Salinas Pueblo Missions, NM	*NA**	Salinas Pueblo Missions, NM	0
Moscow, ID	45	Moscow, ID	0
Rhodope Mts, Bulgaria	*NA**	Rhodope Mts, Bulgaria	0

* indicates no survey performed in given location for that year

Overall, surveys showed rust infection occurred at low frequencies in the Idaho locations with <1% incidence in just one of the three years of survey. Also, it should be noted that between 1999 and 2015 no rust was seen on *B. tectorum* near Moscow, Idaho, although there were no formal surveys completed. Idaho surveys taken in 2017 showed the only incidence of rust with low severity as the most diseased plants hosted a total of only 15 to 20 uredinial pustules per organism. The majority of plants surveyed in Idaho in 2017 had only a single pustule (*Table 1.2*). No observable infection in the New Mexico populations was the simple result in the 2015, 2018, and 2019 surveys (*Table 1.2*). According to USDA SMML records, of the 71 reported rust fungi infecting *B. tectorum* populations across the world, 2.8% are present in Bulgaria (*Table 1.1*). As shown in *Table 1.2*, no rust was found in a 2019 survey spanning the Rhodope Mountains in the region.

 A simple two-way contingency chi-square was done to test the independence of both native-range and invaded-range fungal pathogens reported on *B. tectorum* (*Table 1.3*). This was done using data from 285 individual reports over both the native and invaded ranges from the USDA SMML database (Appendix A). Results indicate significant difference between reported incidences of rust infections among the native range and the invaded range with a P-value of 0.0000. This test, using data acquired from the USDA SMML database (*Table 1.1*), shows that *B. tectorum* populations in the invaded range are not in a state of release from fungal pathogens, which is contrary to the findings of the simple surveys we performed in Idaho and New Mexico.

TABLE 1.3 Chi Square

	USDA SMML Reported Pathogens of *Bromus tectorum*		
	Observed	**Expected**	
Native Range	79 *110.75* (9.10)	142 *110.75* (9.10)	**221**
Invaded Range	206 *174.25* (5.79)	142 *174.25* (5.79)	**348**
	285	**285**	**570**

$$\chi^2 \;=\; 29.775, \quad df \;=\; 1, \quad \chi^2/df \;=\; 29.77\,, \qquad P(\chi^2 > 29.775) \;=\; 0.0000$$

Two-way contingency Chi-square test using 285 data points from the USDA SMML database. Test compares reported incidence between the native and invaded ranges of Bromus tectorum.

To explore the occurrence of rust pathogens within both the native and non-native range, a species indicator analysis was conducted. This was performed using the Dufrêne & Legendre analysis with binary input on PC-ORD software (Dufrêne & Legendre 1997; McCune & Mefford 2015; Peck 2010). This analysis gives insight to the abundance of each USDA SMML reported pathogen, grouped at the genus level, between the native range and invaded range of *Bromus tectorum*. Results of the species indicator test are shown in *Table 1.4*. P-values were corrected for multiple hypothesis testing at alpha = 0.05 to control the family-wise error rate, which is a common occurrence when conducting multivariate tests (Holm, 1979). P-values for each test result in failure to reject the null hypothesis, however important information can still be gleaned from the dataset. As shown, the relative abundance for reported incidence of many genera are 100% for much of the invaded range due to such small sample sets for many species. It should also be noted that *Puccinia* has a reported abundance of 57% in the invaded range as compared to 43% in the native. The

indicator values (IV) are given for each genus within each range, however the absolute value of the IV depends upon the relative abundance, which in turn depends upon the number of analyzed groups (in this case native and invaded). The relative difference between each group IV is the final number used as the indicator of a genus to a particular group.

TABLE 1.4 Indicator Species Analysis

Genus	Observed Frequency		Indicator Value		IV Difference	P value	Corrected P Value*
	Native	Invaded	Native	Invaded			
Ascochyta	0.0	100.0	0	5	5	1.0000	0.0500
Bipolaris	0.0	100.0	0	5	5	1.0000	0.0022
Blumeria	47.0	53.0	15	18	3	1.0000	0.0023
Cercosporella	0.0	100.0	0	5	5	1.0000	0.0024
Chalastospora	0.0	100.0	0	5	5	1.0000	0.0025
Cladosporium	100.0	0.0	5	0	5	0.4783	0.0019
Claviceps	0.0	100.0	0	5	5	1.0000	0.0026
Coniothyrium	100.0	0.0	5	0	5	0.4783	0.0020
Erysiphe	0.0	100.0	0	5	5	1.0000	0.0028
Fusarium	0.0	100.0	0	5	5	1.0000	0.0029
Gloeosporium	0.0	100.0	0	5	5	1.0000	0.0031
Helminthosporium	0.0	100.0	0	5	5	1.0000	0.0033
Hendersonia	0.0	100.0	0	5	5	1.0000	0.0036
Magnaporthe	0.0	100.0	0	5	5	1.0000	0.0038
Ophiobolus	0.0	100.0	0	5	5	1.0000	0.0042
Pellicularia	0.0	100.0	0	5	5	1.0000	0.0045
Pleospora	51.0	49.0	3	2	1	1.0000	0.0050
Polymyxa	0.0	100.0	0	5	5	1.0000	0.0056
Puccinia	43.0	57.0	23	40	17	0.3245	0.0019
Pyrenophora	81.0	19.0	17	1	16	0.1800	0.0018
Pythium	0.0	100.0	0	5	5	1.0000	0.0063
Rhizoctonia	0.0	100.0	0	5	5	1.0000	0.0071
Septoria	51.0	49.0	3	2	1	1.0000	0.0083
Stagonospora	0.0	100.0	0	5	5	1.0000	0.0100
Stemphylium	100.0	0.0	5	0	5	0.4783	0.0021
Tilletia	0.0	100.0	0	20	20	0.1130	0.0017
Typhula	0.0	100.0	0	5	5	1.0000	0.0125
Ustilago	51.0	49.0	24	22	2	1.0000	0.0167
Wojnowicia	0.0	100.0	0	5	5	1.0000	0.0250

* Holm-Bonferroni correction at alpha = 0.05

Both *Puccinia* and *Tilletia* indicate the invaded range with a difference of 17 and 20, respectively, whereas *Pyrenophora* indicates the native range with a difference of 16.

Using the data from *Table 1.1*, a bipartite plot (*Figure 1.6*) was produced to show the abundance and interaction between fungus at the genus level and sites within both the native and invaded ranges of *B. tectorum*. This method was performed using R, version 3.6.1 with the internal bipartite package and plotweb function (Dormann et al. 2019). As shown, the United States is heavily weighted, showing strong interactions with many different fungi known to infect *B. tectorum*. *Puccinia* is almost equally distributed across both ranges as presented in the indicator species analysis shown on *Table 1.4*.
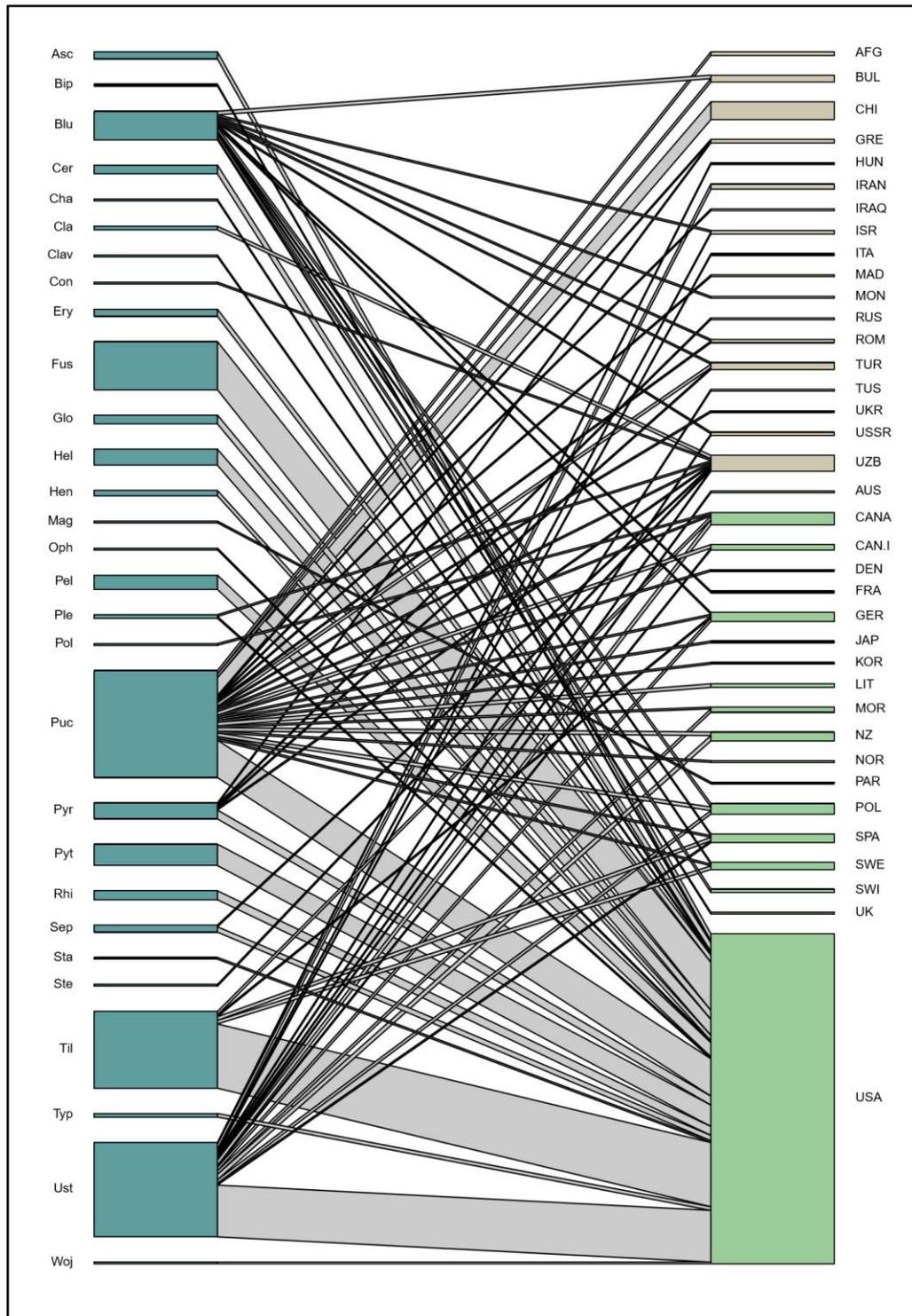
Figure 1.7. Bipartite graph showing interactions between SMML USDA fungal parasites on Bromus tectorum between the native and invaded ranges. Top row indicates sites with brown representing the native range and green representing the invaded range. The bottom row denotes fungal species. All sites and species can be found on **Table 1.5.**

TABLE 1.5 Bipartite Legend

Genus	Abbreviation	Country	Abbreviation
Ascochyta	Asc	Afganistan	AFG
Bipolaris	Bip	Bulgaria	BUL
Blumeria	Blu	China	CHI
Cercosporella	Cer	Greece	GRE
Chalastospora	Cha	Hungary	HUN
Cladosporium	Cla	Iran	IRAN
Claviceps	Clav	Iraq	IRAQ
Coniothyrium	Con	Israel	ISR
Erysiphe	Ery	Italy	ITA
Fusarium	Fus	Madagascar	MAD
Gloeosporium	Glo	Montenegro	MON
Helminthosporium	Hel	Russia	RUS
Hendersonia	Hen	Romania	ROM
Magnaporthe	Mag	Sicily	SIC
Ophiobolus	Oph	Turkey	TUR
Pellicularia	Pel	Tusinia	TUS
Pleospora	Ple	Ukraine	UKR
Polymyxa	Pol	USSR	USSR
Puccinia	Puc	Uzbekistan	UZB
Pyrenophora	Pyr	Australia	AUS
Pythium	Pyt	Canada	CANA
Rhizoctonia	Rhi	Canary Islands	CAN I
Septoria	Sep	Denmark	DEN
Stagonospora	Sta	France	FRA
Stemphylium	Ste	Germany	GER
Tilletia	Til	Japan	JAP
Typhula	Typ	Korea	KOR
Ustilago	Ust	Lithuania	LIT
Wojnowicia	Woj	Morroco	MOR
		New Zealand	NZ
		Norway	NOR
		Paraguay	PAR
		Poland	POL
		Portugal	POR
		Spain	SPA
		Sweden	SWE
		Switzerland	SWI
		United Kindgom	UK
		United States	USA

Discussion

The literature and data review regarding the status of rust presence in North America depicts a dichotomy between USDA SMML records and field findings. According to the USDA SMML (Farr & Rossman, retrieved 2018), rust and other fungal pathogens are common occurrences within North American populations of *B. tectorum*. The unofficial surveys taken in Idaho and northern New Mexico showed infections that were at most miniscule, but mostly absent (*Table1.2*). The most infected plants in Idaho had between 15 and 20 uredinial pustules per organism, whereas most plants surveyed had no infection. In the ten years of unofficial surveys between 1999 and 2018 in Idaho, no serious outbreaks of rust were witnessed in *B. tectorum*. The three years of *B. tectorum* surveys in New Mexico displayed zero indication of rust on any plants identified.

The results from the Bulgarian survey also showed no evidence of significant infection in *B. tectorum* populations. The empirical data of all performed surveys indicate that complied records on fungal infections across the globe may not accurately represent the state of release in many locations. While this may seem obvious, these records are often used when gathering statistics on infections and affected species and/or vicinities. This representation could be misleading to anyone searching for input on fungal presence in specific locales or among specific species.

The USDA SMML records suggest no release from rust fungi within the United States (or the invaded range of *B. tectorum* as a whole) and thus no driving need for rusts as classical biocontrols. A two-way contingency chi-square showed a significant difference in fungal infections between the native range of *B. tectorum* and the invaded range at $P = 0.000$, further indicating the invaded range is not in a state of pathogen release. This result was best

displayed as an informative visual representation via the bipartite graph of the data presented within the USDA SMML, which further illustrates that according to these online data, *B. tectorum* populations in the United States are not in a state of release from rust pathogens. It should also be noted that of all fungal pathogens listed on the USDA SMML database, 73% are located within the United States and within that, 29% are reported in Idaho, yet no observed outbreaks have been reported in the last ten years at a minimum.

The species indicator analysis based on USDA SMML data (*Table 1.4*) shows that while the relative abundance for reported incidence of many genera are 100% for much of the invaded range, the genus *Puccinia* has a reported abundance of 57% in the invaded range as compared to 43% in the native. The indicator values for *Puccinia* resulted in a total indicator value (IV) of 40, with a difference of 17 between the two groups (native and invaded), which indicates the invaded range. The genus *Tilletia* indicates the invaded range with a total indicator value of 20 and a difference of 20. *Pyrenophora* is indicative of the native range with a total indicator value of 17 and a difference of 16 between the two groups.

While large regions within the native range of *B. tectorum* may not show signs of rust infection (e.g. the Rhodope Mountains), damaging rust parasites in other areas within the range have been confirmed. *Puccinia madritensis* is known to negatively affect local cheatgrass populations in Israel, giving signs that rust release expectations may not be met range-wide. This could also be true for the invaded range of *B. tectorum*, however in the critical areas surveyed, rust is not present. While it is possible the invaded range mirrors the native in a patchy distribution of rust infections, the key point is the contrast between available fungal-plant records and the field findings of this thesis. The absence of rust is especially important within the Inland Pacific Northwest, where much of our surveys

occurred, as this area has been the most encroached upon by the invasive grass. This observed release in areas with extreme *B. tectorum* coverage, and the known presence of damaging *Puccinia madritensis* in Israel, leads to the conclusion that this rust fungus is still worthy of consideration for introduction into the invaded range of the United States as a biological control for *B. tectorum.*

The use of fungi as a method of biological control has precedence and in many cases has shown to be successful (Day et al. 2013; Day & Riding 2019). Research regarding *Pyrenophora semeniperda*, a fungal pathogen affecting the seeds of numerous species within the *Poaceae* family, has been extensively examined (Beckstead et al. 2007, Stewart 2009), however has not yet been approved by the USDA. Meta-analyses studies that quantified the impact of classical biological control at the level of individual target plants, target populations, and non-target vegetation presented reductions in plant size, plant mass, flower production, and seed production (Clewley et al.2012; Stiling & Cornelissen, 2005). Significant reductions in target plant density also demonstrated impacts at the population level along an increase in non-target species diversity (Clewley et al. 2012). While the work performed in this thesis shows promise for a biological control of *B. tectorum* in North America, further research needs to be completed. The potential implications of any new species should be explored in detail and from various angles before confidence of no further destruction to local ecosystems is sufficient. The development of clearly stated objectives and a monitoring program for individual *B. tectorum* communities would also need to be devised for proper observation if *P. madritensis* treatment were to be applied.

The discrepancy between empirical data and documented occurrences of rusts on *B. tectorum* in North America and Eurasia is significant. While reported incidence of rust in

North America appears to be greater, the lack of evidence shows there is room for further investigation. If *B. tectorum* populations in North America are in fact in a state of release despite records indicating otherwise, the introduction of native range *P. madritensis* could be a viable option for biological control of the grass. Methodical surveys performed in the future could give better insight to the frequency of rust in North American *B. tectorum* populations as compared to compiled records and could very likely support the findings of this thesis.

Conclusions and Management Implications

The state of *B. tectorum* in North America is critical for many habitats cross the continent, especially within the Intermountain West. Since the introduction of the grass in the 19th century, it has invaded countless ecosystems and produced habitat changes outside of historical variation. Altered fire regimes and loss of sagebrush along with other key species, are some of the many consequences of the introduction of *B. tectorum*.

The need to discover effective methods of control for *B. tectorum* is imperative for the health of many habitats across North America. The invasive grass has repeatedly proven how destructive it can be to almost every aspect of the habitats upon which it encroaches. To date, no classical biological control has been approved by the USDA and mechanical and chemical methods have shown difficult to administer or not entirely effective. The deliberate introduction of *P. madritensis* from the *B. tectorum* native range may be a valid method of biological control. This thesis shows evidence that populations of *B. tectorum* in Idaho and New Mexico are in a state of release from rust infection, despite superficial records from the USDA SMML and USDA Plants Database indicating otherwise. While data analysis based

on USDA records *(Bipartite graph, indicator species analysis, etc)* indicate that rust is more prevalent in North America *B. tectorum* populations than the native range, unofficial field surveys performed show a discrepancy. The lack of any rust infections on the North American *B. tectorum* populations puts us in a prime position to pursue the introduction of *P. madritensis*. This study is merely the first step towards the potential use of *P. madritensis* as a classical biocontrol of North American *B. tectorum,* with future research intended to answer unresolved questions.

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Chapter 2: Host Specificity and Susceptibility of North American *Bromus tectorum* to Eurasian *Puccinia madritensis*

Abstract

To determine whether the introduction of *Puccinia madritensis* into North American *Bromus tectorum* L. populations would be successful, the host specificity of the fungus must first be assessed in regards to species within the *Bromus* genus. The vulnerability of alternate aecial hosts must also be explored. The susceptibility of North American *B. tectorum* genotypes to *P. madritensis* infection should be determined as well. This chapter presents an outline of possible inoculation studies that would need to be performed to determine the susceptibility of North American genotypes of *B. tectorum* to the native *Puccinia madritensis* rust of Israel. Inoculations should also be performed to determine whether *P. madritensis* displays narrow-host specificity to species occurring in the *Genea* section of the *Bromus* genus. This section is comprised of *Bromus sterilis* L.*, Bromus madritensis* L.*, Bromus rubens* L.*, Bromus diandrus* Roth.*, Bromus rigidus* Roth.*, Bromus fasciculatus*, and *Bromus tectorum* L.. If the specificity of *P. madritensis* infection to the section *Genea* is confirmed, the fungus would present little danger of infecting native grasses.

Introduction

B. tectorum is native to southeastern Europe, northern Africa, and central Asia (Huttanus 2011). Obligate rust species in the genus *Puccinia* are present within native *B. tectorum* populations of the Eurasian range where infected plants show increased mortality rates (Plummer et al. 1990; Cummins & Hiratsuka 2003). *Puccinia madritensis* has a heteroecious life cycle, with five distinct spore stages developed on two separate and unrelated hosts (Kolmer et al. 2009; Cummins & Hiratsuka, 2003). To safely introduce any

species of exotic rust fungus into North America there needs to be a certainty that native vegetation will not be negatively impacted.

The narrow range of hosts displayed by the majority of rust species proves advantageous when researching the usefulness of introduction into North America. The verification of the host specificity of *P. madritensis* to the *Genea* section of the *Bromus* genus must be proven before the rust can be considered a potential biocontrol agent. The seven species within the *Genea* section of genus *Bromus* are *Bromus sterilis, Bromus madritensis, Bromus rubens, Bromus diandrus, Bromus rigidus, Bromus fasciculatus*, and *Bromus tectorum* (Williams et al. 2010). None of the seven species within *Genea* are native to North America, which is significant when determining the impact of non-target effects of *P. madritensis*. If *P. madritensis* telial host specificity is confined to *Genea*, there should be no threat of rust infection transferring to further grass or cereal species.

The vulnerability of North American *B. tectorum* populations to native strains of *P. madritensis* is an additional concern when determining the rust's usefulness as a biological control. Studies have shown alterations in the genomes of North American *B. tectorum* populations compared to native Eurasian populations (Novak and Mack 1991). It has also been determined that various ecotypes exist for North American *B. tectorum* via common garden experiments (Hulbert 1955). This alteration in genotypes could affect the ability of *P. madritensis* to act as a successful biological control, thus a study on the specific susceptibility of North American *B. tectorum* should be performed.

Clematis spp. **Susceptibility to** *Puccinia madritensis*

The aecial host is key to the life cycle of *P. madritensis* (Bachecioglu & Kabaktepe 2012; Gobelez 1962). Aecial hosts generally have a higher survivability rate when compared to the telial host, especially true in the case of Gramineae rusts alternating to woody species (Anikster & Whal 1979; Critopoulos 1947), which is the case for *P. madritensis*. *Clematis cirrhosa* is an evergreen woody climber native to the Mediterranean Basin. In its native environment, *C. cirrhosa* grows in USDA hardiness zones 7 to 9 and favors rocky, well-drained soils. This is at odds with many areas of the great Basin, especially where *B. tectorum* is common as these regions are typically at USDA hardiness zones of 6 or below (*Figure 2.2*). The Great Basin has a mosaic of precipitation patterns with averages between 20 cm of annual rainfall in the west 75 cm in higher elevations (Tilley, retrieved 2019). The Mediterranean Basin has an annual average precipitation of 50.8 cm with average low temperatures of approximately 4.5º C. These moisture values indicate an overlap in the needed precipitation for *C. cirrhosa* survival, showing room for further exploration of the area in terms of fitness for introduction.

Figure 2.1. *Stock photo of Clematis cirrhosa var. balearica. Image retrieved from:*
https://www.wyevalegardencentres.co.uk/product/clematis-cirrhosa-var-balearica/p0850021965

Fortunately, numerous rust fungi are known to have more than one alternate host within a given genus (Anikster & Whal, 1979; Ezzahiri et al. 1992), as evolutionary pressures on aecial hosts show less species fidelity that results in hosts with higher taxonomic variability (Aime et al. 2018). Along with *C. cirrhosa*, select supplementary species of the genus *Clematis* may act as the secondary aecial host of *P. madritensis.* A summary of hardiness zones for all possible Clematis spp. can be seen in *Table 2.1*.

Clematis flammula L., *Clematis recta* L., and *Clematis vitalba* L. may also be introduced aecial hosts for *P. madritensis* in North America as all three species occur in USDA hardiness zones of 6 and below. These three *Clematis* species have been identified as (potentially) existing aecial hosts for *P. madritensis* (Plant Parasites of Europe, retrieved 2019). Both *C. recta* and *C. vitalba* have been documented as able to grow in parts of North America; *C. recta* primarily in New England and Ontario and *C. vitalba* with a slightly larger range in the northwestern United States, British Columbia, and Ontario (USDA Plants Database, 2018). Further research would need to be performed to assess the plausibility of introducing one of these *Clematis* species as the secondary host for *P. madritensis*. Not only in terms of ability to thrive, but also in relation to possible effects on the native environment. None of the mentioned species of *Clematis* have been reported as invasive in North America (CABI, retrieved 2019), but the possibility still needs to be explored. An evaluation of the plants' functional roles in their native range in reference to potential interactions within

ecosystems of the Great Basin Desert should be performed before any of these species are

considered for introduction.
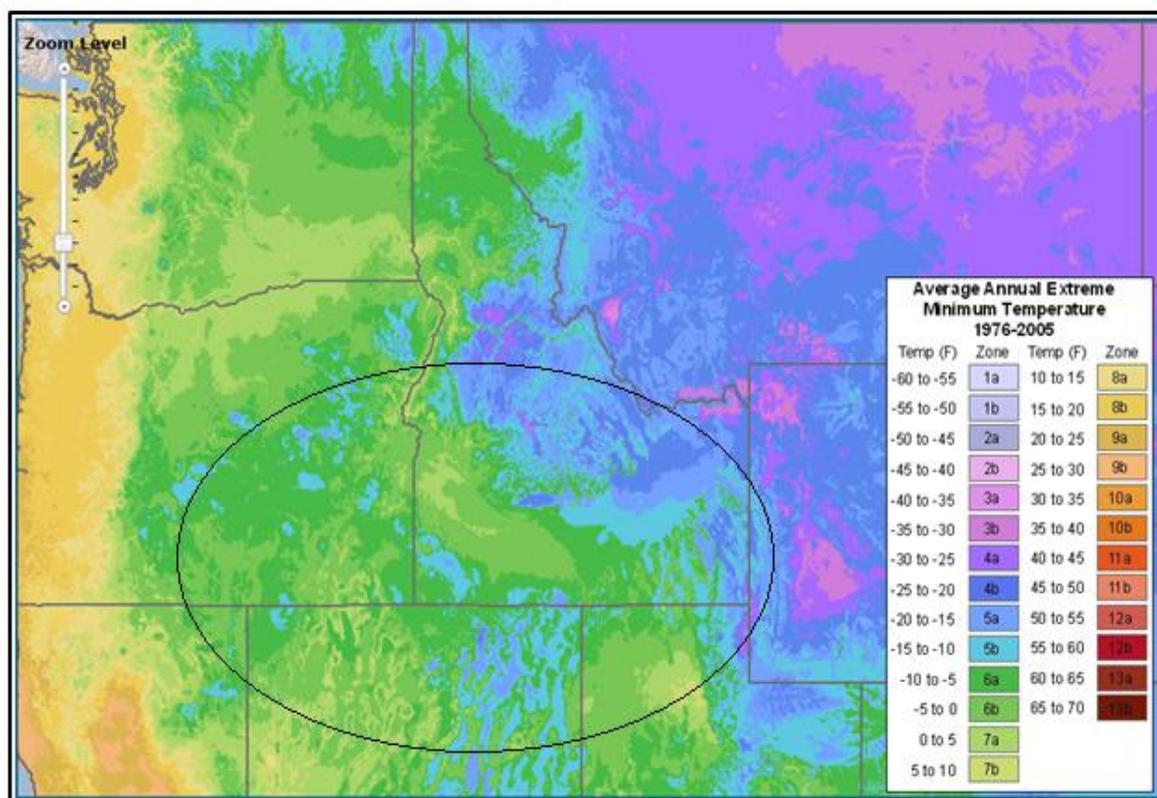
Figure 2.1. USDA Plant Hardiness Zone Map indicates hardiness zones of Washington, Oregon, Idaho, and parts of California, Nevada, Utah, Montana, and Wyoming. The area of the northern Great Basin has been approximately indicated inside the circle. Retrieved from: USDA Plant Hardiness Zone Map, 2012. Agricultural Research Service, U.S. Department of Agriculture. Accessed from https://planthardiness.ars.usda.gov/.

Clematis ligusticifolia Nutt., a native species to southern British Columbia,

California, the Dakotas, and both sides of the Oregon Cascades, is another possible aecial

host. This species has a cold hardiness between 5-7 and is already common in big sage

habitats (Geyer, 1846; Davis, 1952; Stannard & Crowder, retrieved 2019). Another factor

that may be considered when identifying suitable aecial hosts in North America is the

phenology of the genus *Clematis*. *Clematis cirrhosa* is categorized as a Clade unto itself

with two variations; *Clematis cirrhosa var. cirrhosa* and *Clematis cirrhosa var. balearica* L.

(Lehtonen et al. 2016). A closely related species located within the Clade below *C. cirrhosa* is *Clematis columbiana* that also includes two variations: *Clematis columbiana var. columbiana* Nutt. and *Clematis columbiana var. tenuiloba* A. Gray (Lehtonen et al. 2016). Not only are both variations of *C. columbiana* closely related to *C. cirrhosa* within the respective phylogenetic tree, but both are native to North America. The USDA hardiness rating for *C. columbiana var columbiana* ranges from 2 to 5 whereas *C. columbiana var. tenuiloba* falls within the hardiness zone of 3.

TABLE 2.1 Comparison of Clematis species

Clematis Species	Native to North America	USDA Hardiness Zone
Clematis cirrhosa	N	7
var. cirrhosa		
var. balearica		
Clematis flammula	N	6*
Clematis vitalba	N	6*
Clematis recta	N	6*
Clematis ligusticifolia	Y	5-7*
Clematis columbiana	Y	2-5*
var. columbiana		
var. tenuiloba		

* indicates overlap with hardiness zone of North American
B. tectorum populations

While the topic of aecial host issues for *P. madritensis* introduction still need exploration, optimistic results with other rust species of the genus *Puccinia* have been successfully used as agents for biological control. In Papa New Guinea, the rust *Puccinia spegazzinii* De Toni was released in 2008 to target the invasive and destructive vine, *Mikania micrantha* Kunth (Day et al. 2013). The rust was able to establish and spread and by 2012 the population of *M. micrantha* was reduced by 50% in some areas (Day & Riding 2019). The rust fungus *Puccinia araujiae* Lév is currently being studied as a potential biological

control agent for the invasive plant *Araujia hortorum* E. Fourn, which is native to South America, but prevalent in New Zealand (Anderson et al. 2016). These are just two examples of studies performed to initiate *Puccinia* species as biocontrols for various invasive plants across the globe.

Telial Host Specificity to *Genea* in *Puccinia Madritensis*

Establishing *P. madritensis* telial host specificity to grasses that have become invasive to North America or are absent altogether is imperative. Ensuring that target-effects do not distress native flora should always be a major concern for any biological control. The establishment of narrow telial host specificity in *P. madritensis* must be determined before introduction can be considered and future studies in this area should be performed as thoroughly and as soon as possible.

There are six grass species within the *Genea* section of the *Bromus* genus that are recommended for testing to determine host specificity to the *P. madritensis* rust. The six species are *Bromus sterilis, Bromus madritensis, Bromus rubens, Bromus rigidus, and Bromus fasciculatus*. If any of these six species in the *Genea* section show resistance to inoculation, then susceptibility outside *Genea* would seem unlikely as rust fungi are very often narrowly host-specific in regards to telial hosts.

North American *Bromus tectorum* Susceptibility to *Puccinia madritensis*

North American samples of *B. tectorum* from numerous locations should also be confirmed for susceptibility to *P. madritensis* using standard procedures for spray and rub inoculations (Sørensen 2016). Genotypic and ecotypic variations within North American *B. tectorum* populations can vary greatly from those in the native range, specifically cheatgrass

populations in Israel where *P. madritensis* is already established. Some exploratory research regarding this matter has begun and is projected to be added to the project in the near future.

Puccinia madritensis spores were obtained from known isolates harvested near Tivon, Israel via Dr. Yoeshua Anikster at Tel Aviv University in Israel.Initial analysis of three accessions of North American *B. tectorum* (from Utah, Idaho, and Montana) and the two accessions of control samples from *B. tectorum* native ranges in Israel and Greece were tested for vulnerability to the collected *P. madritensis* isolates and categorized into an infection type using the Stakman scale, as seen in *Table 2.2* (Stakman 1962). This unpublished data was provided by Dr. Anikster, a well-known researcher in the field of obligate rust fungi. Both North American and Eurasian samples of *B. tectorum* were shown to be moderately susceptible to *P. madritensis*.

TABLE 2.2. Accessions and cultivars of *Bromus tectorum* surveyed as possible telial hosts for *P. madritensis*[a].

Species	Origin	Cultivar or no. of accessions	Compatibility with *P. madritensis*[b]
Bromus tectorum L.	Utah	8	3
Bromus tectorum L.	Idaho	36	3
Bromus tectorum L.	Montana	18	3
Bromus tectorum L.	Israel[c]	14	3
Bromus tectorum L.	Greece	16	3[+]

[a] Based on Stakman scale: 0-IMMUNE; (0);-NEARLY IMMUNE; 1-VERY RESISTANT; 2-MODERATELY RESISTANT; 3-MODERATELY SUSCEPTIBLE; 4-VERY SUSCEPTIBLE; X-HETEROGENEOUS

[b] Isolate 6332 collected in Tivon, northern Israel

[c] Collected in Heftzieba, Israel

As shown in *Table 2.2*, all preliminary testing of North American cheatgrass genotypes from Utah, Idaho, and Montana showed moderate susceptibility (3) to native-

range *P. madritensis.* These are promising results, indicating the successful introduction of a native-range rust fungus is a possibility. If subsequent inoculation studies are performed, these results could be key in moving forward with the proposed biocontrol method.

Discussion

Puccinia madritensis is a heteroecious fungi, meaning it needs two separate and unrelated hosts to complete its life cycle. The primary telial host, *B. tectorum*, displays reduced productivity and fecundity with rust infection, which is emphasized by the ability of the urediniospores to cause repeated infections, spreading rapidly to cause epidemics. As the growing season wanes, uredinia on the secondary host are converted to telia and produce teliospores that are resistant to cold or desiccation and serve as the over-wintering phase. For *P. madritensis*, this phase occurs on *C. cirrhosa,* the alternate aecial host. During this phase, fertilization occurs between spermatia and receptive hyphae, resulting in the formation of a functionally diploid aecium that produce aeciospores. These aeciospores go on to infect telial hosts, completing the rust fungus life cycle (*Figure 1.2*).

The key to completing this life cycle in North America is finding a suitable alternate host for *P. madritensis*. *Clematis flammula*, *C. recta*, and *C. vitalba* may all be viable options that could be introduced along with *P. madritensis*. *Clematis recta* and *C. vitalba* have already been documented as growing in North America, however the precipitation and soil needs may not align with the climate of *B. tectorum* dominated ranges of the Great Basin. *Clematis ligusticifolia* is a native resident of big-sagebrush habitat and may be a better alternative to the introduction of any exotic species of *Clematis*. The susceptibility of this species to *P. madritensis* infection would need to be verified to determine whether it is a

suitable aecial host. One of the closet relatives of *C. cirrhosa*, *C. columbiana,* is native to North America. Either of the *C. columbiana* variations may also be a viable candidate as the phylogeny of the two species is closely associated, however both variations of this species occur in cold hardiness zones of 5 and below. This distinction has some overlap with the *B. tectorum* locations of typically 6 and below. If *Clematis* species native to North America are determined to be viable candidates as aecial hosts, the probability of negative impact due to the rust is relatively low. The survival rate of aecial hosts is typically greater than their telial counterparts, especially when the rust alters between a grass and a woody species. The first step towards solving this problem is determining aecial host susceptibility to *P. madritensis*.

Another strong driving force behind *P. madritensis* as a biological control for *B. tectorum* is verifying the telial host specificity of *P. madritensis.* This includes an inoculation study within the *Genea* section of the genus *Bromus*, which includes a total of seven grass species. If *P. madritensis* has as narrow telial host range as most other *Puccinia* species, the probability of outside infection is extremely minimal. An additional consideration for determining specificity is that each species within the *Genea* section is either absent from North America or invasive, meaning indigenous grass species would not be negatively impacted.

Conclusion and Management Implications

The narrow host-range for rusts in general make them advantageous biocontrol agents. The host-specificity study proposal in this thesis is aimed to show that *P. madritensis* is a good candidate as a biological control for North American populations of *B. tectorum*. Determining the susceptibility of aecial host range among the genus *Clematis* is key to

introducing *P. madritensis* into North America. While there is much work to be done in this regard, a number of possible species have been identified. Among these, *C. ligusticifolia* and *C. columbiana* are both native to North America. *Clematis columbiana* is also closely related to *C. cirrhosa*, the known aecial host for *P. madritensis*, making it a prime candidate for further study in this area.

A study defining telial host range to grasses within *Genea* would be ideal, as none of these species are native to North America and the only member present in large amounts, *B. tectorum*, is the target species. If any of the six grass species present in the *Genea* section of the *Bromus* genus prove to be resistant to *P. madritensis*, the probability of non-target infection would be diminutive. Preliminary results from an inoculation study indicate that North American accessions of *B. tectorum* are susceptible to native range *P. madritensis* isolates from Israel. This is another important concept, as North American population of *B. tectorum* have become genetically differentiated from native rage populations where *P. madritensis* is known to cause infection.

Given that the rust occurrence in North American *B. tectorum* populations is low as discussed in Chapter 1, and any existing infections have seemingly negligible effects on overall productivity, the introduction of *P. madritensis* from Israel may be a serious contender as a biological control agent in North American populations.

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Appendices

Appendix A

U.S. National Fungus Collections Fungus-Host Database. Jun 06, 2019
285 records were found using the criteria: Host Name = bromus tectorum

Ascochyta graminicola:
Bromus tectorum: Wisconsin - 847

Ascochyta sorghi:
Bromus tectorum: Minnesota - 814, 1061; Virginia - 32148

Bipolaris sorokiniana:
Bromus tectorum: Virginia - 32148

Blumeria graminis:
Bromus tectorum: Bulgaria - 13300, 37557; France - 13300; Germany - 13300; Israel - 44443;
    Montenegro - 39162; Poland - 13300, 44009; Romania - 13300; Spain - 13300; Sweden - 13300;
    Switzerland - 13300, 41110; Turkey - 13300; United Kingdom - 13300; USSR - 13300

Cercosporella herpotrichoides - (Oculimacula yallundae):
Bromus tectorum: Idaho - 1061; Oregon - 94, 1061; Washington - 94, 1061

Chalastospora gossypii:
Bromus tectorum: United States - 43573

Cladosporium graminum - (Cladosporium herbarum):
Bromus tectorum: Uzbekistan - 10250

Cladosporium herbarum:
Bromus tectorum: Uzbekistan - 10250

Claviceps purpurea:
Bromus tectorum: Kansas - 38481

Coniothyrium graminum:
Bromus tectorum: Uzbekistan - 10250

Erysiphe graminis - (Blumeria graminis):
Bromus tectorum: Nebraska - 94, 1061; Washington - 94, 1061

Fusarium acuminatum:
Bromus tectorum: Idaho - 1061; Montana - 1061; North Dakota - 1061; Nebraska - 1061; Oregon - 1061;
    South Dakota - 591, 1061; Washington - 1061; Wyoming - 1061

Fusarium culmorum:
Bromus tectorum: Idaho - 1061; Oregon - 94, 1061; Washington - 94, 1061

Fusarium equiseti:
Bromus tectorum: North Dakota - 94; South Dakota - 94

Fusarium graminearum:
Bromus tectorum: South Dakota - 591, 1061

Fusarium nivale - (Monographella nivalis):
Bromus tectorum: Idaho - 1061; Oregon - 94, 1061; Washington - 94, 1061

Fusarium oxysporum:
Bromus tectorum: South Dakota - 591, 1061

Fusarium scirpi var. acuminatum - (Fusarium acuminatum):
Bromus tectorum: North Dakota - 94; South Dakota - 94

Gloeosporium bolleyi - (Microdochium bolleyi):
Bromus tectorum: Montana - 1061; North Dakota - 1061; Nebraska - 1061; South Dakota - 1061;
    Wyoming - 1061

Helminthosporium bromi - (Pyrenophora bromi):
Bromus tectorum: Washington - 94, 1061; Wisconsin - 1061, 3015

Helminthosporium sativum - (Bipolaris sorokiniana):
Bromus tectorum: North Dakota - 94, 1061; Nebraska - 1061; South Dakota - 591, 1061

Hendersonia crastophila - (Septoriella hirta):
Bromus tectorum: Idaho - 1061; Oregon - 1061; Washington - 1061

Magnaporthe oryzae - (Pyricularia oryzae):
Bromus tectorum: Paraguay - 52805

Ophiobolus graminis - (Gaeumannomyces graminis):
Bromus tectorum: Idaho - 1061
Pellicularia filamentosa - (Rhizoctonia solani):
Bromus tectorum: Idaho - 1061; Montana - 1061; North Dakota - 1061; Nebraska - 1061; Oregon - 1061;
South Dakota - 1061; Washington - 1061; Wyoming - 1061
Pleospora infectoria - (Alternaria infectoria):
Bromus tectorum: Uzbekistan - 10250
Pleospora sp.:
Bromus tectorum: Oklahoma - 2410
Polymyxa graminis:
Bromus tectorum: Canada - 43328
Puccinia bromina - (Puccinia recondita):
Bromus tectorum: Denmark - 39293; Lithuania - 34393; Norway - 10330; Poland - 11280, 44009;
Romania - 12208; Spain - 40282; Sweden - 39293; Ukraine - 36202; Uzbekistan - 10250
Puccinia coronata:
Bromus tectorum: Bulgaria - 29774; Lithuania - 34393
Puccinia coronata var. hordei - (Puccinia coronati-hordei):
Bromus tectorum: Minnesota - 33945; North Dakota - 33945; South Dakota - 33945
Puccinia graminis - (Puccinia graminis subsp. graminis):
Bromus tectorum: California - 94, 25284; China - 8097, 38669, 39922; Colorado - 94; Madagascar -
8516; Turkey - 45814; Washington - 94, 38654
Puccinia graminis f. sp. avenae:
Bromus tectorum: California - 94; Colorado - 94; Washington - 94
Puccinia graminis f. sp. phlei-pratensis - (Puccinia graminis subsp. graminicola):
Bromus tectorum: California - 94; Colorado - 94; Washington - 94
Puccinia graminis f. sp. secalis:
Bromus tectorum: California - 94; Colorado - 94; Washington - 94
Puccinia graminis f. sp. tritici - (Puccinia graminis subsp. graminis):
Bromus tectorum: California - 94; Colorado - 94; Washington - 94
Puccinia graminis subsp. graminis:
Bromus tectorum: Japan - 6973
Puccinia madritensis - (Puccinia recondita):
Bromus tectorum: Morocco - 10446
Puccinia recondita:
Bromus tectorum: Afghanistan - 6855, 39281; Bulgaria - 29774; Canada - 7870; Canary Islands - 6922,
15488; China - 38669, 39922; Germany - 7172; Greece - 5985; Iraq - 23619; Korea - 39283; New
Zealand - 6224, 34286; Turkey - 45814
Puccinia rubigo-vera - (Puccinia recondita):
Bromus tectorum: Western states - 94
Puccinia tsinlingensis:
Bromus tectorum: China - 6907, 8097, 38669, 39922, 48545
Pyrenophora bromi:
Bromus tectorum: Uzbekistan - 10250
Pyrenophora semeniperda - (Pyrenophora seminiperda):
Bromus tectorum: Colorado - 44653; Idaho - 44653; North America - 44653; Utah - 44653; Washington
- 44653
Pyrenophora seminiperda:
Bromus tectorum: Greece - 43954; Turkey - 43954
Pyrenophora tritici-repentis:
Bromus tectorum: Russia - 52336
Pythium debaryanum - (Globisporangium debaryanum):
Bromus tectorum: Idaho - 1061; Nebraska - 1061; Wyoming - 1061
Pythium graminicola:
Bromus tectorum: Nebraska - 1061; South Dakota - 591, 1061; Washington - 1061
Pythium irregulare - (Globisporangium irregulare):
Bromus tectorum: North Dakota - 94; Nebraska - 94, 1061

Pythium perniciosum:
Bromus tectorum: Washington - 94
Pythium proliferum - (Globisporangium middletonii):
Bromus tectorum: Nebraska - 94
Rhizoctonia oryzae - (Waitea circinata):
Bromus tectorum: Idaho - 1061
Rhizoctonia solani:
Bromus tectorum: Minnesota - 94; South Dakota - 591
Rhizoctonia sp.:
Bromus tectorum: Idaho - 94; Washington - 94
Septoria graminum:
Bromus tectorum: Uzbekistan - 10250
Septoria jacucella:
Bromus tectorum: Oregon - 2705; Washington - 94, 1061
Stagonospora montagnei:
Bromus tectorum: Virginia - 32148
Stemphylium ilicis - (Ulocladium consortiale):
Bromus tectorum: Uzbekistan - 10250
Tilletia bromi:
Bromus tectorum: California - 25284; Idaho - 32951, 33636, 34353; Oregon - 34353; Spain - 6777, 30843; United States - 40105; Utah - 32951, 33636; Washington - 32951, 33636, 42050, 44314; Wyoming - 32951
Tilletia bromi-tectorum - (Tilletia bromi):
Bromus tectorum: Wyoming - 3646
Tilletia caries:
Bromus tectorum: California - 25284; Colorado - 94, 571; Idaho - 94, 571; Montana - 94, 571; Nevada - 571; Utah - 94, 571; Washington - 94, 571
Tilletia fusca:
Bromus tectorum: California - 7985; Canada - 6892, 7985; Colorado - 7985; Germany - 7846; Idaho - 7985; Montana - 7985; Oregon - 7985; Spain - 7985; Washington - 7985; Wyoming - 7985
Tilletia fusca var. bromi-tectorum:
Bromus tectorum: Idaho - 30880, 32657; Washington - 32657
Tilletia guyotiana - (Tilletia bromi):
Bromus tectorum: Spain - 6059
Typhula idahoensis - (Typhula ishikariensis var. idahoensis):
Bromus tectorum: Washington - 1061
Typhula itoana - (Typhula incarnata):
Bromus tectorum: Washington - 1061
Typhula sp.:
Bromus tectorum: Washington - 94
Ustilago bromi - (Tilletia bromi):
Bromus tectorum: Germany - 7846
Ustilago bromivora:
Bromus tectorum: California - 2705, 6059; Canada - 6059; China - 39922; Colorado - 2; Germany - 6059; Idaho - 6059; Iran - 46539; Israel - 50166; Morocco - 6059, 12968; Montana - 6059; New Zealand - 37467, 37532; Nevada - 6059; Oregon - 6059; Portugal - 6914; Tunisia - 12968; USSR - 36213; Utah - 6059; Uzbekistan - 10250; Washington - 6059; Wyoming - 2, 6059
Ustilago bullata:
Bromus tectorum: Australia - 6765; Arizona - 2, 571; California - 571, 3395, 25284; Canada - 7870, 8376; Canary Islands - 8323; Colorado - 571; Idaho - 571; Iran - 8464, 36142; Italy, Sicily - 45968; Montana - 571; North Dakota - 571; Nebraska - 571; New Zealand - 6224; Nevada - 571; Oregon - 571; Portugal - 38086; South Dakota - 571, 591; Spain - 38086; Utah - 571; Washington - 571; Western states - 94; Wyoming - 571
Ustilago hypodytes - (Tranzscheliella hypodytes):
Bromus tectorum: Hungary - 38248
Wojnowicia graminis - (Septoriella hirta): Bromus tectorum: Oregon –

Appendix B

Matrices used in PC-ORD, binary output.
Indicator Species Analysis results report generated via PC-ORD.

Main Matrix PC-ORD																													
	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q
	Asc	Bip	Blu	Cer	Cha	Cla	Clav	Con	Ery	Fus	Glo	Hel	Hen	Mag	Oph	Pel	Ple	Pol	Puc	Pyr	Pyt	Rhi	Sep	Sta	Ste	Til	Typ	Ust	Woj
AFG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
BUL	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
CHI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
GRE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
HUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
IRAN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
IRAQ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
ISR	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ITA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
MAD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
MON	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
ROM	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
SIC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
TUR	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
TUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
UKR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
USSR	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
UZB	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1	0
AUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CANADA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0
CAN ISL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
DEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
FRA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GER	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0
JAP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
KOR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
LIT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
MOR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
NZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
NOR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
PAR	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POL	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
POR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SPA	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0
SWE	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
SWI	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UK	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
USA	1	1	0	1	1	0	1	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1

Second Matrix PC-ORD

	c	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q	q
	Group	Asc	Bip	Blu	Cer	Cha	Cla	Clav	Con	Ery	Fus	Glo	Hel	Hen	Mag	Oph	Pel	Ple	Pol	Puc	Pyr	Pyt	Rhi	Sep	Sta	Ste	Til	Typ	Ust	Woj
AFG	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
BUL	Native	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
CHI	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
GRE	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
HUN	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
IRAN	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
IRAQ	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
ISR	Native	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ITA	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
MAD	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
MON	Native	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RUS	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
ROM	Native	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
SIC	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
TUR	Native	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
TUS	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
UKR	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
USSR	Native	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
UZB	Native	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0
AUS	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CANADA	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0
CAN ISL	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
DEN	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
FRA	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GER	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0
JAP	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
KOR	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
LIT	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
MOR	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
NZ	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
NOR	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
PAR	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POL	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
POR	Invaded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SPA	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0
SWE	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
SWI	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UK	Invaded	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
USA	Invaded	1	1	0	1	1	0	1	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1

```
PC-ORD, 7.07
 7 Jun 2019, 10:04:05

 Project file:
  Main matrix: C:\Users\lione\OneDrive\Documents\PCORD\Indicator Species
Analysis\Matrix 1.mjm
Second matrix: C:\Users\lione\OneDrive\Documents\PCORD\Indicator Species
Analysis\Matrix 2.mjm
```

Indicator values calculated with method of Dufrêne, M. & P. Legendre.
1997.
Species assemblages and indicator species: the need for a flexible
asymmetrical approach. Ecological Monographs 67:345-366.
ISA 060719

        Groups were defined by values of: Group
        Input data has: 39 Sites by   29 Species

RELATIVE ABUNDANCE in group, % of perfect indication
(average abundance of a given Species in a given group of Sites
over the average abundance of that Species in all Sites

expressed as a %)
NOTE: Group identifiers use first 10 characters of categorical variables

					Group	
			Sequence:		1	2
			Identifier:		Native	Invaded
			Number of items:		19	20
	Column	Avg	Max	MaxGrp		
1	Asc	50	100	2	0	100
2	Bip	50	100	2	0	100
3	Blu	50	53	2	47	53
4	Cer	50	100	2	0	100
5	Cha	50	100	2	0	100
6	Cla	50	100	1	100	0
7	Clav	50	100	2	0	100
8	Con	50	100	1	100	0
9	Ery	50	100	2	0	100
10	Fus	50	100	2	0	100
11	Glo	50	100	2	0	100
12	Hel	50	100	2	0	100
13	Hen	50	100	2	0	100
14	Mag	50	100	2	0	100
15	Oph	50	100	2	0	100
16	Pel	50	100	2	0	100
17	Ple	50	51	1	51	49
18	Pol	50	100	2	0	100
19	Puc	50	57	2	43	57
20	Pyr	50	81	1	81	19
21	Pyt	50	100	2	0	100
22	Rhi	50	100	2	0	100
23	Sep	50	51	1	51	49
24	Sta	50	100	2	0	100
25	Ste	50	100	1	100	0
26	Til	50	100	2	0	100
27	Typ	50	100	2	0	100
28	Ust	50	51	1	51	49
29	Woj	50	100	2	0	100
	Averages	50	91		22	78

```
RELATIVE FREQUENCY in group, % of perfect indication
(% of Sites in given group where given Species is present)
NOTE: Group identifiers use first 10 characters of categorical variables
                            Group
                    Sequence:        1         2
                    Identifier:   Native    Invaded
              Number of items:      19        20
        Column    Avg Max MaxGrp
     1 Asc          3   5    2        0         5
     2 Bip          3   5    2        0         5
     3 Blu         33  35    2       32        35
     4 Cer          3   5    2        0         5
     5 Cha          3   5    2        0         5
     6 Cla          3   5    1        5         0
     7 Clav         3   5    2        0         5
     8 Con          3   5    1        5         0
     9 Ery          3   5    2        0         5
    10 Fus          3   5    2        0         5
    11 Glo          3   5    2        0         5
    12 Hel          3   5    2        0         5
    13 Hen          3   5    2        0         5
    14 Mag          3   5    2        0         5
    15 Oph          3   5    2        0         5
    16 Pel          3   5    2        0         5
    17 Ple          5   5    1        5         5
    18 Pol          3   5    2        0         5
    19 Puc         61  70    2       53        70
    20 Pyr         13  21    1       21         5
    21 Pyt          3   5    2        0         5
    22 Rhi          3   5    2        0         5
    23 Sep          5   5    1        5         5
    24 Sta          3   5    2        0         5
    25 Ste          3   5    1        5         0
    26 Til         10  20    2        0        20
    27 Typ          3   5    2        0         5
    28 Ust         46  47    1       47        45
    29 Woj          3   5    2        0         5
       Averages     8  11             6        10
```

```
 INDICATOR VALUES (% of perfect indication,
 based on combining the above values for relative abundance
 and relative frequency)
 NOTE: Group identifiers use first 10 characters of categorical variables
                             Group
                          Sequence:      1        2
                         Identifier:   Native   Invaded
                     Number of items:    19       20
           Column     Avg Max MaxGrp
         1 Asc          3   5     2        0        5
         2 Bip          3   5     2        0        5
         3 Blu         17  18     2       15       18
         4 Cer          3   5     2        0        5
         5 Cha          3   5     2        0        5
         6 Cla          3   5     1        5        0
         7 Clav         3   5     2        0        5
         8 Con          3   5     1        5        0
         9 Ery          3   5     2        0        5
        10 Fus          3   5     2        0        5
        11 Glo          3   5     2        0        5
        12 Hel          3   5     2        0        5
        13 Hen          3   5     2        0        5
        14 Mag          3   5     2        0        5
        15 Oph          3   5     2        0        5
        16 Pel          3   5     2        0        5
        17 Ple          3   3     1        3        2
        18 Pol          3   5     2        0        5
        19 Puc         31  40     2       23       40
        20 Pyr          9  17     1       17        1
        21 Pyt          3   5     2        0        5
        22 Rhi          3   5     2        0        5
        23 Sep          3   3     1        3        2
        24 Sta          3   5     2        0        5
        25 Ste          3   5     1        5        0
        26 Til         10  20     2        0       20
        27 Typ          3   5     2        0        5
        28 Ust         23  24     1       24       22
        29 Woj          3   5     2        0        5
           Averages     5   8              3        7
```

```
MONTE CARLO test of significance of observed maximum
indicator value for Species
  4999 permutations.
Random number seed:        549
```

```
----------------------------------------------------------------
                                          IV from
                              Observed   randomized
                              Indicator    groups
    Column              Maxgrp Value (IV)  Mean  S.Dev    p *
-------------------- -------- -------- ----- ------ -------
       1 Asc            2        5.0    5.1   0.15  1.0000
       2 Bip            2        5.0    5.1   0.15  1.0000
       3 Blu            2       18.4   23.8   5.76  1.0000
       4 Cer            2        5.0    5.1   0.15  1.0000
       5 Cha            2        5.0    5.1   0.15  1.0000
       6 Cla            1        5.3    5.1   0.15  0.4783
       7 Clav           2        5.0    5.1   0.15  1.0000
       8 Con            1        5.3    5.1   0.15  0.4783
       9 Ery            2        5.0    5.1   0.15  1.0000
      10 Fus            2        5.0    5.1   0.15  1.0000
      11 Glo            2        5.0    5.1   0.15  1.0000
      12 Hel            2        5.0    5.1   0.15  1.0000
      13 Hen            2        5.0    5.1   0.15  1.0000
      14 Mag            2        5.0    5.1   0.15  1.0000
      15 Oph            2        5.0    5.1   0.15  1.0000
      16 Pel            2        5.0    5.1   0.15  1.0000
      17 Ple            1        2.7    6.4   3.78  1.0000
      18 Pol            2        5.0    5.1   0.15  1.0000
      19 Puc            2       40.0   37.6   5.33  0.3245
      20 Pyr            1       17.0   12.2   4.46  0.1800
      21 Pyt            2        5.0    5.1   0.15  1.0000
      22 Rhi            2        5.0    5.1   0.15  1.0000
      23 Sep            1        2.7    6.4   3.78  1.0000
      24 Sta            2        5.0    5.1   0.15  1.0000
      25 Ste            1        5.3    5.1   0.15  0.4783
      26 Til            2       20.0   10.1   4.69  0.1130
      27 Typ            2        5.0    5.1   0.15  1.0000
      28 Ust            1       24.3   30.2   5.68  1.0000
      29 Woj            2        5.0    5.1   0.15  1.0000
----------------------------------------------------------------
     Averages                 8.1327   8.26   1.27  0.8639
----------------------------------------------------------------
* proportion of randomized trials with indicator value
  equal to or exceeding the observed indicator value.
  p = (1 + number of runs >= observed)/(1 + number of randomized runs)
Maxgrp = Group identifier for group with maximum observed IV
```

```
Number of indicator species at three significance levels (alpha)
--------------------------
 alpha    signif.  expected
 0.050         0       1.5
 0.010         0       0.3
 0.001         0       0.0
--------------------------


Randomization test for sum of IVmax
    235.85     = observed sum of IVmax across all Species
          2817 = number of randomization runs with sum of IVmax >=
observed value
          4999 = number of randomization runs
      0.56371 = p

************************ Indicator values finished
************************
 7 Jun 2019, 10:04:06
```