

AN ABSTRACT OF THE THESIS OF

Tyler C. Gordon for the degree of Master of Science in Botany and Plant Pathology presented on September 16, 2011.

Title: Overwintering Survival of Stem Rust on Perennial Ryegrass: Construction of a Simulation Model, and Effects of the Mycoparasite *Sphaerellopsis filum*.

Abstract approved:

William F. Pfender

Stem rust, caused by the fungus *Puccinia graminis* subsp. *graminicola*, can cause yield reductions exceeding 90% on perennial ryegrass (*Lolium perenne* L.) crops grown for seed if not treated with fungicide in the spring. There is evidence that late-spring stem rust epidemics are initiated by populations that survived the winter. In addition, a previously developed stem rust severity model indicates that the late-spring epidemic severity is proportional to the stem rust population that overwintered. In this study, a mathematical model was constructed to describe the biological processes of stem rust overwintering as a function of meteorological conditions.

The model was validated with two years of stem rust severity data collected at two field sites. After model validation, the effects of changing the sporulation or daily infection conditions on overwintering stem rust foci were quantified. The model indicated that reducing the infection conditions or sporulation rate over the winter would reduce the stem rust population in the spring. A mycoparasite of stem rust, *Sphaerellopsis filum* (Biv.-Bern ex Fr.) Sutton (teleomorph *Eudarluca caricis* (Fr.) Eriksson), is known to infect the uredinia of other rust species and to lower spore production capacity. The effects of *S. filum* on individual stem rust uredinia and on overwintering populations were quantified. *S. filum* and stem rust were inoculated under controlled conditions in the glasshouse. In the field, *S. filum* was applied to plots that were naturally infected with stem rust at two sites in two years. *S. filum* infected stem rust uredinia in the glasshouse and field under conditions typical of an Oregon winter, and reduced spore production capacity. Furthermore, winter applications of *S. filum* or one winter application of a fungicide reduced the stem rust overwintering population at several field sites on first-year stands of perennial ryegrass. Treatment effects were not observed on second-year stands of perennial ryegrass, which had lower levels of disease severity than first-year stands during both years. These studies suggest several IPM strategies that could be utilized to reduce the severity of stem rust epidemics on perennial ryegrass. These strategies might include lowering the spore production capacity of stem rust by introducing effective strains of *S. filum*, or applying a fungicide to first-year stands over the winter.

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Overwintering Survival of Stem Rust on Perennial Ryegrass: Construction of a Simulation Model,
and Effects of the Mycoparasite *Sphaerellopsis filum*

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Tyler C. Gordon, Author

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Dr. William Pfender and Sheila Seguin collected data for Chapter 2: Predicting overwintering stem rust populations on perennial ryegrass using a mechanistic, meteorologically-driven model, including observations of leaf growth and tillering in 2004-2007, pustule longevity from 2000-2005, and field disease severity for model training from 2004-2006. Without these contributions, this chapter would be incomplete.

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Overwintering Survival of Stem Rust on Perennial Ryegrass: Construction of a Simulation model, and Effects of the Mycoparasite *Sphaerellopsis filum*

GENERAL INTRODUCTION

Perennial ryegrass grown for seed

Perennial grasses grown for seed thrive in the mild climate and high rainfall region of the Willamette Valley in Oregon, USA. These grasses are preferred by farmers for their lack of planting costs after the first year, ability to reduce soil erosion, and easy digestibility and high nutrient content that they provide to livestock as forage (Wilkins and Humphreys, 2003).

Over half of the grass seed acres in the Willamette Valley are planted to perennial ryegrass (*Lolium perenne* L.) or tall fescue (*Festuca arundinacea* Schreb.). In 2010 an estimated 91,570 acres of perennial ryegrass were harvested which accounted for 146,590,000 lbs of seed, and \$72,569,000 of gross sales. An estimated 117,080 acres of tall fescue were harvested accounting for 186,363,000 lbs of seed and \$58,734,000 gross sales (Young, 2010). The most damaging disease incurred by growers of these grass seed crops is stem rust caused by the fungus *Puccinia graminis* Pers. subsp. *graminicola* Z. Urb. which infects the leaves, stems and inflorescence, and can give rise to severe epidemics in May and June, reducing seed yields by up to 90% if the crop is not treated with fungicides (Pfender, 2004b).

The rusts

The rusts are a diverse group of biotrophic, plant-pathogenic fungi which infect vascular plants including Pteridophytes, Gymnosperms and Angiosperms. The rusts are represented by some 7,000 species in genera such as *Puccinia*, *Hemileia*, *Uromyces*, and *Gymnosporangium* all in the order Uredinales (Webster and Weber, 2007). Various rusts have been associated with cereal grains since antiquity, being unearthed from primitive wheat remains 3,300 years old

(Kislev, 1982), and referenced by the Romans who held a Robigalia agricultural festival on April 25 to appease the rust gods (Webster and Weber, 2007).

Rusts go through a complex lifecycle that utilizes both a principal host where its hyphae are dikaryotic, and an alternate host where the hyphae are monokaryotic. A dikaryotic aeciospore lands on a susceptible principal host where it germinates and forms uredinia which give rise to urediniospores. These urediniospores have a thick wall and can survive long distance wind dispersal of hundreds of miles, but frequently re-infect the original host plant (Lannou et al., 2008; Mundt, 2009; Pfender, 2004a). After repeated uredinial cycles, and in response to colder temperatures in the fall, uredinia become telia whose teliospores, upon germination, go through meiosis to produce basidiospores. Basidiospores can only germinate on the alternate host to produce spermogonia which are fertilized by spermatia and go through the process of dikaryotization to make an aecium (Agrios, 2005). The lifecycle is repeated when the aecium gives rise to aeciospores that can infect the principal host once again.

A given rust species can have all five spore stages, termed macrocyclic, it can be demicyclic and not have the uredinial stage, or be microcyclic and not have the uredinial and aecial stages (Webster and Weber, 2007). Stem rust is macrocyclic, and during the uredinial cycle it infects economically important crops including wheat, barley, oats and perennial ryegrass. A single uredinium produces hundreds of thousands of urediniospores which can rapidly re-infect the graminaceous crop during the growing season, giving rise to the epidemic stage of the disease.

Puccinia graminis Pers. is the causal agent of stem rust, also known as black rust or summer rust. It is a devastating fungal disease of the cereal grains, and is able to infect 365 species of plants in 54 genera (Webster and Weber, 2007). The species concept is difficult to apply in the rusts as isolates from different plant hosts are often morphologically identical, but

only infect a specific host range. Therefore, subcategories of the species are given by the *formae speciales* (*f.sp.*) or subspecies (*subsp.*) designation. Some examples of this designation include *P. graminis* *f.sp. tritici* which infects wheat, *P. graminis* *f.sp. avenae* infecting oat, and *P. graminis* *subsp. graminicola* (also called *P. graminis* *f. sp. lolii* in some accounts) which infects perennial ryegrass, tall fescue and several other grasses.

Host plant genotypes are susceptible to stem rust if their host response mechanisms are not able to detect or respond to the rust infection process vigorously enough. This virulence coincides with the rust fungus having lost the alleles that express proteins originally identified as foreign by the plant defense mechanisms. Different genes confer different types of resistance against stem rust in wheat. For instance, when challenged with the appropriate race, the Sr6 gene confers hypersensitivity in the wheat plant, Sr5 confers immunity, the presences of Sr8 and Sr22 coincide with a reduction in the number of uredinospores produced, and Sr2 confers adult slow-rusting non-specific resistance (Roelfs and Bushnell, 1985). As a result of the rust fungus overcoming a series of wheat resistant genes, severe stem rust epidemics occurred in the Midwestern US during the 1878, 1904, 1916, 1923, 1925, 1935, 1937, 1953 and 1954 growing seasons (Roelfs and Bushnell, 1985). These epidemics prompted substantial funding for wheat stem rust resistance research programs, and the creation of an eradication program of *Berberis vulgaris*, the alternate host of *P. graminis* (Roelfs and Bushnell, 1985).

These intensive breeding efforts have prevented serious stem rust epidemics in the US since 1954. In 1999, however, a race of wheat stem rust known as Ug99, or race TTKS, arose in Africa and was able to overcome many of the established resistance genes which represent some 25% of the world's wheat germplasm. As of 2011, Ug99 had not reached the US; however, the alarm caused by this race in Africa and the Middle East has led to renewed wheat breeding efforts (Singh et al., 2006).

Unlike wheat, the perennial grasses have only recently been introduced to agriculture, and have not been extensively selected for disease resistance (Welty and Barker, 1992; Welty and Barker, 1993; Wilkins and Humphreys, 2003), although some limited work has been done breeding for resistance to gray spot, crown and stem rust in perennial ryegrass (Curley et al., 2005; Dumsday et al., 2003; Muylle et al., 2005; Rose-Fricker et al., 1986). Other measures of rust suppression, including use of fungicides and cultural planting practices, are currently utilized to control rust on the perennial grasses.

Managing plant disease

Fungicide control of stem rust. Fungicides are the most common method of controlling stem rust on perennial ryegrass and tall fescue seed crops. One to five fungicide applications are recommended starting in May with the first detection of the disease, and then at 14-21 day intervals. Several of the most common fungicides, their active ingredients, and their recommended application rates are: Bravo[®] (chlorothalonil 54%) up to 24 oz per acre; Tilt[®] (propiconazole 41.8%) 4 oz per acre; Quilt[®] (azoxystrobin 7.0%, propiconazole 11.7%) up to 27.5 oz per acre; Headline[®] (pyraclostrobin 23.6%) 6-12oz per acre; Folicur[®] (tebuconazole 38.7%) 8oz/acre; Laredo[®] (myclobutanil 25.5%) 8-12 oz/acre (Ocamb, 2010).

These fungicides have a variety of mechanisms that prevent and/or treat established fungal disease in the field. Strobilurin fungicides (azoxystrobin, myclobutanil and pyraclostrobin) inhibit mitochondrial respiration by binding to the cytochrome b complex involved in electron transfer (Bartlett et al., 2002). Triazole fungicides (propiconazole and tebuconazole) bind to heme proteins and inhibit demethylation in the ergosterol biosynthesis pathway. Chlorothalonil depletes thiols and disrupts glycolysis in germinating spore cells (Tomlin, 2003).

Both propiconazole and azoxystrobin show considerable protective and curative properties against *P. graminis* f.sp. *graminicola* when applied to perennial ryegrass plants. Propiconazole protects plants when applied up to 1.2 latent periods before spores land on susceptible leaf tissue, and significantly reduces disease severity when applied up to 0.8 latent periods after infection (Pfender, 2006). Azoxystrobin protects plants when applied up to 1.6 latent periods before spore arrival, and reduces disease severity when applied up to 1.2 latent periods after infection (Pfender, 2006). For both fungicides, efficacy is greatest when applied at the time of infection, and decreases with time before or after infection.

Fungicides are commonly utilized and are often the only suitable control measure for fungal plant diseases. Yet they have several notable disadvantages including their cost, environmental impacts, and the selection pressure that can lead to fungal insensitivity to their modes of action. The purchase price per gallon of three fungicides commonly used to control stem rust epidemics are listed: Bravo (\$34), Tilt (\$126) and Quilt (\$119) (Quote from Wilbur Ellis Chemical Co., 2011). Added to this are the costs associated with application (\$6 to \$8 an acre). This makes the cost of one fungicide application range from \$12 to \$33 per acre depending on the fungicide and rate used.

The recent introduction of soybean rust *Phakopsora pachyrhizi* to the US has given researchers an area in which to document the environmental effects of the triazole and strobilurin fungicides. A year after soybean rust arrived in 2006, upper Midwest soybean farmers increased fungicide application from one to four percent on the soybean crop (Battaglin et al., 2010). To obtain baseline water quality data, samples from 29 streams in 13 states were collected in 2005-06 in areas where large amounts of soybeans were produced. Azoxystrobin was found in 45% of the streams with a maximum concentration of 1.13 µg/L, propiconazole was found in 17% of streams with a maximum concentration of 1.15 µg/L, and chlorothalonil was

infrequently detected in the streams (Battaglin et al., 2010). There was significant correlation between the azoxystrobin and propiconazole content in the streams, and estimated application rates in upstream drainage basins (Battaglin et al., 2010).

Several recent studies showed harmful effects to freshwater primary producers when exposed to low levels of these fungicides. For instance, the common green alga, *Pseudokirchneriella subcapitata*, showed impaired growth at 32 µg/l of azoxystrobin and 6.8 µg/l of propiconazole after 72hrs (Ochoa-Acuna et al., 2009). Sixteen µg/l of pyraclostrobin and 2 µg/l of propiconazole caused 10-80% of *Daphnia magna*, a small fresh water crustacean, to die after 96hrs of exposure (Ochoa-Acuna et al., 2009). The Great Plains Toad, *Bufo cognatus* suffered 100% mortality when directly exposed to 15 µg/l of pyraclostrobin (Belden et al., 2010). The LC₅₀ of azoxystrobin on rainbow trout, *Oncorhynchus mykiss*, is 0.47mg/l (European Food Safety Authority, 1998).

The European Food Safety Authority determined these chemicals have moderately long half-lives in biological systems. Azoxystrobin has a half-life of 34-57 days, whereas propiconazole has a half-life of up to 636 days in water (European Food Safety Authority, 2003).

Aside from the environmental risks, there are many cases of pathogenic fungi becoming resistant to the triazole and strobilurin fungicides. *Sclerotinia homoeocarpa*, the fungus that causes dollar spot (Miller et al., 2002), *Blumeria graminis* f. sp. *hordei*, the fungus that causes powdery mildew on barley (Blatter et al., 1998), *Mycosphaerella fijiensis*, the causal agent of black sigatoka on banana (Romero and Sutton, 1997), and *Monilinia fructicola*, the fungus that causes brown rot on soft fruits (Luo et al., 2008) have become resistant to triazole demethylation mechanisms. *Alternaria* spp. on pistachio (Ma et al., 2003), *Pyricularia grisea*, gray spot on perennial grasses (Vincelli and Dixon, 2002), *Blumeria graminis* f.sp. *tritici*, powdery mildew of wheat (Bäumler et al., 2003), *Podosphaera fusca*, powdery mildew on cucumber, and

Pseudoperonospora cubensis, downy mildew on cucumber (Ishii et al., 2001) have overcome strobilurin electron transfer interruption activity. *Botrytis cinerea*, the cause of gray mold, has overcome the broad protective inhibition caused by chlorothalonil (Barak and Edgington, 1984).

Epidemiology and modeling. Models for an array of fungal diseases, including stem rust, have been developed to not only reduce the number and cost of fungicide applications, but also to identify potential IPM strategies for reducing disease severity (Coakley et al., 1988; Kropff et al., 1995; Savary et al., 2006). The models give a disease risk assessment based on biological and meteorological data. Quantitative plant disease models have been developed to integrate knowledge of crop and pathogen biology into disease management decision aids (Berger et al., 1995; Coakley et al., 1988; Kushalappa et al., 1984). A review by Savary et al. (2006) addresses the four types of decisions that could be influenced by modeling. They include: (i) short-term within-season decisions, (ii) between-season decisions, (iii) long term breeding programs and development of IPM strategies, and (iv) research prioritization decisions. Both empirical and simulation models can be used as decision tools in these areas.

Empirical models are derived by regression analysis, and can relate meteorological conditions and crop development, to crop damage or yield loss (Kropff et al., 1995). Empirical models relating disease severity and weather conditions to yield loss through regression analysis have been developed for rust diseases in several studies. A predictive model developed for bean rust caused by *Uromyces appendiculatus* included parameters for basic infection rate, environmental favorability and change of host susceptibility (Berger et al., 1995). A regression model for wheat leaf rust (*Puccinia recondita*) severity included parameters for dispersal, temperature and hours of free water. The regression explained 70% of field disease severity in a post-hoc analysis (Eversmeyer and Burleigh, 1970). A barley leaf rust model accurately correlated disease severity with yield loss using regression analysis (Teng et al., 1979). Yield loss

on wheat caused by stripe rust was accurately predicted by using stepwise regression to correlate monthly temperature and precipitation predictors that could be integrated into a comprehensive overwintering model for the Pacific Northwest (Coakley et al., 1988). In another study, maximum leaf rust severity on wheat was accurately predicted by coefficients and equations representing cultivar, planting date and winter meteorological conditions (Moschini and Pérez, 1999). These empirical models can predict the damage threshold of a particular disease over one year, but often overlook the underlying dynamics between the crop and pathogen, which are crucial for development of long-term strategies.

Another method of modeling mathematically describes the subcomponents of a disease process based on experimental data. These processes can be integrated into a cohesive model which not only allows for the determination of crop loss, but can also be used to simulate how changes in the subcomponent processes will affect other factors in the model. Simulation models can also be used as decision support tools for long-term projects such as developing IPM strategies (Savary et al., 2006).

Examples of simulation models include those for soybean rust (Yang et al., 1991) and leaf rust on wheat (Rossi et al., 1997). The soybean rust model is driven by cumulative physiological days (heat units), and daily meteorological conditions including hours of dew and average night temperatures. Subcomponents in the model describe infected and susceptible plant area, pustule latent periods and senescence. The model accurately predicted soybean rust severity over the course of the epidemic with regression B_1 and r^2 coefficients generally higher than 0.85. The leaf rust model simulated the proportion of rusted leaf area by using the same subcomponents that were described in the soybean rust model, with the addition of stochastic variation incorporated into latent period and proportion of spores causing new infections.

Other examples of simulation models include a model for apple scab (*Venturia inaequalis*) which incorporated factors affecting ascospore dispersal, germination, appressorial formation and penetration for ascospores and conidia. The model was developed to estimate disease severity, and to appropriately time fungicide applications (Xu et al., 1995). Another soybean rust (*Phakopsora pachyrhizi*) model, which includes information about infection efficiency, latent period, and daily spore production was used to predict the first occurrence of the disease from overwintering locations in the deep south (Pivonia and Yang, 2006).

A simulation model has been developed also for stem rust on perennial ryegrass caused by the fungus *Puccinia graminis* subsp. *graminicola* Z. urb. (*Pgg*) (Pfender, 2001b; Pfender, 2003). The model accurately predicted *Pgg* disease severity based on daily spring meteorological data inputs, and allowed perennial ryegrass growers to apply fewer fungicides during an epidemic (Pfender, 2001b; 2003; 2004a). Over a five-year trial period this predictive model saved an average of \$96 per hectare in demonstration field tests (Pfender et al., 2008).

The stem rust model also predicts that lower *Pgg* severity in early spring (March) will give rise to less severe epidemics in the late spring (June). This implies that overwinter plant growth dynamics and meteorological conditions, if they affect rust population survival, will impact the severity of the spring epidemic. Other studies have demonstrated that summer stripe rust epidemics are influenced by meteorological conditions over the preceding winter (Coakley et al., 1982; Coakley et al., 1988; Sharma-Poudyal and Chen, 2011). There is also evidence that localized stripe rust infection centers spread from leaf to leaf during winter months and give rise to the spring population (Shaner and Powelson, 1973). Further support of overwintering stem rust populations having an effect on stem rust epidemic severity was demonstrated in a study where late plantings of perennial ryegrass had significantly lower stem

rust severity in summer than those stands planted early in the fall, presumably because late plantings had less fall infection than did early plantings (Pfender, 2004b).

Cultural control. Another method of slowing fungal epidemic disease development is through the use of cultural controls. Agrios (2005) defines cultural control as eradicating or reducing the amount of pathogen through avoiding the pathogen or creating environmental conditions unfavorable to the pathogen. One cultural control that works to slow epidemic development of fungal pathogens is to plant mixtures of different cultivars in the same field. Cultivar mixtures reduce stripe rust (*Puccinia striiformis*) severity by 13-97%, and in the presence of disease, increase yields from 8 to 13% (Finckh and Mundt, 1992). Multiline cultivar mixtures slow the progress of a variety of diseases including *Magnaporthe grisea*, *Blumeria graminis*, *Phytophthora infestans*, *Uromyces appendiculatus*, *Mycosphaerella graminicola*, *Melampsora* spp. and *Puccinia* spp. (Mundt, 2002).

Planting date also affects the severity of disease epidemics. Planting date affects the severity of powdery mildew (*Blumeria graminis*) on winter wheat (Frank et al., 1988), Sclerotinia crown and stem rot in alfalfa (Sulc and Rhodes, 1997), leaf rust on wheat (Moschini and Pérez, 1999) and stem rust on grass seed crops (Pfender, 2004b). In the perennial ryegrass cropping system, planting in early November gives rise to only 2 to 4% as much stem rust during the epidemic as planting in mid-September, however these late plantings produce 23% less seed at harvest in early July if there is no disease pressure (Pfender, 2004b).

Biological control of fungal plant pathogens

Viral, insect, bacterial, and fungal organisms can suppress fungal plant pathogens using several general mechanisms including competition, antibiosis, induced plant resistance, and/or mycoparasitism (Johnson, 1994; Xu et al., 2010). A recently developed predictive model

estimates the efficacy of the various biological controls, not by taxonomy, but by focusing on the mode of action (Xu et al., 2010). The model demonstrates that biological control organisms with mechanisms involving competition and induced resistance are the most effective at controlling epidemics. The model predicted that mycoparasitism would suppress disease development, but not satisfactorily for commercial agriculture, unless these agents are frequently applied or combined with a biological control agent that utilized another mode of action (Xu et al., 2010). Nonetheless, biological control agents using all of the modes of action listed suppress diseases caused by fungal plant pathogens (Koc and Defago, 1983; Kuhlman and Matthews, 1976; Milgroom and Cortesi, 2004; Sundheim, 1982; Thomashow and Weller, 1988).

Unconventional biological control. Recent viral and entomological studies have shown the effectiveness of utilizing unconventional biological organisms to control fungal diseases on plants. A virus has been used to produce hypovirulent strains of the chestnut blight fungus *Cryphonectria parasitica*, and controls the disease in Europe and Michigan (Milgroom and Cortesi, 2004). Epiphytic Tydeid mites (*Orthotydeus lambi*) were shown to graze on and significantly suppress powdery mildew (*Uncinula necator*) on grapes (English-Loeb et al., 2007; Melidossian et al., 2005).

Bacteria as biological control agents. Bacterial biological controls typically work to suppress fungal disease through antibiosis, but some bacteria employ a variety of mechanisms. *Pseudomonas fluorescens*, which produces an antibiotic derivatives of phenazine, controls *Gaeumannomyces graminis* and *Pyrenophora tritici-repentis*, the causal agents of take-all and tan spot in wheat, respectively (Pfender et al., 1993; Thomashow and Weller, 1988). *P. fluorescens* strains suppress foliar pathogens also, including powdery mildew *Leveillula taurica* and fruit rot *Colletotrichum capsici* in chili peppers (Anand et al., 2010). *P. fluorescens* also

induces host defense mechanisms enabling the suppression of the Tobacco Necrosis Virus (Maurhofer et al., 1994).

Another bacteria that works to control fungal diseases through competition, antibiosis and induced host resistance, *Bacillus subtilis*, has been shown to suppress gray mold (*Botrytis cinerea*), damping-off (*Rhizoctonia solani*), and several powdery mildews (Paulitz and Belanger, 2001). *Bacillus* spp. lyse spores and reduce the number of uredinia *in vitro* when spores of bean, leaf or stem rusts land on healthy host tissue (Gowdu and Balasubramanian, 1988). *Erwinia uredovora* also inhibits the germination of urediniospores and lyses spores of *P. graminis*, *P. recondata*, *P. coronata* and *Uromyces appendiculatus* (Gowdu and Balasubramanian, 1988). *Pantoea agglomerans* and *Stenotrophomonas maltophilia* isolates suppress bean rust uredinial development in the greenhouse (Yuen et al., 2001).

Fungi as biological control agents. Fungi are also antagonistic to plant pathogenic fungi, suppressing germination and sporulation, and competing for space and nutrients. *Trichoderma harzianum* uses competition and antibiosis mechanisms to control *Rhizoctonia solani* and *Fusarium oxysporum* in the rhizosphere (Paulitz and Belanger, 2001). *Trichoderma virens* secretes fungitoxic compounds and suppresses the growth of *Pythium ultimum* and *Rhizoctonia solani* in a hydroponic system (Paulitz and Belanger, 2001). Yeast species including *Trichosporon pullulans*, *Cryptococcus laurentii*, *Rhodotorula glutinis*, and *Pichia membranefaciens* are effective at outcompeting major postharvest fungal pathogens including *Alternaria alternata*, *Penicillium expansum*, *Botrytis cinerea*, and *Rhizopus stolonifer* for space and nutrients on sweet cherries (Qin et al., 2004). *Sporothrix flocculosa* reduces the disease progress of *Sphaerotheca fuliginea* (causal agent of cucumber powdery mildew) by 20 to 50% through antibiosis (Dik et al., 1998).

Helminthosporium sativum, *Myrothecium roridum*, *Alternaria alternata*, *Botrytis cinerea*, and *Cladosporium cladosporioides* filtrates and spores suppressed the germination of *P. graminis* urediniospores *in vitro* (Gowdu and Balasubramanian, 1988). *Cladosporium tenuissimum* reduced germination of *Uromyces appendiculatus*, *Melampsora pinitorqua* and *Puccinia sorghi* urediniospore by up to 21% *in vitro*, and on detached leaves (Assante et al., 2004). *C. tenuissimum* was found to produce extracellular β -1,3-glucanase, and filtrates of this fungus were able to control bean rust caused by *Uromyces appendiculatus* under controlled conditions (Assante et al., 2004).

Mycoparasitic fungi, which inhabit and obtain nutrients from other fungi, colonize plant pathogenic fungi and can suppress the spread of the diseases they cause (Elad et al., 1998; Falk et al., 1995b; Verhaar et al., 1999b). *Coniothyrium minitans* decreases the amount of sclerotia produced in the soil by *Sclerotinia sclerotiorum* and *S. minor* by the same degree as standard fungicide treatments (Chitrampalam et al., 2011; Chitrampalam et al., 2008; Paulitz and Belanger, 2001). *Aphanocladium album*, *Sporothrix rugulosa*, *Tilletiopsis minor*, and *Verticillium lecanii* parasitized up to 90% of the colony area of *Sphaerotheca pannosa* (causal agent of rose powdery mildew), but only *V. lecanii* is effective at colonization when applied at lower (70%) humidity (Verhaar et al., 1999a).

In the phyllosphere mycoparasitism has been highlighted with the interaction between powdery mildews and the mycoparasite *Ampelomyces quisqualis* (Angeli et al., 2009; Jarvis and Slingsby, 1977; Kiss, 1998). *A. quisqualis* is composed of two distinct clades in the order Pleosporales, and is a generalist mycoparasite which infects 65 species of powdery mildew in eight genera all in the order Erysiphales (Kiss et al., 2004). It invades the powdery mildew hyphae through appressorial-like penetration, and extracellular lytic enzymes including β -1,3-glucanase (Kiss et al., 2004). High relative humidity ranging from 90 to 95% for 10-20 hours

favors *A. quisqualis* spore release and germination (Kiss et al., 2004). Application of *A. quisqualis* inoculum at the start of a powdery mildew epidemic, using a unique application strategy of wick cultures hung from grape vines, significantly reduced epidemic development during a wet year but not during a dry year (Falk et al., 1995a). Commercially available spores of this mycoparasite are nearly as effective as many commercial fungicides (Daoust and Hofstein, 1996). *A. quisqualis* also works in concert with certain fungicides as it is not adversely affected by ergosterol biosynthesis inhibitors (Kiss et al., 2004).

There are several examples of mycoparasites colonizing rusts which, like powdery mildews, are a diverse and economically important group of fungal plant pathogens (Grabski and Mendgen, 1985; Koc and Defago, 1983; Pei and McCracken, 2005). *Simplicillium lanosoniveum* has been found to control soybean rust *Phakopsora pachyrhizi* under controlled conditions (Ward et al., 2010). *Verticillium lecanii* has the ability to parasitize the uredinia of *Uromyces appendiculatus* and *Puccinia striiformis* under high (>80%) relative humidity (Grabski and Mendgen, 1985; Mendgen, 1981). *Aphanocladium album* incubated under high (90-95%) humidity penetrates *P. graminis* urediniospores and germ tubes, and covers rust sori in a white mycelial mat (Koc et al., 1981). *A. album* invades more than 80% of the spores in many rust species including seven species of *Puccinia*, four species of *Uromyces*, *Phragmidium* sp., *Hemileia* sp., and *Melampsora* sp. (Koc and Defago, 1983). *A. album* also induces early development of telia in *Puccinia* spp. under high humidity (Biali et al., 1972). TEM (Transmission Electron Microscopy) analysis reveals that rust urediniospore cytoplasm is destroyed, and sori cavities are overrun with the hyphae of *A. album* (Koc et al., 1981).

The mycoparasite *Sphaerellopsis filum*

Taxonomy, morphology and host range. *Sphaerellopsis filum* (Biv.-Bern ex Fr.) Sutton [Darlucalucal filum (Biv.-Bern. ex. Fr.)] is the most widely researched mycoparasite of rust fungi (Driessen et al., 2004; Keener, 1934; Liesebach and Zaspel, 2004; Paningbatan and Bastasa, 2000; Pei et al., 2010; Szunics and Vajna, 1992; Yuan et al., 1999). *S. filum* is the anamorph of the rarely observed ascomycete *Eudarlucal caricis* (FR.) Eriksson (Ascomycotina, Dothideomycetidae, Pleosporales, Venturiaceae) (Yuan et al., 1998).

Pycnidia of *S. filum* are black, 90-200 μm in diameter, and have distinct ostioles which excrete hyaline 13-18 x 3-5 μm conidiospores in mucilaginous chains known as cirrhi (Plachecka, 2005). Spatial clustering of genotypes in the field, as well as a morphology characterized by gelatinous appendages on the ends of the conidia, indicate that conidia are splash dispersed (Bayon et al., 2007). Each pycnidium produces between 0.8×10^3 and 4.3×10^3 conidiospores (Pei et al., 2010).

The rare teleomorph, *E. caricis*, was well described when it was found on the blackberry rust, *Phragmidium violaceum*, and consists of black-brown stromata with 120-260 μm pseudothecia occurring in locules of varying sizes near pycnidia (Yuan et al., 1998). The bitunicate asci measure 55-90 x 7-11 μm , and ascospores are 1-3 septate, pale yellow and measure 15-24 x 3.5-5 μm (Yuan et al., 1998). It is not certain whether the fungus regularly undergoes sexual recombination, or what triggers it to enter the teleomorphic stage, but there is considerable genetic variation in the population (Bayon et al., 2007; Pei and McCracken, 2005). An extensive two year search on *Melampsora larici-epitea* in England and Germany found no asci or ascospores of *E. caricis*, even though the population underwent a genetic shift during the study, and background levels of *Melampsora* spp. infected with *S. filum* pycnidia were as high as 26% (Bayon et al., 2006).

An extensive host list of *S. filum* shows the mycoparasite colonizing 369 rust species including *Cronartium strobilinum* on *Quercus minima*, *Melampsora larici-epitea* on *Salix* spp., *Phragmidium violaceum* on *Rubus* spp, and *Puccinia graminis* on *Triticum* spp. (Kranz and Brandenburger, 1981). The hosts are all in the order Uredinales, growing from gymnosperm and angiosperm hosts.

As *S. filum* infects such a wide host range there has been an effort to understand its genetic diversity through morphological, genetic and host specificity observations and experiments (Bayon et al., 2007; Bayon et al., 2006; Keener, 1934; Liesebach and Zaspel, 2004; Nischwitz et al., 2005; Pei et al., 2010; Pei et al., 2003; Yuan et al., 1999; Zaspel and Liesebach, 2004). When 19 rust genera from different host species were inoculated with 11 *S. filum* isolates there was no correlation between the *S. filum* host genera of rust, and the rust host it was later able to parasitize (Keener, 1934). More recent studies, however, demonstrate *S. filum* specificity (Bayon et al., 2007; Liesebach and Zaspel, 2004; Pei et al., 2010). Isolates from different hosts varied in their ability and degree to which they colonized other hosts; only six of nine isolates obtained from various rust genera were able to colonize *Melampsora epitea* (Yuan et al., 1999).

ITS (internal transcribed spacer) phylogenetic analysis reveals that there is a significant genetic difference between isolates taken from *Melampsora* spp., and those taken from *Puccinia* spp., but not amongst isolates from the same host (Nischwitz et al., 2005). *E. caricis* falls into four Loculoascomycete-Pleosporalean clades in a grouping that also contains *Leptosphaeria* spp, *Alternaria* spp. and *Ampelomyces* spp. (Nischwitz et al., 2005). Isolates of *S. filum* obtained from various rust hosts show no distinguishing morphological features, and there has been no evidence indicating a geographic or ecological distribution of these isolates (Nischwitz et al., 2005; Pei and McCracken, 2005). These studies indicate that there are specific

species or subspecies of *S. filum*, but these groups are difficult to identify through traditional means such as morphological identification.

Mode of parasitism. All of the *S. filum* isolates, regardless of their taxonomic relationships, have been termed destructive biotrophic mycoparasites (Carling et al., 1976). SEM (Scanning Electronic Microscopy) analysis reveals that urediniospores are penetrated by *S. filum* hyphae, and uredinial cell walls are slightly degraded with enzymes (Carling et al., 1976). In another SEM analysis *S. filum* hyphae was interwoven in the uredinia, and specialized hyphal appendages of *S. filum* resembling appresoria were attached to the surface of urediniospores which had been disintegrated and divested of spikes (Plachecka, 2005). *S. filum* releases enzymes which degrade starch, but not pectin or cellulose (El-Shatter, 1982). Chitinase and xylanase and 1,3- β -glucanase are correlated with the mycelia weight of *S. filum* (El-Shatter, 1982; Liesebach and Zaspel, 2005).

Cultivation and Storage. Artificial solid media containing malt extract, arabitol, mannitol, potato dextrose agar, or lima bean agar support growth and sporulation of *S. filum* (Calpouzios et al., 1957; El-Shatter, 1982; Kuhlman et al., 1978; Pei and McCracken, 2005; Rambo and Bean, 1970; Swendsrud and Calpouzios, 1970). *S. filum* has been shown to germinate on leaf tissue in the absence of uredinia or other additives, but leaf rust urediniospores significantly increase the germination percentage of *S. filum* conidia (Stähle and Kranz, 1984; Swendsrud and Calpouzios, 1970). In place of spores, nitrogen, in the form of ammonium sulfate or biotin, increased mycelial weight of *S. filum*, and was required for its sporulation in artificial medium (El-Shatter, 1982; Rambo and Bean, 1970). To prevent contamination on the media by common saprophytic bacteria, antibiotics including chloamphenicol (10 μ g/ml) and oxytetracyclin (15 μ g/ml) are added to the media (Zaspel and Liesebach, 2004).

Cultures begin to produce cirrhi after incubation at 15°C for two to three weeks under continuous UV light (Liesebach and Zaspel, 2005). A black plectenchyma forms after several

months in culture at 15°C, and sporulation ceases (Lieseback and Zaspel, 2004). Long term spore viability is achieved by storing isolates on uredinia on leaves at -15°C (Lieseback and Zaspel, 2005).

The optimum germination temperature for *S. filum* conidiospores is between 20 and 30°C; however, germination occurs at 8, 15, 20, 25, 30 and 35°C (Rambo and Bean, 1970; Swendsrud and Calpouzos, 1970). In another study spores failed to germinate below 10 or above 40°C, or on media with a pH below 4 or above 9 (El-Shatter, 1982).

Ideal infection conditions. Controlled inoculation and subsequent growth of *S. filum* on rust uredinia is well documented, but the optimal rust age required for maximum infection efficiency remains unclear (Keener, 1934; Pei et al., 2003; Swendsrud and Calpouzos, 1972). The proportion of *Cronartium fusiforme* uredinia that are infected by *S. filum* with 24 hours of free water increases with rust age, from 0% when the two fungi are applied to oak leaves simultaneously, to 85% when *S. filum* was applied to 21 day old rust (flecking was visible 4-6 days after inoculation) (Kuhlman et al., 1978). With 15 days of free water *S. filum* infects 76% of *P. recondita* uredinia when applied simultaneously with the urediniospores, 67% when applied three days after the spores, and 9% when applied three days before the spores are applied to wheat leaves (Swendsrud and Calpouzos, 1972). A different dynamic occurs on *Melampsora epitea*. *S. filum* reduces spore production on *M. epitea* by 64 to 98% when simultaneously applied with spores, but only reduces spore production by 53 to 73% when applied 10 days after the rust spores (pustules erupted after 6-7 days) (Yuan et al., 1999). Whereas applications of *S. filum* severely reduce urediniospore production in established uredinia, *S. filum* has no effect on urediniospore germination when placed together on agar plates (Stähle and Kranz, 1984; Swendsrud and Calpouzos, 1970).

Unlike the variability associated with rust age and *S. filum* colonization of uredinia, longer periods of free water consistently increase the efficacy of the mycoparasite (Kuhlman et al., 1978; Swendsrud and Calpouzos, 1972). Between five and 12 percent of *C. fusiforme* sori are colonized by *S. filum* after four hours of mist duration, and 59 to 67% of sori are infected after 24 hours of moist incubation at 18-24°C (Kuhlman et al., 1978). Prolonging the mist period from three to 15 days at 16-21°C increased the proportion of *P. recondita* uredinia colonized by *S. filum* pycnidia from 26% to 76% (Swendsrud and Calpouzos, 1972). Others report that free water or high humidity is essential for *S. filum* infection, but these observations are not quantified (Keener, 1934; Pei and McCracken, 2005; Yuan et al., 1999).

Effects of *S. filum* on rusts. Once *S. filum* colonizes a rust pustule it is able to reduce sporulation and shorten the life span of the pustule (Kuhlman et al., 1978; Pei et al., 2003). *S. filum* infected 30 to 100% of *Melampsora epitea* uredinia and reduced overall uredinospore production on willow by 64 to 98% (Yuan et al., 1999). On *Melampsora larici-epitea* overall uredinospore production was reduced by up to 78% after 13 days and up to 94.5% after 23 days of incubation with *S. filum* on willow leaf discs (Pei et al., 2003). The overall basidiospore production dropped from 1,525 to 535 per telial pustule infected with *S. filum* (Kuhlman et al., 1978). The lifespan of *C. fusiforme* uredinia and telia are shortened by *S. filum* infection (Kuhlman and Matthews, 1976). *S. filum* on *Puccinia recondita* decreases uredinial life expectancy by 7-10 days in the greenhouse (Szunics and Vajna, 1992).

Reduced rust sporulation and pustule longevity lead to disease suppression on pines, oak, willows and corn in the field. *S. filum* suppressed one rust epidemic caused by *Melampsora epitea* in an established plantation of willow without artificial introduction of the mycoparasite (Yuan et al., 1999). Pycnidia were observed to colonize 91% of the *Cronartium fusiforme* uredinia and 3% of telia in some uninoculated stands of *Quercus minima* (Kuhlman and

Matthews, 1976; Kuhlman et al., 1978). One field application of *S. filum* reduces *Puccinia polysora* severity from 46% to 25% and increases corn yield from 4.7 to 7.3 tons/ha (Paningbatan and Bastasa, 2000). A model based on wheat, *Puccinia recondita* and *S. filum* biology simulated that the mycoparasite could reduce the severity of a leaf rust epidemic by over 80% (Hau and Kranz, 1978).

Potential for biological control. *S. filum* has the potential to become an effective biological control agent. It readily infects a wide range of rust hosts, and its mycelium and pycnidia occupy a large percentage of the rust sori and produce thousands of conidia. It also demonstrates the ability to decrease the life span of a sorus, and significantly reduces urediniospore production. However, most studies have been limited in scope, confined to the controlled conditions of the laboratory or greenhouse, and have not integrated the overall effects of *S. filum* on a rust epidemic in a field setting.

Thesis objectives

The purposes of this research are to quantify (i) the dynamic processes of stem rust survival over the winter on perennial ryegrass, and (ii) the effects that *S. filum* has on the stem rust overwintering dynamics. The specific objectives include obtaining empirical data on perennial ryegrass and stem rust biology to construct a model that simulates the effects of changing biological or meteorological conditions on the overwintering stem rust population. The effects of *S. filum* on stem rust sporulation, viability and pustule longevity are quantified and incorporated into the stem rust overwintering survival model. The model is validated against stem rust overwintering severity observations taken in the field.

PREDICTING OVERWINTERING STEM RUST POPULATIONS ON PERENNIAL RYEGRASS USING A MECHANISTIC, METEOROLOGICALLY-DRIVEN MODEL

Abstract

Stem rust caused by the fungus *Puccinia graminis* subsp. *graminicola* (*Pgg*) can cause wide-spread yield losses on perennial ryegrass grown for seed. There is evidence that the economically important uredinial stage of the fungus (like stripe and leaf rust fungi in some regions) survives over the winter in the Willamette Valley. These overwintering populations are theorized to give rise to epidemics in the spring. In this study we developed a simulation model using data collected from the field that describes the component processes of a *Pgg* population surviving the winter. The model mathematically describes perennial ryegrass plant growth, and *Pgg* infections, pustule latent period, longevity and sporulation dynamics. These processes are integrated with meteorological conditions. We validated the model from datasets obtained at two field locations during two winters. The model was able to predict the general trend of the *Pgg* population over the winter in both years. In the winter of 2009-10, the model accurately predicted springtime *Pgg* populations ($P > 0.05$) at two field sites on the susceptible cultivar. In 2010-11 the model overestimated the observed springtime severity. In an informal sensitivity analysis, spore production and infection condition parameters were varied to simulate their effects on the overwintering *Pgg* population. When pustule spore production was reduced by 20, 40 and 60%, the *Pgg* population was reduced by 74, 92 and 99%, respectively. When infection conditions were constant at 0, 0.125 or 0.25 of the ideal conditions for two latent periods in January, the *Pgg* population was reduced by 82, 46 and 0.0%, respectively from that observed in model calculations over the winter of 2009-10.

Introduction

Quantitative plant disease models have been developed to integrate knowledge of crop and pathogen biology into disease management decision aids (Berger et al., 1995; Coakley et al., 1988; Kushalappa et al., 1984). A review by Savary et al. (2006) addresses four types of decisions that could be influenced by modeling. They include: (i) short-term, within-season decisions, (ii) between-season decisions, (iii) long-term breeding programs and development of IPM strategies, and (iv) research prioritization decisions. Both empirical and simulation models can be used as decision tools in these areas.

Empirical models are derived by regression analysis, and can relate meteorological conditions and crop development, to crop damage or yield loss (Kropff et al., 1995). Empirical models relating disease severity and weather conditions to yield loss through regression analysis have been developed for rust diseases in several studies. A barley leaf rust model accurately correlated disease severity with yield loss using regression analysis (Teng et al., 1979). Yield loss on wheat caused by stripe rust was accurately predicted by using stepwise regression to correlate monthly temperature and precipitation predictors that could be integrated into a comprehensive overwintering model for the Pacific Northwest (Coakley et al., 1988). In another study, maximum leaf rust severity on wheat was accurately predicted by coefficients and equations representing cultivar, planting date and winter meteorological conditions (Moschini and Pérez, 1999). These empirical models can predict the damage threshold of a particular disease over one year, but often overlook the underlying dynamics between the crop and pathogen, which are crucial for development of long-term strategies.

Another method of modeling, simulation, describes the subcomponents of a disease process mathematically based on experimental data. These processes can be integrated into a cohesive model which not only allows for the determination of crop loss, but can also be used to

simulate how changes in the subcomponent processes will affect other factors in the model. Simulation models can also be used as decision support tools for long-term projects such as developing IPM strategies (Savary et al., 2006).

Examples of simulation models include those for soybean rust (Yang et al., 1991) and leaf rust on wheat (Rossi et al., 1997). The soybean rust model is driven by cumulative physiological days (heat units), and daily meteorological conditions including hours of dew and average night temperatures. It uses subcomponents that describe infected and susceptible plant area, pustule latent periods and senescence. The model accurately predicted soybean rust severity over the course of the epidemic with regression B_1 and r^2 coefficients generally higher than 0.85. The leaf rust model simulates the proportion of rusted leaf area by using the same subcomponents that were described in the soybean rust model, with the addition of stochastic variation incorporated into latent period and proportion of spores causing new infections.

A simulation model has been developed also for stem rust on perennial ryegrass caused by the fungus *Puccinia graminis* subsp. *graminicola* Z. urb. (*Pgg*) (Pfender, 2001b; Pfender, 2003). The model accurately predicted *Pgg* disease severity based on daily spring meteorological data inputs, and allowed perennial ryegrass growers to apply fewer fungicides during an epidemic (Pfender, 2001b; 2003; 2004a). Over a five-year trial period this predictive model saved an average of \$96 per hectare in demonstration field tests (Pfender et al., 2008).

The stem rust model also predicts that lower *Pgg* severity in late winter (March) will give rise to less severe epidemics in the late spring (June). This implies that overwinter plant growth dynamics and meteorological conditions if they affect rust population survival will impact the severity of the spring epidemic. Other studies have demonstrated that summer stripe rust epidemics are influenced by meteorological conditions over the preceding winter (Coakley et al., 1982; Coakley et al., 1988; Sharma-Poudyal and Chen, 2011). There is also evidence that

localized stripe rust infection centers spread from leaf to leaf during winter months and give rise to the spring population (Shaner and Powelson, 1973). Further support of overwintering stem rust populations having an effect on *Pgg* epidemic severity was demonstrated in a study where late plantings of perennial ryegrass had significantly lower stem rust severity in late spring than those stands planted early in the fall presumably because late plantings had less fall infection than did early plantings (Pfender, 2004b).

The purpose of this study is to derive a mechanistic model that describes the dynamics of stem rust and perennial ryegrass biology as affected by weather conditions over the winter. The model allows for predictions of the effects of management techniques and weather conditions on the overwintering stem rust population and the severity of the subsequent spring epidemic. The equations driving the model are derived by quantifying (i) plant dynamics including the growth of leaves and tiller formation, (ii) pustule dynamics including pustule longevity and spore production, (iii) spore dispersal, and (iv) weather conditions favorable for infection and pustule development. Growth dynamics for both the host and the pathogen were integrated with weather data into a comprehensive model that was validated with field observations from several experiments.

Methods

Plant growth dynamics. Perennial ryegrass seeds (cv. Morningstar) were planted at Site 1, the Oregon State University Hyslop Experiment Farm in Corvallis (44° 38' N, 123° 12' W), on 28 September 2004, 29 September 2005, and 12 October 2006. Plot planting and maintenance procedures have been described previously (Pfender, 2004b). As plants emerged, a leaf on each of 6 individual replicate plants was tied with colored thread in order to identify the same leaf on future score dates. Leaf length and width (1/3 of the way from the tip) were measured for all leaves of each plant weekly. Weather conditions, including temperature and precipitation data,

were collected with a data logger (CR10X, Campbell Scientific, Logan, UT). The area of each leaf was calculated by multiplying the length and width by an empirically derived correction factor of 0.837 (Pfender, 2004a).

Tiller formation rates were obtained by counting the number of tillers on individual perennial ryegrass plants (cvs. Jet and Morningstar) randomly sampled several times a month. Data were obtained from plants seeded in field plots on 29 September 2005 and 21 October 2006 and from plants seeded in the glasshouse on 1 April 2009.

Inoculation procedure. Urediniospores were obtained each year from multiple-cultivar perennial ryegrass field plantings at Site 1. Spores were dried overnight, stored at -60°C , and heat shocked at 43°C for 1.5 min before use (Pfender, 2004a). A spore suspension was made for each inoculation by adding 1 mg of spore per ml of light mineral oil (Isopar M[®]; ExxonMobile Chemical Co., Houston, TX). Each 500 μl aliquot was mixed immediately before being sprayed onto seven plants (four months old) with a handheld Venturi atomizer operating at 21 kPa. After the oil dried, inoculated plants were placed in a dew chamber at $20 \pm 4^{\circ}\text{C}$ with a 15 h dark period immediately followed by a 4 h light period. Mist was run for 15 min at 2 h intervals during the dark period, and continuously during the light period to keep the leaves consistently moist. At the time of inoculation, a 50 μl sample of the suspension was placed on water agar, and spore germination percentage was determined after 24 h of dark incubation at 23°C .

***Pgg* pustule longevity.** Seeds of perennial ryegrass cv. Morningstar were planted at Site 1 in mid-October in 2000, 2002, 2003, 2004, 2005 and 2010. Field plants at least at the four leaf stage were dug, placed in 10 cm by 10 cm pots, inoculated with *Pgg* spores suspended in a light mineral oil (Soltol[®] 170; Chevron Phillips Chemical Co; The Woodlands, TX), and incubated under ideal infection conditions previously described (Pfender, 2001a). Plants were out-planted to the field the next day and monitored for *Pgg* pustule development over the winters. Leaves with

one or two pustules emerging were tied with a colored string in order to identify the same leaf on future score dates. Scoring dates in different years began throughout the late fall and into the spring. Start dates ranged from 27 October in 2005 to 21 April in 2002. Individual marked leaves and pustules (5 or more individual pustules erupting on the same day were scored each year) were repeatedly observed every 3 to 5 days and given a developmental score ranging from 1 to 4. A score of 1 indicated that the pustule was just emerging, whereas a leaf with a score of 1 was not fully extended. A pustule with a score of 2 was fully eruptent and sporulating, while a leaf with a score of 2 had fully extended. A score of 3 indicated that a pustule had a black necrotic ring forming around its periphery, while a leaf with a score of 3 had started to senesce. When the pustules or leaves appeared to be dead, they were given a score of 4. Only pustules that reached a score of 4 while the leaf was still alive were used in the final analysis of pustule longevity.

Spore production and viability. Plants raised in a glasshouse were vegetatively propagated so that four copies of each of four clones were obtained. All cloned plants were inoculated with a *Pgg* spore suspension and incubated under conditions ideal for *Pgg* infection for 18 h. Spore collection began after inoculated plants were maintained on a glasshouse bench for six days. In the glasshouse, six days generally corresponded with 0.7 *Pgg* latent periods, and flecks of pustules were beginning to show (a *Pgg* latent period equals the heat units required between when an infection was initiated and when it erupted) (Pfender, 2001b). The temperature and leaf wetness was monitored (Watchdog A130, Spectrum Technologies, Plainsfield, IL) to insure leaf wetness conditions were not conducive for additional infections so all pustules would have been initiated on the same day. Spores from open pustules were collected every 2 to 3 days into gelatin capsules fitted on a small vacuum spore sampler. Spores from four pustules from each

plant were collected into one capsule, and spores from four plants from the same clone were combined as one replication; there were four replications in each experiment.

A 100 μ l aliquot of light mineral oil (Isopar M[®]; ExxonMobile Chemical Co., Houston, TX) was added to each capsule, thoroughly mixed with the spores, and the resulting suspension was placed in a hemacytometer (Fisher Scientific, Pittsburg, PA). The spores were counted, and the total number of spores produced per pustule per day was calculated. An additional aliquot (20 μ l) of the spore suspension was spread on a water agar plate, and the spore germination percentage was determined by microscopic observation after 24 h of incubation at 23°C in the dark. Two trials of the experiment were completed. A Weibull (4-parameter) curve was fit to the spore production data using SigmaPlot (SigmaPlot v. 11.0, Systat Software, San Jose, CA).

Spore dispersal. Alloinfection and autoinfection spore dispersal experiments were conducted. The purpose of the alloinfection experiments was to determine the proportion of spores from mother pustules that would cause daughter lesions on nearby plants. The purpose of the autoinfection experiments was to determine the proportion of spores from a mother pustule that would cause daughter lesions on the same leaf and plant that had the mother lesion. The alloinfection experiments were conducted at Site 1. The autoinfection experiments were conducted outdoors near the USDA NFSPRC glasshouse in Corvallis.

Both dispersal experiments rely on information about favorability of weather conditions for infection (Pfender, 2003). *Pgg* spores do not germinate at temperatures ≤ 2 or ≥ 32 °C, and germinate at a maximum rate when they are at 23°C. Leaf wetness is also required for spore germination. Twenty uninterrupted degree-hours of wetness at night combined with over 5 uninterrupted degree-hours of wetness in the morning are ideal for infection. This information was integrated into an equation for proportion of maximum infection = $1 - e^{(-.0031) \times (\text{DHWIndex})}$, where

DH_w Index is the product of morning and overnight wet degree-hours, multiplied by a correction factor of 0.64 if the wet period is interrupted (Pfender, 2003).

Seeds of perennial ryegrass cv. Jet, highly susceptible to stem rust, were planted on soil (Sunshine Growing Mix; Sun Gro Horticulture Inc., Bellevue, WA) in pots ("Cone-tainer", 2.5 x 12 cm; Stuewe & Sons Inc., Corvallis, OR), one seed per pot. They were kept constantly moist until three leaves emerged, after which the pots were irrigated by placing them in water for two days every week, and fertilized with a solution of 6 mg N, 5.4 mg P₂O₅ and 6 mg K₂O in 4 liters water every two weeks. Plants were grown in a glasshouse maintained at day and night temperatures of 20°C and 15°C, respectively, with 12 h days, and periodic trimming to a height of 10cm until time of inoculation 2 to 5 months after planting.

Plants with five or more leaves and beginning to tiller were used in the alloinfection experiment. These plants were inoculated with a *Pgg* spore suspension of approximately 1×10^5 spores per ml. One inoculated plant with between 5 and 15 emerging lesions was placed in the center of a rack of non-inoculated plants just before lesions emerged (Fig. 1). Non-inoculated plants were placed next to the inoculated plant and one another in the rack so that the center of each plant was approximately 3 cm from the center of the next plant. Additional non-inoculated plants were placed in a circle (radius of 30.5 cm) around the inoculated plant with a cross pattern of plants running through the center of the circle. Black plastic was stretched across each rack, around the plants, so there would be no air movement between the openings in the rack. Each trial (three replications) was paired with three replications of check racks that had a non-inoculated plant in the center. The racks were placed in holes dug in the field so that the experimental plant base was level with the field plant base. Potting soil was placed on the plastic around the racks so that the plants in the trays had soil conditions similar to the field plants. Each trial was left in the field for three to seven days and weather data were collected

(CR10X, Cambell Scientific, Logan, UT). After the 3 to 7 days exposure, and before transporting from the field, erumpent pustules on the source plant were covered with water-based glue with a toothpick (Elmer's wood glue, Elmer's products Inc., Columbus, OH) to immobilize the spores, and the center plant was removed and discarded. Plants were placed under ideal infection conditions in a dew chamber and scored for lesions after approximately 1.5 *Pgg* latent periods. Four trials of the experiment were conducted (15 December, 26 January, 17 March and 8 April) over the winter of 2010-11.

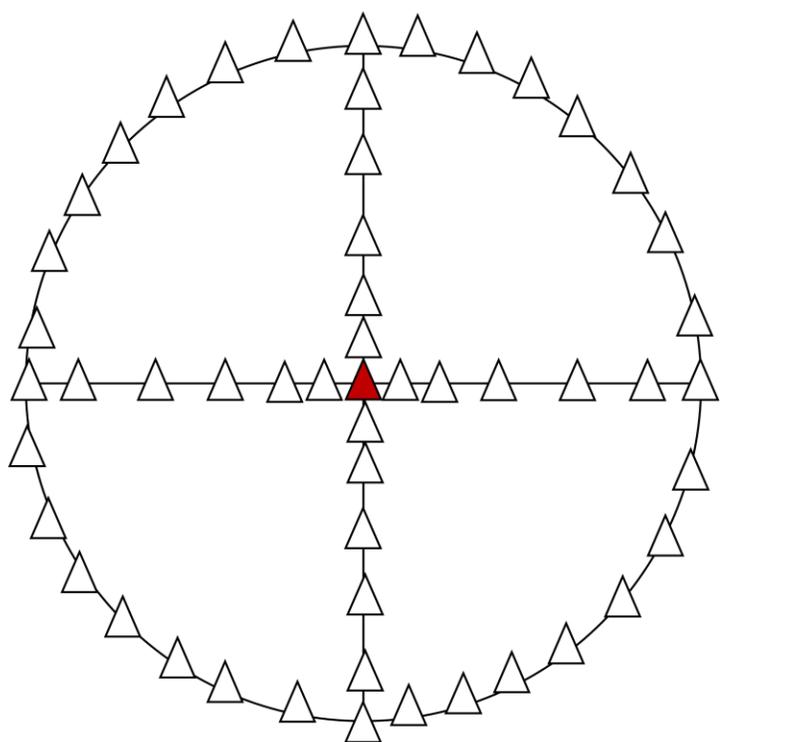


Fig. 1. Diagram of the alloinfection experimental layout. A perennial ryegrass plant (cv. Jet) was inoculated with a *Puccinia graminis* subsp. *graminicola* spore suspension and incubated under ideal infection conditions. The inoculated plant was placed on a glasshouse bench until 5-15 pustules were seen emerging. The inoculated plant was then placed in the center (solid triangle) of a rack with non-inoculated plants (open triangles). Remaining open spaces in the rack were covered with a sheet of plastic to prevent air flow. The rack was placed in the field before the inoculated plant's pustules erupted. The bar is equal to 30.5 cm.

The purpose of the autoinfection experiment was to determine the proportion of spores that caused infections on the leaf that held the mother lesion. Plants with five or more leaves were inoculated with a *Pgg* spore suspension of approximately 1×10^4 spores per ml. A small painter's brush was used to inoculate one spot on one leaf of each plant. The plants were then placed under ideal infection conditions. Just before lesions emerged, one inoculated plant with one emerging lesion was placed in a row of non-inoculated plants in a rack (Fig. 2).

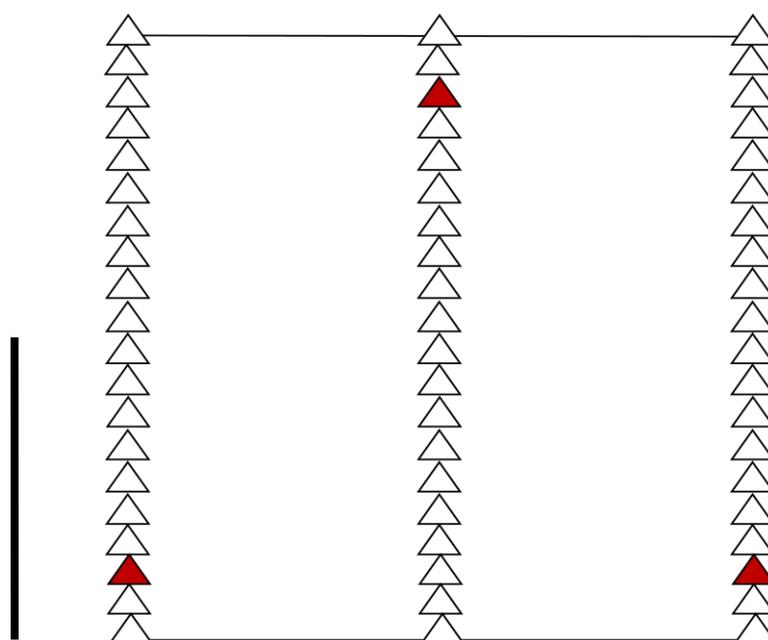


Fig. 2. Diagram of the alloinfection experimental layout. A perennial ryegrass plant (cv. Jet) was inoculated with a *Puccinia graminis* subsp. *graminicola* spore suspension using a small artist brush so that only one spot on one leaf was inoculated. The plant was then incubated under conditions ideal for *Pgg* infection. The inoculated plant was placed on a glasshouse bench until 1 pustule was seen emerging. The inoculated plant (solid triangle) was then placed in a rack with non-inoculated plants (open triangles). Remaining spaces in the rack were covered with a sheet of plastic to prevent air flow. The rack was placed in the field before the inoculated plant's pustules erupted. The bar is equal to 30.5 cm.

Each plant was placed approximately 3 cm from the center of the next plant. Each row of non-inoculated plants was 30.5 cm apart from one another, and black plastic was stretched

between the rows to prevent air flow. Each trial consisted of three replications of one inoculated plant in a center of a row of non-inoculated plants. A non-inoculated plant was placed in the center of three additional rows to act as a control. Each trial was placed outdoors in a hole dug in the ground so the base of the experimental plants was level with the soil. Trials were brought in to the glasshouse after weather conditions conducive to *Pgg* infection were recorded, and plants were maintained on the bench under conditions that did not permit further infections. Trials were scored after 1.5 *Pgg* latent periods. Experimental trials were initiated on 23 June and 16 August, 2011.

Integrating the model. Equations were derived that described the observed dynamics of plant growth, pustule longevity and spore production. These equations were integrated into a spreadsheet (Microsoft Excel 2007, Redmond, WA) with additional functions for infection favorability (Pfender, 2003) and spore dispersal. Model details are described in the Results section and in Appendix A.

Model training. The model was calibrated on *Pgg* severity data taken from six inoculation experiments, each done on a different month, over the 2002-03 winter. Perennial ryegrass seeds of the rust-susceptible cv. Morningstar were planted in the field in rows on 26 September. At monthly intervals (23 October, 25 November, 3 January, 28 January, 25 February, and 25 March), plants (6 replications of 10 cm of row) were dug from the field, placed in pots, inoculated with a *Pgg* spore suspension (6mg spores per ml of Soltrol® 170), and incubated under conditions favorable for infection in a dew chamber. Inoculated plants (10 cm of row) and non-inoculated control plants were out-planted (30.5 cm apart) into existing rows in a field of cv. Manhattan 4 (moderately resistant to *Pgg* infection) the day after they were inoculated. Weather data including leaf wetness and temperature measurements were taken on a datalogger (CR10X, Campbell Scientific, Logan, UT) during the winter. Individual plants were

scored in place repeatedly for rust severity weekly. Care was taken not to disturb the pustules by touching the plants. *Pgg* severity plotted against *Pgg* latent periods since planting were the observed data sets to be modeled. The model was run using inputs of weather data and the size of the first cohort of erumpent pustules observed in the field. The model parameters (latent period, pustule longevity, spore production, alloinfection and autoinfection, and the number of initial infections on each leaf cohort) were adjusted so that the model output number of erumpent pustules approximated the number of erumpent pustules observed in the field. The adjusted parameters of each of the six trials were averaged to produce the parameter values for latent period, alloinfection and autoinfection.

Model validation. The model was tested with *Pgg* severity data taken at two field sites over the winters of 2009-10 and 2010-11. Site 1 is described above. Site 2 was a commercial perennial ryegrass field in Junction City, Oregon (2009-10 at 44° 23' N, 123° 20' W, and in 2010-11 at 44° 29' N, 123° 20' W). Perennial ryegrass plants (10 cm of row) were dug from Site 1 in November, inoculated with *Pgg*, incubated overnight under ideal infection conditions, and then replanted in the field with an equal number of plants that were non-inoculated and acted as controls.

Individual plants (cvs. Jet and Morningstar) were scored weekly for *Pgg* severity. At Site 1 each replication consisted of two inoculated plants spaced 0.6 m (two rows) apart from one another (four replications planted 1.5 m apart), and out-planted into cv. Jet in 2009 and Manhattan 5 in 2010. At Site 2, a replication consisted of one plant (six replications). Each replication was spaced 5 m apart, and out-planted into cv. Silver Dollar in 2009 and 2010.

Modeled effects of changing *Pgg* spore production and infection conditions. Once validated, the model was run to simulate the effects of altered spore production and infection conditions on the *Pgg* population over the 2009-10 winter at Site 1. To model the effects of reduced *Pgg* spore production, the model was run with spore production curves that were set at 0.8, 0.6 and

0.4 of the curve calculated in Fig. 5. To test the effects of weather conditions on the overwintering *Pgg* population, the model was run with infection conditions set at a constant daily infection value of 0, 0.125, 0.25, or 0.5 of that expected under ideal infection conditions for two continuous *Pgg* latent periods starting on 15 January. These weather conditions were chosen to simulate the infection spectrum between dry, cold (unfavorable for infection) and moist, warm (favorable for infection) winter conditions that can occur in the Willamette Valley.

Data analysis. Field observations and the model output were plotted against time. The area under the disease progress curve (AUDPC) between 1 March and 15 April was calculated in order to compare the model output with the observed *Pgg* population. The area was chosen to analyze instead of a slope or point measurement because of the variability noted from observation to observation in this data set. Compared to the single-time measurements the area gives a more general measure of population size in the spring at the start of the cropping-season epidemic. AUDPC comparisons between the model output and the observed values were done by constructing 95% confidence intervals around the difference of the means (modeled minus observed).

Results

Plant growth dynamics. Individual leaf areas from 1st-year planted perennial ryegrass cv. Morningstar were calculated from measurements taken over three winters. These areas were used to mathematically describe leaf growth and senescence from planting date as a function of cumulative *Pgg* latent periods. Curves derived from each of the three years were averaged (Fig. 3A) so that each leaf cohort was described by a single equation (Fig. 3B). Tiller formation was observed on two cultivars in two years. Secondary tillers formed 2.67 *Pgg* latent periods after planting. An exponential curve (r^2 of 0.60) was fit to the observed number of tillers plotted against cumulative *Pgg* latent periods (Fig. 4).

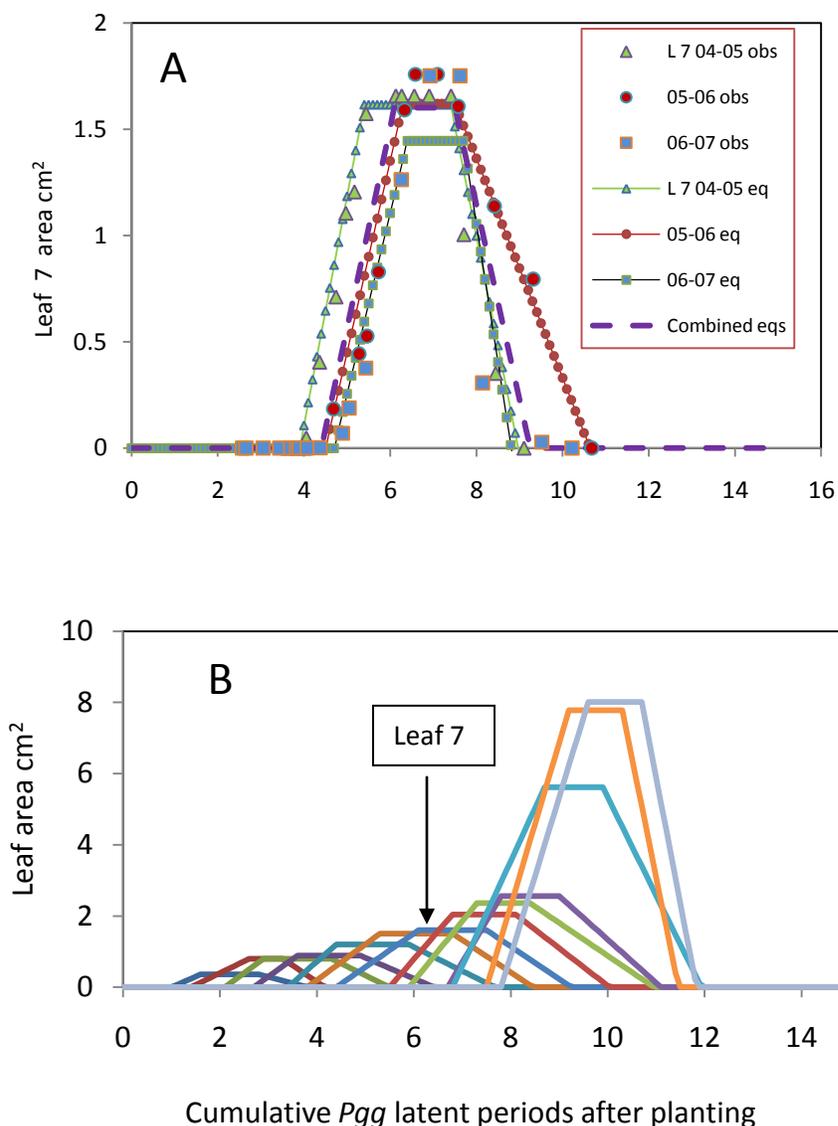


Fig. 3. Area of individual perennial ryegrass leaves as a function of time expressed in *Puccinia graminis* subsp. *graminicola* (*Pgg*) latent periods. **Fig. 3A** shows leaf 7 (L7) as an example of how leaf growth equations were calculated. Seeds of cv. Morningstar were planted in the field in late September or early October in 2004, 2005 and 2006. Leaf lengths and widths from each leaf cohort (6 replications) were measured for the newly-seeded plants over the winters of 2004-05, 2005-06 and 2006-07. Observed data (symbols in 3A) were used to calculate leaf emergence, expansion and senescence equations in 2004-05, 2005-06 and 2006-07 (solid lines with symbols in 3A). The average of the parameter estimates of the equations specific to each year were used to calculate a mean combined equation for each leaf (thick dotted line in 3A). The mean equations for leaf cohorts 1 through 13 (leaf 7 is shown with an arrow) representing plant growth over the winter since planting are plotted against *Pgg* latent periods (**Fig. 3B**).

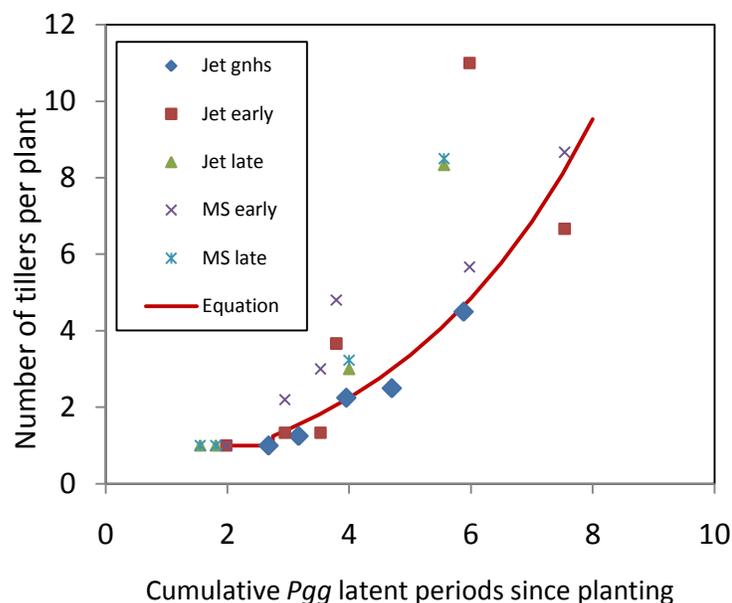


Fig. 4. Observed values and a derived curve showing the cumulative number of tillers on a single perennial ryegrass plant. Seeds of cv. Jet and Morningstar (MS) were planted early (September) or late (October) in the field (2005-06) or glasshouse (2009) and randomly-sampled plants (4 replications) were observed for the number of tillers every few weeks. Symbols represent the means from each trial. An exponential curve ($\exp^{(.2943x)}$), which starts at 2.67 *Puccinia graminis* subsp. *graminicola* (*Pgg*) latent periods, is fit to all tiller data (adjusted r^2 0.60) and is shown by the solid line. Data are plotted against *Pgg* latent periods since planting.

Pustule dynamics. *Pgg* pustules on perennial ryegrass were rated for developmental stage in 11 experiments (between 5 and 20 pustules were scored during each experiment) over six winters. On average, pustules lived 1.63 ± 0.06 *Pgg* latent periods after they erupted (Fig. 5). The average spore viability across the life of the pustule was $94 \pm 2.2\%$, and no significant ($P > 0.05$) trend was observed across sampling times. The product of spore production and viability is plotted against cumulative *Pgg* latent periods, and a Weibull (4-parameter) curve is fit to the data (Fig. 6). The standard form for the Weibull curve is $f(x) = a * ((c-1)/c)^{((1-c)/c)} * (\text{abs}((x-x_0)/b + ((c-1)/c)^{(1/c)}))^{(c-1)} * \exp(-\text{abs}((x-x_0)/b + ((c-1)/c)^{(1/c)})^c + (c-1)/c)$ only if $(x \leq x_0 - b * ((c-1)/c)^{(1/c)})$. The parameter estimates for the *Pgg* spore curve are: $a=5000$, $b=5.44$, $c=1.12$ and $x_0= 1.4$. The

parameter estimates for the *Pgg*-only treatment for the spore curve multiplied by spore viability are $a = 4000$, $b = 0.4$, $c = 1.1 \times 10^3 = 1.02$. The parameter estimates for the *S. filum* treatment for the rust spore curve multiplied by spore viability are $a = 3200$, $b = 0.19$, $c = 1.5 \times 10^3 = 1.01$. A pustule produced $3.9 \times 10^4 \pm 3.3 \times 10^3$ spores during its lifetime, and 84% of those spores were produced within the first latent period after erupting. There was no significant effect of plant genotype on pustules spore production ($P = 0.64$).

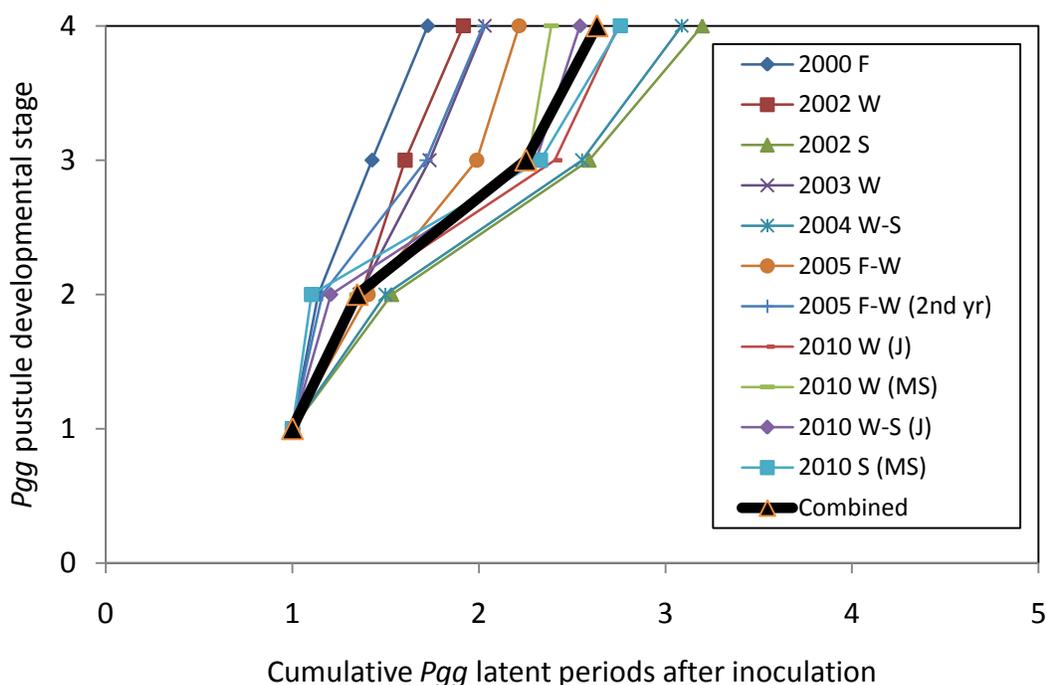


Fig. 5. *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustule longevity plotted as a function of *Pgg* cumulative latent periods since infection. Perennial ryegrass plants cvs. Jet (J) and Morningstar (MS) were inoculated with a low concentration *Pgg* spore suspension, incubated under ideal infection conditions, and placed outside during the fall (F), winter (W) or spring (S) in several different years between 2000 and 2010. There were between 5 and 20 pustules per experiment. Inoculated pustules on leaves with 1 to 2 pustules were observed several times a week, and scored for pustule and leaf condition. The pustule condition categories are: 1=just emerging; 2=well developed, healthy; 3=black edges around the pustule perimeter, starting to die; and 4=black with no detectable spore production. Pustules that died after senescence of the host leaf tissue were omitted from the analysis. Symbols represent the means from each trial, and the heavy line represents the average of all of the trials.

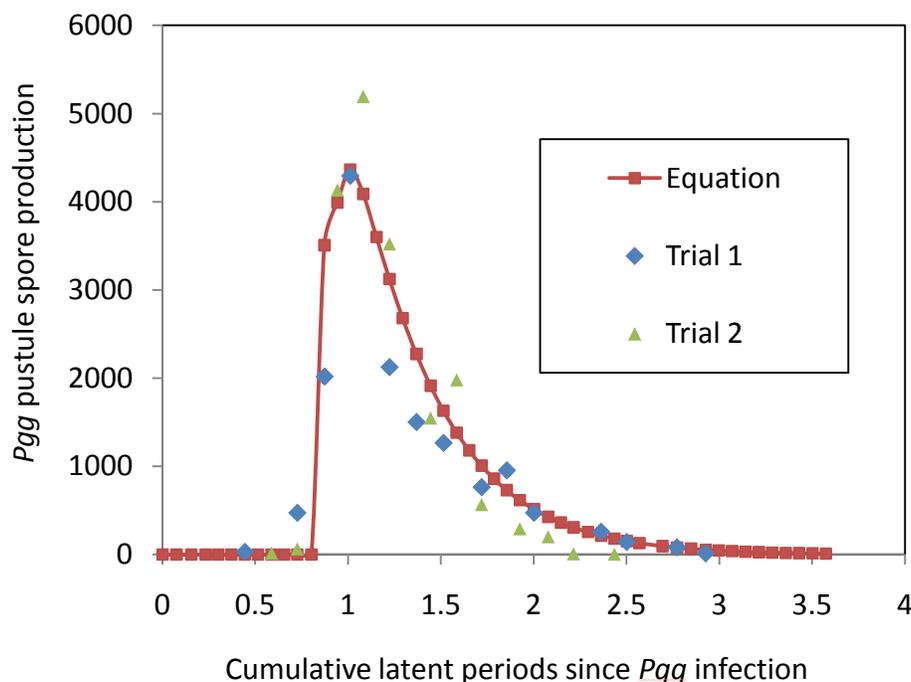


Fig. 6. *Puccinia graminis* subsp. *graminicola* (*Pgg*) spore production plotted as a function of cumulative latent periods since infection. Perennial ryegrass cv. Jet plants were inoculated with rust and incubated for seven days (approx. 0.7 rust latent periods). Spores were collected from individual *Pgg* pustules every two to three days, counted and tested for viability. Data points are the mean (two trials with four replicates of four plants) of the daily spores produced per pustule multiplied by the spore viability. A Weibull (4-parameter) curve (see text) was fit to data normalized with the number of cumulative latent periods that pustules lived under winter field conditions (Fig. 5). Symbols represent the mean sporulation for each trial; lines represent the average Weibull curve.

Spore dispersal. Plants with many *Pgg* pustules (alloinfection experiments) or one *Pgg* pustule (autoinfection experiments) were placed in the field with non-inoculated plants. The number of spores produced from individual mother lesions was estimated from the number of *Pgg* latent periods the pustule(s) were in the field and the spore production curve (Fig. 6). The proportion of spores giving rise to pustules (number of daughter pustules divided by estimated number of spores) was calculated and plotted against distance from the mother lesion. Some 84% of the pustules originated on the same plant as the mother lesion (Fig. 7). The proportion of spores

that caused infections on the same leaf as the mother lesion was $4.3 \pm 2.6 \times 10^{-4}$ pustule per spore produced, and the proportion of spores that caused infections on the same plant as the mother lesion, but not on the same leaf was $2.0 \pm 1.7 \times 10^{-4}$ pustules per spore produced.

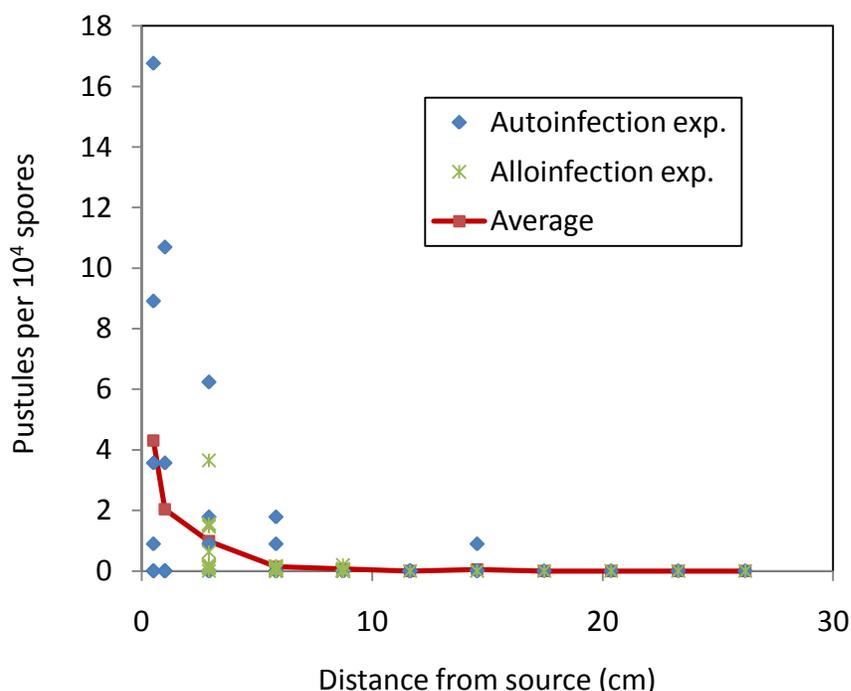


Fig. 7. The proportion of *Puccinia graminis* subsp. *graminicola* (*Pgg*) spores causing erumpent infections as a function of distance from a source. Perennial ryegrass plants cv. Jet were inoculated with a spore suspension, and incubated under ideal infection conditions overnight. In the alloinfection experiments (star symbols) plants with 5 to 15 *Pgg* pustules beginning to emerge (3 replications) were placed in the center of a rack of non-inoculated plants (Fig. 1). The plants were placed in the field for several days, then recovered and placed under ideal infection conditions overnight. These alloinfection experiments were repeated four times. In the autoinfection experiments (diamond symbols) inoculated plants with one emerging pustule (3 replications) were placed in rows of non-inoculated plants (Fig. 2) until overnight conditions were conducive for *Pgg* infection. Plants were brought into the greenhouse where conditions were not conducive to additional infection. The autoinfection experiment was repeated twice. The number and location of pustules was determined 1.5 *Pgg* latent periods after the plants were brought into the greenhouse. A spore production curve (Fig. 6) was used to calculate how many spores were produced during each trial. This number was used to calculate the proportion of spores that were able to cause infections at different distances away from the source pustules. For the autoinfection experiments a correction was made also for the relative favorability for infection of the overnight weather.

Integrating model parameters. Plant and pustule dynamics, spore dispersal and meteorological parameters obtained from experimental data were integrated into a deterministic model and are described (Appendix 1). The model was run on a daily time-step; it was initiated with the infections on each leaf on the inoculation date, and weather data (temperature and leaf wetness) were input as driving variables. The input number of initial infections was calibrated so that the first cohort of erumpent pustules from the model output matched that of the observed number of erumpent pustules on 10 cm of row, accounting for the fact that leaf senescence causes some infections to die before becoming erumpent.

After initiating the model run, the model first determined how many spores were produced on each leaf by calculating the product of the number of erumpent pustules on each leaf by the number of spores produced by each cohort on that leaf. The spores produced from each pustule cohort was determined independently; as each cohort erupted it produced spores according to the spore production curve (Fig. 6). The number of spores produced on each leaf on each day was used in an equation that described the number of infections initiated on each leaf. The total number of spores produced on each leaf was multiplied by experimentally determined autoinfections or alloinfection coefficients, respectively, providing a relative number of alloinfection or autoinfections (Fig. 7).

The number of infections initiated on each leaf was determined by multiplying the number of alloinfections by the proportion of total leaf tissue that a given leaf comprised on a given day and the relative weather-based infection efficiency on a given day. These infections were added to the number of infections initiated by autoinfection spores. A poisson correction was included in the model to account for the reduced susceptible leaf area occupied by the already established infections and spores that had landed that day on the remaining infection sites. The infected site correction is equal to \exp^{-m} where m is proportion of sites on a leaf

cohort already occupied by an established infection or another spore. Although this equation approximates the potential number of occupied sites, a different equation which first subtracts the number of sites occupied by erumpent pustules will need to be incorporated into future versions of this model. With the current occupied site correction the model will slightly overestimate the number of new infections occurring on any leaf cohort.

Each cohort of infections initiated on each leaf was tracked to determine when they erupted, how many spores they produced and when they died. Each cohort of infections did not emerge until one *Pgg* latent period had elapsed. These infections lived until 2.63 latent periods had elapsed or until the leaf tissue they were on died. The proportion of leaf tissue that senesced in a given cohort on a given day was used as the proportion of pustules that died on that day.

The model was trained on 2002-03 field data from plants inoculated with *Pgg* and observed over the winter (Fig. 8). In training the model, the *Pgg* latent period was extended from 1 to 1.15, and the dispersal coefficients for both alloinfection and autoinfection rates were shifted from 4.3×10^{-4} and 3.27×10^{-4} respectively, to 1.15×10^{-4} , and 5×10^{-5} , respectively.

Model validation. The model was validated with two years of field data on cvs. Jet and Morningstar at Site 1 (Fig. 9) and Site 2 (Fig. 10). The model followed the general population trend observed in both years on both cultivars. The area under the curve was taken from 1 March to 15 April on all data sets. In 2009 the model predicted *Pgg* levels that were not significantly different from those observed on cvs. Jet and Morning star (Table 1). In 2010 the model predicted a population that was higher than those observed; however, this difference could not be analyzed for significance as all observed values were zero.

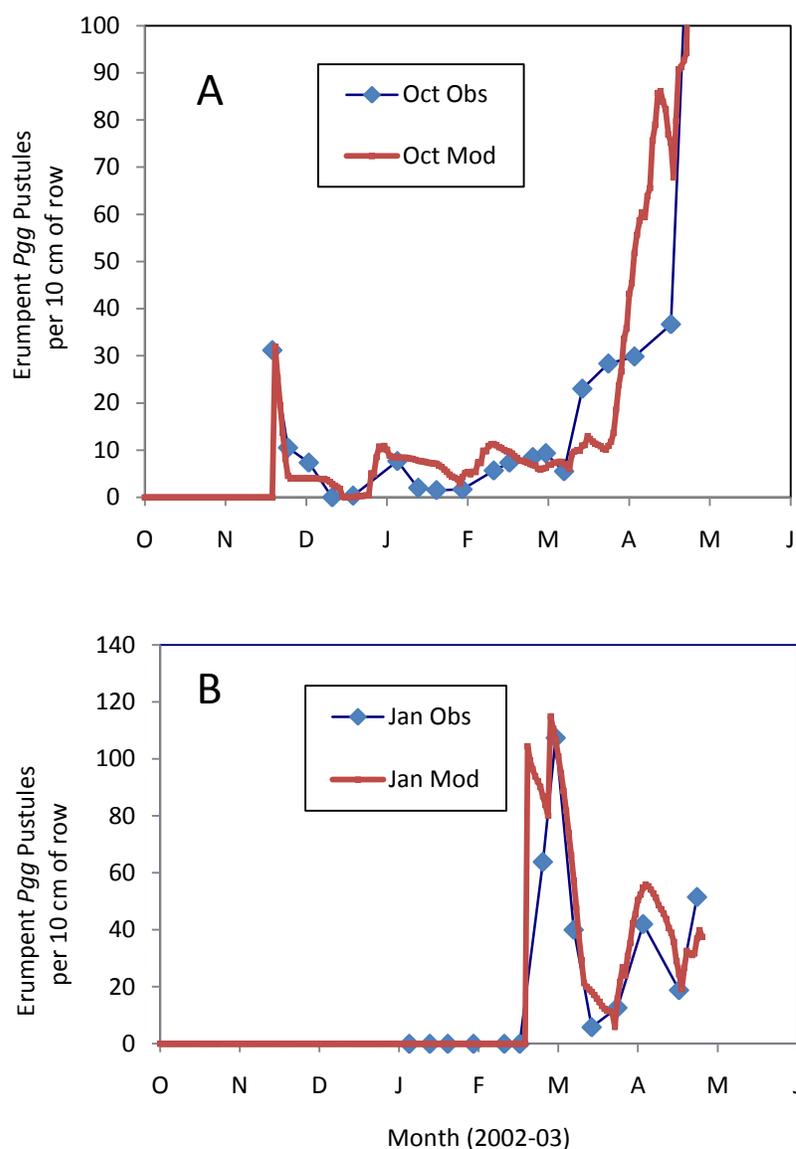


Fig. 8. Model output trained to observed field data. Perennial ryegrass plants cv. Morning Star were dug from the field, inoculated with *Puccinia graminis* subsp. *graminicola* (*Pgg*), incubated overnight under ideal infection conditions and immediately out-planted to the field in October, November, January, February or March of 2002-03 (6 replicate plants on each date). The overwintering model, complete with plant growth, pustule longevity, spore production and spore dispersal parameters was run so that the first cohort of erumpent pustules in the model was approximately equal to the observed number of pustules. Latent period and dispersion parameters were then adjusted so that the model was closely aligned with the observed pustule data. Symbols represent the mean of the observed data, and lines represent the model output starting with inoculations on 23 October (**Fig. 8A**) or 3 January (**Fig. 8B**).

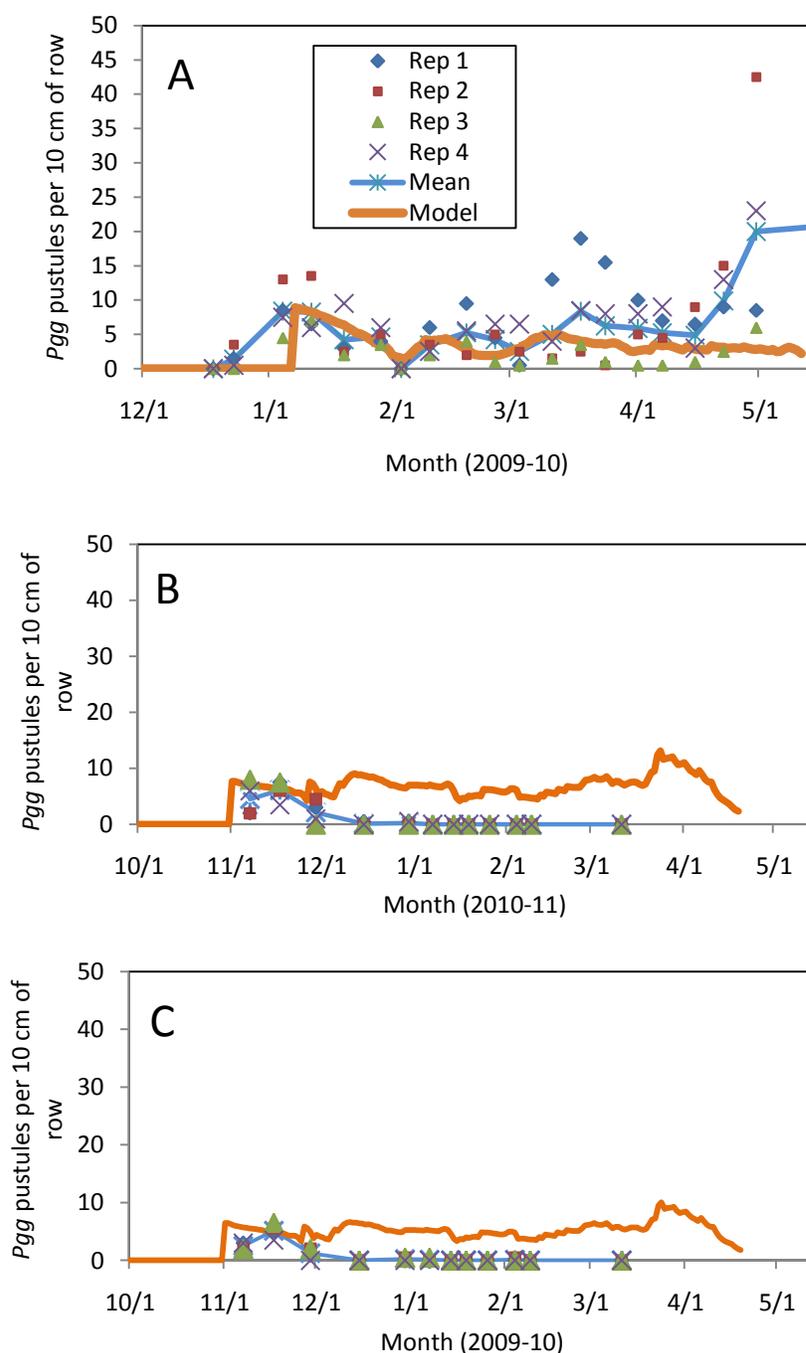


Fig. 9. Overwintering model estimate (heavy solid line) plotted with the observed number of *Puccinia graminis* subsp. *graminicola* (Pgg) pustules at Site 1 in 2009-10 (**Fig. 9A**) and 2010-11 (**Figs. 9B and 9C**). Plants of perennial ryegrass cv. Jet (**Figs. 9A and 9B**) and Morningstar (**Fig. 9C**) growing at Site 1 were dug, inoculated with a Pgg spore suspension, incubated overnight under ideal infection conditions, and out-planted to the field. Four replications of two plants were scored for Pgg severity every week. The model was run with plant growth, pustule longevity, pustule spore production, and spore dispersal parameters that were trained with 2002-03 field data (Fig. 8).

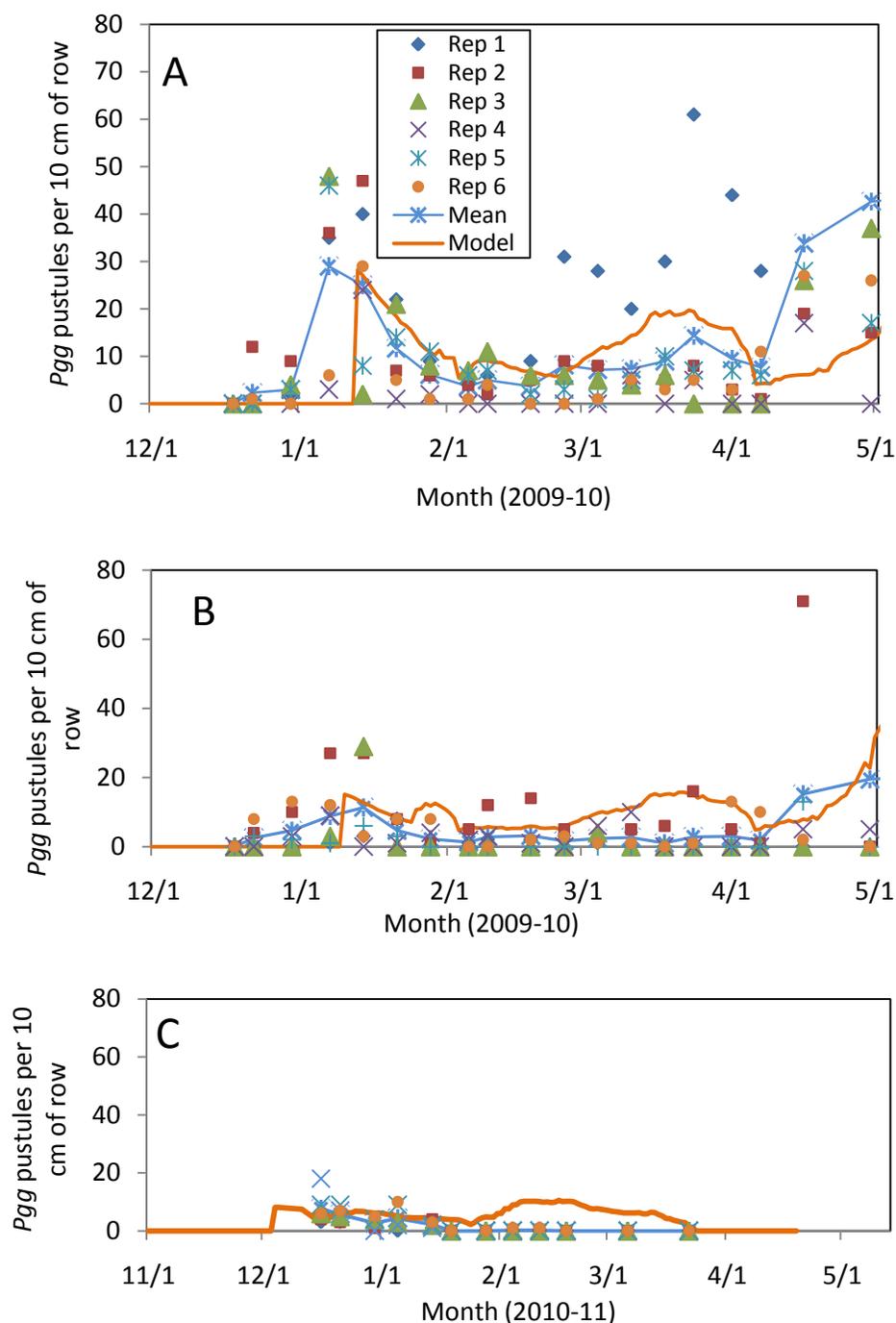


Fig. 10. Overwintering model estimate (heavy solid line) plotted with the observed number of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules at Site 2 in 2009-10 (**Figs. 10A and 10B**) and 2010-11 (**Fig. 10C**). Plants of perennial ryegrass cv. Jet (**Figs. 10A and 10C**) and Morningstar (**Fig. 10B**) growing at Site 2 were dug, inoculated with a *Pgg* spore suspension, incubated overnight under ideal infection conditions, and out-planted to the field. Six replications were scored for *Pgg* severity every week. The model was run with plant growth, pustule longevity, pustule spore production, and spore dispersal parameters that were trained with 2002-03 field data (Fig. 8).

Table 1. Differences associated with the area under the disease progress curve (AUDPC) from 1 March to 15 April calculated from the model output and the observed values. Observed values were taken from plants cvs. Jet and Morning Star (MS) that were inoculated in the fall with a *Puccinia graminis* subsp. *graminicola* (*Pgg*) spore suspension, incubated overnight under ideal conditions and out-planted to the field where they were scored weekly. Data were taken at Site 1 (4 replications) and Site 2 (6 replications) during the winters of 2009-10 and 2010-11. Model values (Fig. 9 and 10) were obtained by running a model that incorporated plant growth, pustule longevity and spore production and dispersal parameters against cumulative *Pgg* latent periods. The mean observed area was subtracted from the model area and 95% confidence intervals were constructed around the observed means to determine significance.

Year	Site	Cultivar	AUDPC observed	AUDPC model	Mean Difference (95% CI)
2009-10	1	Jet	243	151	-91 (-349, 166)
	2	Jet	512	571	60 (-560, 679)
	2	MS	156	481	324 (118, 532)
2010-11	1	Jet	0	197	...
	1	MS	0	149	...
	2	Jet	0	107	...
	2	MS	0	65	...

Effects of changing model parameters. Once validated, the model was used to predict the effect of reduced spore production or unfavorable meteorological conditions on *Pgg* population development. Reducing pustule spore production by 20, 40 or 60% reduced the disease severity from 1 March to 15 April by 74, 92 and 99%, respectively (Fig. 11A).

If infection conditions were changed to 0, 0.125, or 0.25 times that of ideal infection conditions for 2 continuous latent periods starting on 15 January, then the disease severity from 1 March to 15 April was reduced by 82, 46 and 0.0%, respectively (Fig. 11B). If the infection conditions were set at 0.5 times that of ideal infection conditions for 2 continuous latent periods starting on 15 January, then the disease severity was predicted to be 1.4 times greater than that modeled with actual weather data from 2009-10. A theoretical fungicide that did not allow *Pgg* infections to initiate 1 latent period before application and 2 latent periods after application was

also simulated into the model with a 15 January application date. The model predicted that the fungicide would reduce disease severity by 100% between 1 March and 15 April.

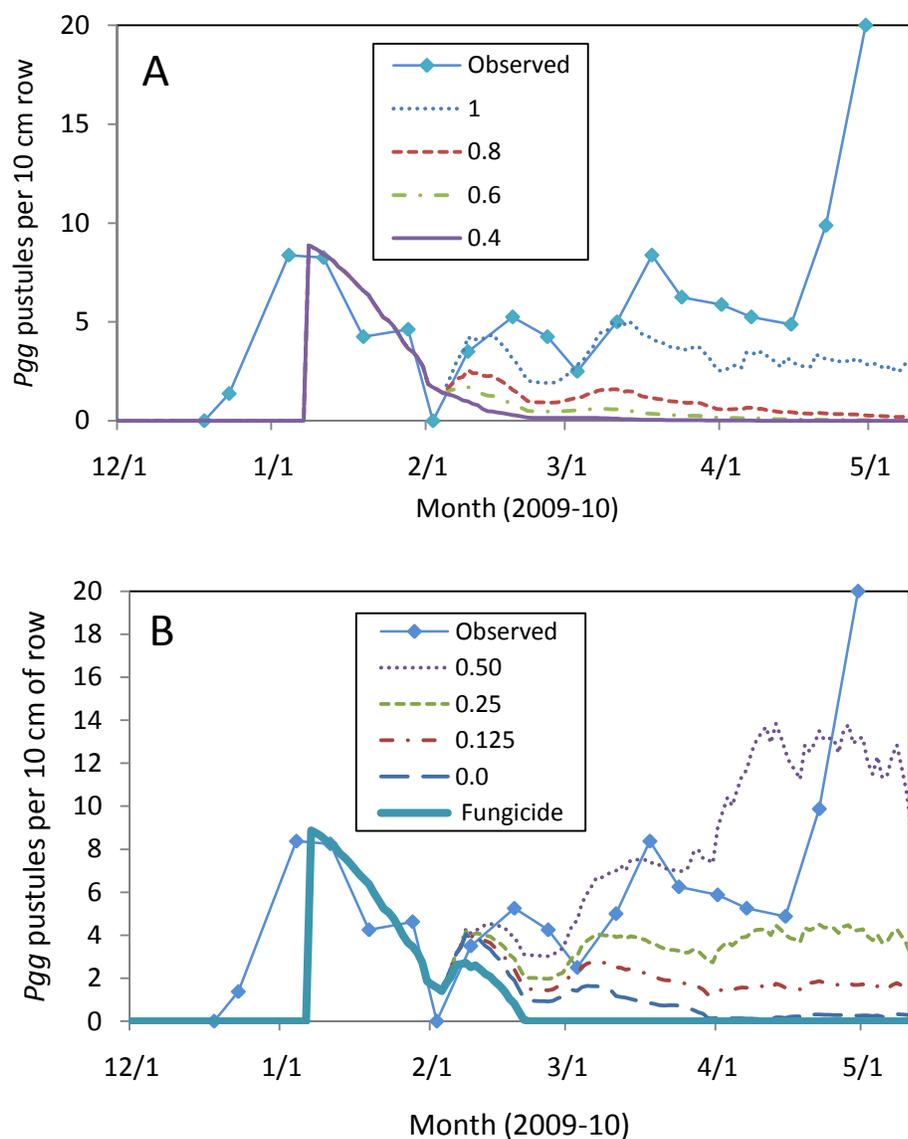


Fig. 11. The effects of changing *Puccinia graminis* subsp. *graminicola* (*Pgg*) sporulation (**Fig. 11A**) or infection condition (**Fig. 11B**) parameters on the modeled overwintering population. An overwintering model was run using 2009-10 disease severity data at Site 1. In **Fig. 11A** the spore production curve in the model was adjusted so that the curve parameters (*a* and *b*) were 1, 0.8, 0.6, or 0.4 times the value used in the parameterized model (Fig. 5). In **Fig. 11B** infection conditions were set to 0.0, 0.125, 0.25, or 0.5 times that of optimal *Pgg* infection conditions for 2 continuous *Pgg* latent periods starting on 15 January. A theoretical fungicide that killed *Pgg* infections that started 1 latent period before application and 2 latent periods after application was also simulated, with a 15 January application date.

Discussion

Empirical and simulation models have been developed that accurately predict cropping-season rust severity (Coakley et al., 1988; Pfender, 2003; Rossi et al., 1997; Yang et al., 1991). In this current study we developed an overwintering *Pgg* population simulation model that integrates the effects of plant and pustule dynamics, spore dispersal and meteorological conditions. The model generally predicted the *Pgg* population trend over the winter. In 2009-10 the model accurately predicted the severity of the *Pgg* population from 1 March to 15 April on cultivar Jet at two sites, and predicted a severity that was higher than expected on the Morningstar cultivar. Although the model correctly predicted the *Pgg* population to be lower in 2011 than in 2010, it nonetheless over-predicted the actual size of the 2011 population at both sites and on both cultivars.

Parameters in the model, except those for spore production and viability, were obtained from winter field studies over a ten year period, and were thought to be representative of the winter field environment. The spore production curve describing an individual *Pgg* lesion was calculated using data taken from pustules in the glasshouse under dry and warm (15-25°C) conditions. We collected spores under these conditions in order to reduce the number of confounding errors associated with winter field conditions, including spores washed away by rain or dispersed by wind. However, the pustules under these conditions lived approximately twice as long (expressed in *Pgg* latent periods) as the pustules observed in the field. We normalized the spore production curve so that it spanned only the 2.63 latent periods observed during winter field conditions. There may be factors other than heat, such as rain or daylight length, that affect pustule longevity and spore production. These factors could be further researched to get a more complete understanding of rust biology and to possibly increase the accuracy of the overwintering model. At its peak, the sporulation per pustule we observed for

stem rust on perennial ryegrass was approximately 1.7 and 2.25 times greater than that calculated for leaf and stripe rusts, respectively on wheat (Sache, 1997; Sache and Vallavieille Pope, 1993). However, pustules of all three rusts lived approximately 35 days after infection and followed a similar spore production pattern. The pustules from which spores were collected in our experiments were alone or with one other pustule on a leaf. They were also the first to erupt and were easy to see. Therefore, these pustules may have been larger than average (personal observation), and may have produced more spores than those that were smaller or on leaves with many other pustules (Newton et al., 1998; Sache, 1997). We therefore suggest that the spore production curve (Fig. 4) will describe a pustule producing more spores than would be produced by small or crowded pustules.

Autoinfection has been shown to play an important role in determining the rate of polycyclic disease progression (Willoquet and Savary, 2004). A single leaf rust (*Puccinia triticina*) lesion on wheat caused an average of 197 daughter lesions on the leaf it was on during the epidemic, but only 16 lesions on all neighboring leaves, thus the autoinfection rate was 12 times greater than the alloinfection rate on neighboring leaves for wheat leaf rust (Lannou et al., 2008). Our results showed that autoinfection for stem rust on perennial ryegrass under winter field conditions was 1.3 times greater than alloinfection when autoinfection is defined as daughter lesions occurring on the same leaf as the mother lesion. Autoinfection rates were 5.1 times greater than alloinfection rates when autoinfection is defined as daughter lesions occurring on the same plant. The plants we studied had not extended, and were beginning to tiller. Many of the leaves were touching one another, and a mother pustule was often closer to leaf tissue on several other leaves than it was to some of the leaf tissue on its host leaf. The leaves on perennial ryegrass are narrow (2 to 3 mm wide). This contrasts with leaves of wheat

that can be 8 to 10 mm wide, and have a larger target of susceptible area on the mother leaf that could give rise to the higher autoinfection rates seen on wheat.

The model overestimated the early-spring disease severity at both sites and on both cultivars in 2010-11. The field observations showed the *Pgg* population reached zero and did not rebound after 10 February. It was noted that leaves of the transplants senesced faster than leaves of other plants, and new leaves were also slow to emerge this year. This absence of available host tissue would have prevented *Pgg* from having the necessary host bridge during the most challenging time for survival. One possible explanation for this phenomenon is that the leaves died from cold exposure, having been in the glasshouse for several days prior to out-planting. These cold shock transplanting events are not accounted for in the model. Future overwintering studies should take care to transplant when conditions are favorable to plant health.

One way to improve the model and account for the variability inherent in these biological systems would be to link each variable with a stochastic factor. Others have linked stochastic environmental variation and spore dispersal to fungal epidemic development (Rossi et al., 1997; Truscott and Gilligan, 2003; Xu and Ridout, 1998). Stochastic processes including leaf longevity and senescence rate will have a predictable mean, but an individual leaf will deviate from that mean by random processes. This randomness will then influence other variables in the model, including how long a *Pgg* lesion lives, and how many spores it produces. Ultimately, these factors influence the probability of foci extinction, and how severe the epidemic will be in the spring. In order to factor in these complex processes, it would first be necessary to write the model into a programming language such as Perl, or C#.

Others have shown that overwintering meteorological conditions and host dynamics are important to the severity of rust epidemics (Coakley et al., 1988; Pfender, 2004b; Shaner and

Powelson, 1973). The research reported here provides additional evidence that plant development plays a crucial role in the ability of rust fungi to survive the winter.

Further research could be done to integrate cultivar and stand-age effects into the model. Other cultivars and plants of different ages might show different leaf growth and senescence rates, spore production or infection efficiencies. Other work might focus on using the model to predict how the reduction of overwinter inoculum using different management strategies affects the stem rust overwintering population. The mycoparasite, *Sphaerellopsis filum*, is capable of reducing stem rust winter inoculum; this reduction in spore production could be incorporated into the model to quantify the effect of the mycoparasite on the overwintering population. The overwintering rust model described here provides an example that might be adapted for other plant pathogens and insects that start epidemics via residual overwintering populations and must overcome yearly bottlenecks.

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QUANTITATIVE EVALUATION OF EFFECTS OF THE MYCOPARASITE *Sphaerellopsis filum*
ON THE OVERWINTERING SURVIVAL OF STEM RUST IN A PERENNIAL RYEGRASS
CROPPING SYSTEM

Abstract

Sphaerellopsis filum is a mycoparasite of *Puccinia graminis* subsp. *graminicola* (*Pgg*), a rust fungus that causes wide-spread crop damage on perennial ryegrass grown for seed. *S. filum* reduces the life-time spore production of a single *Pgg* pustule by half, from 39,000 to 18,000 spores ($P < 0.001$). Mist duration, temperature and *Pgg* pustule age at the time of *S. filum* inoculation have significant effects ($P < 0.001$, $P < 0.05$, and $P < 0.001$, respectively) on the proportion of *Pgg* pustules infected by *S. filum*. Up to 50% of all *Pgg* pustules are infected when *S. filum* is inoculated onto erumpent pustules and incubated above 5°C for 48 h while exposed to mist. Plants inoculated with both fungi under controlled conditions and planted into the field, had a 2.5 times greater proportion of *Pgg* pustules infected with *S. filum* ($P = 0.045$), and a *Pgg* overwintering population one third the size, compared to plants inoculated with *Pgg* only ($P = 0.013$) at two field sites in one year. First-year stands of perennial ryegrass treated with monthly applications of *S. filum* had up to 10 times the proportion of pustules infected with *S. filum* ($P < 0.05$), and up to 0.5 times ($P = 0.02$) as much *Pgg* disease, as the non-treated controls. In comparison, plants with one winter fungicide application had *Pgg* severity up to 0.02 times that of the *Pgg*-only control ($P < 0.001$). There were no effects of *S. filum* application on rust in 2- or 3-year old perennial ryegrass. A recently-developed model for overwintering of the *Pgg* population estimates that *S. filum* would reduce the overwintering *Pgg* disease severity by 62 to 88% from that of a non-treated control when 20 to 60% of the pustules were infected with the mycoparasite.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is a cool-season seed crop intensively cultivated in the Willamette Valley of Oregon with nearly 100,000 acres harvested in 2010 (Young, 2010). The most damaging disease of perennial ryegrass in the Pacific Northwest is stem rust caused by the fungus *Puccinia graminis* subsp. *graminicola* Z. urb (*Pgg*). If untreated with fungicide, stem rust can cause reductions in seed yields exceeding 90% (Pfender, 2009).

Weather conditions and host growth during the cropping season (spring and summer) play a major role in determining the severity of the stem rust epidemic. These factors have been combined into a mathematical model that predicts disease severity based on daily weather inputs, host stage, and initial spring rust severity (Pfender, 2001b; 2003; 2004a). This model also allows for the estimation of epidemic severity given different starting parameters. The model predicts that disease severity during the course of the epidemic is proportional to the initial amount of rust, if all other variables are held constant. Other studies have found that autumn planting date, cultivar and stand age impact spring and summer epidemic severity of other rust diseases in the Pacific Northwest (Coakley et al., 1988; Moschini and Pérez, 1999; Shaner and Powelson, 1973). Late autumn plantings of perennial ryegrass have significantly less stem rust severity during the epidemic than those planted earlier in the fall, probably due to lower overwintering population of the pathogen (Pfender, 2004b). However, when stands are kept free of disease with fungicide in the spring and summer, early planted stands have a 23% greater seed yield. Reducing the overwintering *Pgg* population with a biological control may also lower the severity of the spring epidemic.

Considerable research has been conducted in evaluating biological control strategies for reducing severity of foliar diseases (Falk et al., 1995a; Grabski and Mendgen, 1985; Ward et al., 2010). Of the organisms studied, the mycoparasite *Sphaerellopsis filum* (Biv.-Bern ex Fr.) Sutton

(teleomorph *Eudarlucacaris* (Fr.) Eriksson), has shown the greatest potential in controlling a wide range of rust diseases (Keener, 1934; Kranz and Brandenburger, 1981; Pei et al., 2010; Pei et al., 2003). *S. filum* can infect up to 93% of *Cronartium fusiforme* and 76% of *Puccinia recondita* uredinia when applied and incubated under moist conditions (Kuhlman et al., 1978; Swendsrud and Calpouzos, 1972). When infected with *S. filum*, *Melampsora epitea* urediniospore production is reduced by 64 to 98% (Yuan et al., 1999). A simulation model integrating wheat leaf area, *P. recondita* and *S. filum* biology estimated that leaf rust severity would be reduced by 60 to 80% if *S. filum* infected 40 to 60% of the pustule surface (Hau and Kranz, 1978).

Under scanning electron microscope (SEM) analysis *S. filum* hyphae are seen penetrating urediniospores and interwoven throughout uredinia of both leaf rust (*P. recondita*) and stem rust (*P. graminis* f.sp. *tritici*) (Carling et al., 1976; Plachecka, 2005). The shortened lifespan and reduced spore production of *S. filum*-infected uredinia are thought to be the underlying mechanisms responsible for observed suppression of oak rust (*C. fusiforme*) and willow rust (*M. epitea*) epidemics in the field (Kuhlman et al., 1978; Yuan et al., 1999).

Field studies of *S. filum* infecting rust uredinia have been qualitative and limited in scope. In addition, infection parameters including host age and moisture and temperature conditions required for infection have not been clearly established (Kuhlman et al., 1978; Swendsrud and Calpouzos, 1972). The purpose of this study is to determine if *S. filum* can infect *Pgg* uredinia during the winter in the Willamette Valley, and subsequently reduce *Pgg* severity of the spring epidemic. The experiments seek to quantify (i) the ideal infection conditions for *S. filum* on *Pgg* uredinia on perennial ryegrass plants (ii) the effects of *S. filum* on *Pgg* urediniospore production and viability (iii) the effects of *S. filum* parasitism on the *Pgg* population size over the winter and into the spring epidemic.

Methods

Greenhouse plants. Seeds of perennial ryegrass cultivar (cv.) Jet, highly susceptible to stem rust, were planted in soil (Sunshine Growing Mix; Sun Gro Horticulture Inc., Bellevue, WA) in pots (“Cone-tainer”, 3.8 by 21 cm; Stuewe & Sons Inc., Corvallis, OR), one seed per pot. They were kept constantly moist until three leaves emerged, at which point the pots were irrigated by placing them in water for two days every week, and fertilized every two weeks with aliquots from a solution of 6 mg N, 5.4 mg P₂O₅ and 6 mg K₂O in 4 liters water. Plants were grown in a greenhouse maintained at day and night temperatures of 20°C and 15°C, respectively, with 12 h day length, and periodically trimmed to a height of 8 cm until time of inoculation 2 to 5 months after planting.

Controlled inoculation with *P. graminis* subsp. *graminicola* (Pgg). Urediniospores were obtained each year from multiple-cultivar perennial ryegrass field plantings at Oregon State University Hyslop Experimental Farm (44° 38' N, 123° 12' W). Spores were dried at 30% RH overnight, stored at -60°C, and heat shocked at 43°C for 1.5 min before use (Pfender, 2004a). A spore suspension (approximately 2x10⁵ spores/ml) was made for each inoculation by adding 1 mg of spores per ml of light mineral oil (Isopar M; ExxonMobile Chemical Co., Houston, TX). To document spore viability a 50 µl sample of the suspension was placed on water agar and incubated in the dark at 23 ± 2°C. Spore germination was determined after 24 h by counting the number of spores out of 100 that had a germ tube longer than the spore was wide. Each 500 µl aliquot of spore suspension was mixed immediately before being sprayed onto seven plants with a handheld Venturi atomizer operating at 21 kPa. After the oil dried, inoculated plants were placed in a mist chamber at 20 ± 4°C with a 15 h dark period immediately followed by a 4 h light period. Mist was run for 15 min at 2 h intervals during the dark period, and continuously during the light period to keep the leaves consistently moist.

Controlled inoculation with *S. filum*. *S. filum* conidia were originally harvested from parasitized rust pustules infecting perennial ryegrass leaves in 2003. Three monoconidial isolates, SF1, SF2 and SF3, were obtained from infected rust pustules in the greenhouse, maintained on PDA, and used for the experiments. All of the isolates were maintained separately, but used as a mixture for the experiments. Conidia were stored at -60°C in a 15% glycerol solution. Conidial suspensions were transferred to potato-dextrose agar (PDA) dishes amended with 300 mg Bacto Tryptone and 150 mg Bacto Yeast Extract, and incubated for 2 days under UV light (40 W blacklight, GE, Fairfield, CT) at 23°C and then under gro-lights (40 W, gro-lux, Sylvania, Danvers, MA) at 23°C for 2 to 3 weeks (Pei and Yuan, 2005). Mucilaginous cirrhi of conidia were harvested from pycnidia using a sterile blade, and were placed into centrifuge tubes with 1 ml of 0.05M MgSO_4 in water. After mixing for 30 sec, the suspension was transferred to a larger container and adjusted to 10^5 conidia per ml with 0.05M MgSO_4 . The resulting suspension (1.5 ml) was sprayed on each rust-infected plant with an aerosol sprayer (Preval, Precision Valve Corporation, Yonkers, NY).

Effects of mist duration. Plants were inoculated with *Pgg* ten, five or zero days prior to the day on which all plants were inoculated with *S. filum*. These intervals coincided with *Pgg* latent periods of approximately 1.2, 0.6 and 0.0 as calculated by temperature measurements taken in the greenhouse (Pfender, 2001b). Immediately after the plants dried, they were placed on a bench in a glasshouse under a misting system that kept the leaves wet (misting leaves for 30 min every 2 h) with day and night temperatures of 15 and 20°C , respectively. At 0, 2, 4, 6, 12, 24, 48, 72 and 120 h after *S. filum* inoculation, twelve plants (four replications from each rust-inoculated age group) were transferred to a glasshouse bench where plants were not misted, in order to prevent subsequent infections from occurring. Additional control treatments, not inoculated with *S. filum*, were given 0 or 120 h of mist treatment. Trials were scored for rust and

S. filum severity approximately 1.6 ± 0.2 rust latent periods after *S. filum* inoculation. The experiment was repeated once.

Effects of temperature. Plants were inoculated with *Pgg* and *S. filum* as described in the mist experiment above. Immediately after the plant leaves dried following *S. filum* inoculation, the plants were placed in a growth chamber that misted plants for 2 min every 2 h keeping the leaves wet for 48 h. Treatment temperatures were 5, 10, 15, 20, and 25°C. Because of limited growth chamber availability only one of the listed treatments was conducted at a time, paired each time with a treatment at 15°C to allow normalization of results across trials. Each treatment consisted of 30 plants (5 inoculated with *S. filum*, and 5 non-inoculated plants, from each *Pgg*-inoculated age group). After the 48 h mist exposure, plants were kept on a bench to prevent subsequent infections. The experiment was scored as described in the mist duration experiment and was repeated once.

Effects of *S. filum* on *Pgg* spore production and germination. To reduce variability in rust spore production that might arise from host genetic differences, plants raised in a glasshouse were vegetatively propagated so that twelve copies of each of four clones were obtained. All plants were inoculated with *Pgg* and left for six days (0.7 stem rust latent periods) while the infections incubated. Four plants of each of the four clones were then assigned to each of three treatments; inoculated with *S. filum*, sprayed with the MgSO_4 solution as a check, or left untreated. For analysis there were 4 replicates, each composed of 4 cloned plants, per treatment. Immediately after the plants had dried they were placed under a misting system, as described in the mist duration experiment, for 48 h then transferred to a glasshouse bench. Starting at the time when the misting finished, 4 pustules were identified on each plant for repeated sampling. Spores from open pustules were collected every 2 to 3 days into gelatin capsules fitted on a small vacuum spore sampler, and all spores from the 4 plants (16 pustules)

as a replicate were composited for one measurement. Isopar M (100 μ l) was added to each capsule, thoroughly mixed with the spores, and the resulting suspension (20 μ l) was placed on a hemacytometer (Fisher Scientific, Pittsburg, PA). The spores were counted, and the total number of spores produced per pustule per day was calculated. An additional aliquot (20 μ l) of the spore suspension was spread on a water agar plate, and the spore germination percentage was determined by microscopic observation after 24 h of incubation at 22°C in the dark. Two trials of the experiment were completed. Weibull (4-parameter) curves were fit to the data for spore production, and for spore production multiplied by spore viability for each treatment, using SigmaPlot (SigmaPlot v. 11.0, Systat Software, San Jose, CA).

Field experiments. The effects of *S. filum* on *Pgg* populations in the field over the winter were investigated at two sites with two types of experiments during the 2009-10 and 2010-11 field seasons. Site 1 was at the Oregon State University Hyslop Experiment Farm near Corvallis (44° 38' N, 123° 12' W). Site 2 was a commercial perennial ryegrass field near Junction City, Oregon (44° 23' N, 123° 20' W). In experiment 1 ("controlled inoculation experiment") plants were inoculated with *Pgg* and *S. filum* under controlled conditions, then out-planted to the field. In experiment 2 ("field inoculation experiment") field plants were naturally infected with *Pgg*, and *S. filum* conidia were applied in the field using a backpack sprayer. Weather conditions were collected at both sites with a data logger (CR10X, Campbell Scientific, Logan, UT) that had sensors for temperature and leaf wetness at the canopy level, rainfall and relative humidity.

Field experiment 1: controlled inoculations transplanted to the field. Seeds of perennial ryegrass cvs. Jet, Morningstar and Linn were planted in a soil-filled box outside of the USDA NFSPRC glasshouse in Corvallis on 7 October 2009 and 15 September 2010. Perennial ryegrass plants (10 cm of row) were dug from the box, and placed in pots on 11 December and 6 November for the 2009 and 2010 experiments, respectively. The potted plants were brought

into a glasshouse, and inoculated with *Pgg* and *S. filum* as described in the spore production experiment; however, in this experiment only *Pgg* infections that were 7 days (0.8 *Pgg* latent periods) old were inoculated with *S. filum*. The three treatments were (i) non-inoculated plants, (ii) *Pgg* inoculated plants, and (iii) *Pgg* inoculated plants that stayed on a bench for 7 days and were then inoculated with *S. filum*. After *S. filum* inoculation all treatments were placed under the mist system for 48 h, then out-planted to Sites 1 and 2 (described below). Plants were scored weekly for *S. filum* and *Pgg* severity while in the field.

At Site 1 each experimental unit consisted of two identically-treated plants 0.6 m (two rows) apart from one another. At Site 1 in 2009-10 treatments were applied to plants of cv. Jet plants, and experimental units were set out 11 m apart from one another into a field of cv. Jet, in a 2m by 45m plot area. In 2010-11 treatments were applied to cvs. Jet, Morningstar and Linn, and experimental units were set out 1.2 m apart from one another into a plot of cv. Manhattan 5 (moderately resistant to *Pgg* infection). In both years there were 4 replications.

At Site 2 each experimental unit within a treatment consisted of an individual plant. At Site 2 experimental units were set out 2 m apart within a 35 m by 35 m plot in a commercial field of cv. Silver Dollar. In 2009-10 at Site 2, treatments were applied to plants of cvs. Jet and Morningstar. In 2010-11 treatments were applied to plants of cvs. Jet, Morningstar and Linn. In both years there were 6 replications of repeatedly observed plants. Plants were scored as previously described in the field during both years.

At Site 2 only, additional plants (36 experimental units from each treatment) with the same treatments were out-planted, and dug (6 experimental units from each treatment) every month in 2009-2010, and in alternate months in 2010-2011. These dug plants were potted and brought to the glasshouse, where they were maintained under conditions that would not allow new infections, but would allow latent infections to become erumpent. The plants were scored

immediately after digging and a second time after approximately 1.5 *Pgg* latent periods. These scores were used to get a better assessment of how much disease was in the field. After 1.5 latent periods had elapsed, *Pgg* pustules and pustules infected with *S. filum* were easier to observe.

Field experiment 2: field inoculations. “Field inoculation” experiments were conducted at Site 1 only. During the 2009-2010 season four plots (2 m by 45 m) of perennial ryegrass cv. Jet were planted on 7 October 2009 and were designated as the first-year stand. Second-year stand plots of cv. Jet had been planted on 29 September 2008. For the 2010 season, cvs. Jet and Linn were planted on 15 September, and were designated as first-year stand plots. Third-year stand plots of cv. Jet and Linn had been planted on 29 September 2008. A minimum distance of 4.5 m was maintained between plots to allow access for spraying and disease assessment without disturbing adjacent plots. Plot planting and maintenance procedures following standard agronomic practices have been described previously (Pfender, 2004b).

Plots were split into four treatments each measuring 2.1 m by 11.2 m, and were arranged in a randomized complete block design. The four treatments were (i) a non-treated control (ii) one application of *S. filum* in the winter, (iii) monthly applications of *S. film* from late fall until late spring, or (iv) one application of the fungicide Quilt® (azoxystrobin 7%, propiconazole 11%, Syngenta Inc., Basel, Switzerland) in the winter. A 3 x 2.1 m buffer (sprayed with Quilt® to minimize the spread of disease) was maintained between treatments in 2010-2011.

S. filum was applied by first harvesting conidia from approximately 80 PDA plates into 12 L of sterilized 0.05M MgSO₄ in water to give a final concentration 10⁵ spores per ml. The conidial suspension was applied to plots at a rate of 10⁹ spores per ha (12 L per ha) by means of a CO₂-pressurized backpack sprayer operated at a pressure of 138 kPa (20 psi). The suspension was

applied also to 2 PDA plates to test for contamination and to quantify the germination of the *S. filum* conidia.

One-time applications of *S. filum* were applied on 15 November in 2009 or 30 November in 2010. Monthly applications of *S. filum* were applied on 15 November, 15 December, 24 January, 21 March, 14 April, 3 May and 26 May during the 2009-10 winter, and on 24 October, 30 November, 27 December, 14 February, 7 March and 6 April during the 2010-11 winter. Quilt was applied at a rate of 1462 ml in 75 liters of water per ha at a pressure of 207 kPa (30 psi) on 28 January in the 2010 experiment and 4 February in the 2011 experiment.

Treatment plots were surveyed for *Pgg* and *S. filum* severity in three ways. The number of *Pgg* pustules, and the number of *Pgg* pustules infected with *S. filum*, were counted in each survey method. Some plants (designated plants in two replications per treatment) were observed repeatedly during the experiment. These plants were marked by arbitrarily selecting 10 cm of row in each of 2 treatments, and repeatedly scoring two to four times a month.

A second method of observation, done two to four times every month, was to cut plants (10 cm of row, randomly selected in each of three replications from each treatment) and bring them to the lab for scoring. The third method of observation was to dig randomly selected plants (10 cm of row, and two replications from each treatment), pot them, and bring them to a dry bench in the glasshouse where they were scored once immediately and a second time approximately 1.5 *Pgg* latent periods after they had been dug. These plants were maintained in the glasshouse in a way that prevented new infections, while permitting latent infections to become erumpent and thus visible sooner than they would be in the field. This method of scoring was used to determine the number of latent infections in the field on a particular score date, and allowed for the counting of pustules and pustules infected with *S. filum* that would not have been seen with the other observation methods.

Integrating *S. filum* effects into an overwintering model. A *Pgg* overwintering population model was developed (See Chapter 2) that integrated the effects of plant and *Pgg* growth dynamics, spore production and spore dispersal with daily weather inputs on the daily *Pgg* population over the winter and into the spring. The model was run to estimate the effects of *S. filum* on the overwintering population when 20, 40 or 60% of pustules were infected. To do this, the sporulation rate associated with pustules infected with *S. filum* was incorporated into the model and multiplied by the proportion of pustules infected by the mycoparasite.

Effects of fungicide on *S. filum* germination. The effects of three common fungicides used to prevent *Pgg* epidemic development were tested to see how they would affect the viability of *S. filum* spores. The fungicides used were: Bravo (54% chlorothalonil, Syngenta Inc., Basel, Switzerland), Quadris (azoxystrobin 22.9%, Syngenta Inc., Basel, Switzerland) and Tilt® (propiconazole 41.8%, Syngenta Inc., Basel, Switzerland). Filter discs (5.5 mm dia. Whatman 42, ashless filter paper, Kent, UK) were placed in a dilution series (1, 1:10, 1:100, 1:500, 1:1000 and a control) of each of the fungicides. The fungicide-soaked discs were placed on water agar plates (three replications of each dilution) that had been sprayed with a conidial suspension of *S. filum* in 0.05M MgSO₄ (as described in the controlled inoculation experiments above). The plates were incubated for two days under ultraviolet light at 23°C, and three days under gro-lights at 23°C. The plates were scored for the zone of inhibition where germination was less than 10%. This was determined by counting the number of spores that had a germ tube longer than the spore was wide and dividing this number by the total number of spores counted. The distance between the edge of the fungicide filter disc and the delineated inhibition edge was measured with a ruler.

Data analysis. The mist duration and temperature experiment scores of *Pgg* pustules or *Pgg* pustules infected with *S. filum* were expressed as total number of *Pgg* pustules or the proportion of *Pgg* pustules infected with *S. filum*. The zero-day old rust treatment, which was

rarely infected by the mycoparasite, was omitted from analysis in the mist duration experiment. Mist duration, *Pgg* pustule age at time of *S. filum* inoculation and trial were factors in the three-way ANOVA for mist duration experiment. In the temperature experiment, data was normalized with a 15°C standard treatment run simultaneously with each temperature treatment. Temperature, *Pgg* pustule age at time of *S. filum* inoculation and trial were factors in the three-way ANOVA for the temperature experiment.

Spore production and viability data were analyzed by three-way ANOVA. The factors in the analysis were treatment, host genotype, trial, and for the germination experiment, days after inoculation. Counts of rust pustules in the field experiment were plotted against time, and the area under the disease progress curve (AUDPC) between 1 March and the end of the observations (11 June, 2010 and 2 June, 2011) was calculated. This was done to determine any treatment differences that were manifested during the spring when stem rust is a problem for seed growers. The proportion of *Pgg* pustules with *S. filum* AUDPC values were obtained from data across the entire sample period (14 November to 11 June in 2009-10 and 11 October to 2 June in 2009-10). Data were analyzed using three-way ANOVA. The factors were stand age, treatment and year. Multiple comparisons were made using Tukey's HSD method. Analysis was conducted using R (R Development Core Team [2011]. R Foundation for Statistical Computing, Vienna, Austria).

Results

Effects of mist duration. Mist duration and *Pgg* pustule age at the time of *S. filum* inoculation had significant ($P < 0.001$) effects on the proportion of *Pgg* pustules infected with *S. filum* (Fig. 1). The interaction of mist duration and pustule age was statistically significant. More than 6 h of leaf wetness allowed *S. filum* to infect 10% more *Pgg* pustules than the treatments

incubated with 0, 2, 4, or 6 h of mist ($P < 0.05$). There was no significant difference among treatments incubated with more than 6 h of mist. The treatment effect across all mist durations was that *S. filum* infected 11% more *Pgg* pustules that were 10 days old, than those that were 5 days old when inoculated with *S. filum* ($P < 0.001$). The proportion of *Pgg* pustules infected with *S. filum* with 6 h of mist was 13, 4 and 0% for 10-day-old, 5-day-old and 0-day-old pustules, respectively. With 12 h of mist the proportion of *Pgg* pustules increased to 26, 15 and 0% for 10-day-old, 5-day-old and 0-day-old pustules, respectively.

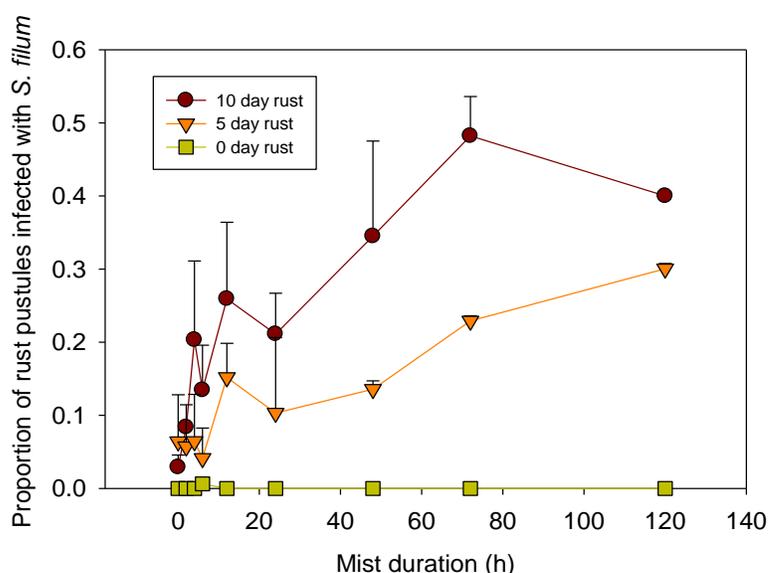


Fig. 1. The effects of pustule age and leaf wetness on the proportion of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules infected with *S. filum*. Perennial ryegrass plants were inoculated with *Pgg* 10, 5 or 0 days before they were treated with a conidial suspension of *S. filum*. The plants were then incubated at day and night temperatures of 15 and 20°C, respectively. Treatments (four replications) were misted for 30 min every 2 h to maintain wet leaves for the durations shown, and scored after 1.5 *Pgg* latent periods. Each data point is the mean and SEM between two experiments. Non-inoculated controls (incubated with zero or 120 h of mist) for each treatment had no infection of rust pustules by *S. filum*.

Effects of temperature. The temperature at which *S. filum* was incubated did have a significant effect ($P = 0.03$) across all ages on the proportion of *Pgg* pustules infected with *S. filum*.

However, this difference could not be attributed to any specific temperature. *Pgg* pustule age at the time of *S. filum* inoculation had a significant effect ($P < 0.001$) across all temperatures on the

proportion of *Pgg* pustules infected with *S. filum* (Fig. 2). The overall effect of pustule age averaged across temperatures was that *S. filum* infected 13% more 10-day-old *Pgg* pustules than 5-day-old pustules and 36% more 10-day-old pustules than 0-day-old pustules ($P < 0.001$). There was no interaction between temperature and age ($P = 0.40$), or trial and temperature ($P = .72$), or trial and age ($P = 0.74$).

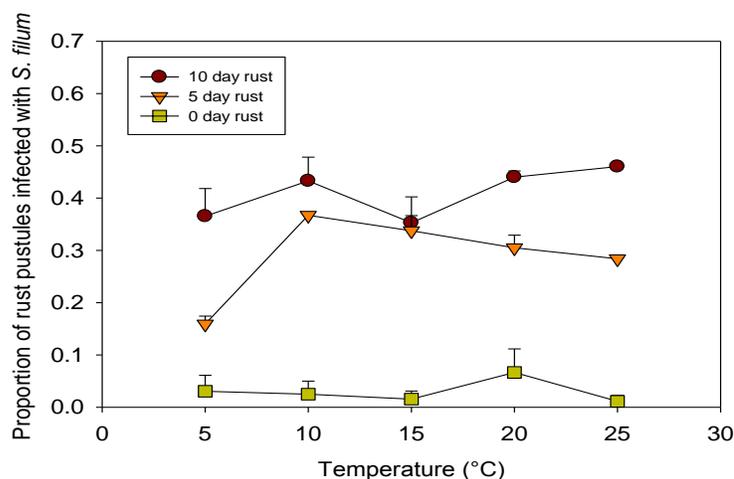


Fig. 2. Effect of temperature on the proportion of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules infected with *S. filum*. Perennial ryegrass plants were inoculated with a conidial suspension of *S. filum* 10, 5 or 0 days after they were inoculated with *Pgg*. The plants were placed in growth chambers at the constant temperatures shown with 48 h of mist. After 1.5 *Pgg* latent periods, plants were scored for *Pgg* and *S. filum* severity. Data points represent the mean and SEM between two trials of the experiment. Each trial consisted of five treated and five non-treated plants at each treatment. *S. filum* was not detected on controls that were not inoculated with *S. filum*.

Rust spore production and germination. Non-treated *Pgg* pustules produced 39,000 spores over the lifetime of the pustule, more than double ($P < 0.001$) the 18,000 produced when infected with *S. filum* (Fig. 3A). There was no effect of host genotype on the number of spores produced ($P = .064$), and no interaction between host genotype and treatment ($P = 0.99$). *S. filum* reduced the overall average viability of spores by 4.75% ($P < 0.001$). Spore viability was determined, multiplied by daily spore production, and plotted against days since *Pgg* inoculation (Fig. 3B). Weibull (4-parameter) curves were fit to each treatment and plotted with the averages

of each trial. The standard form for the Weibull curve is $f(x) = a \cdot ((c-1)/c)^{(1-c)/c} \cdot (\text{abs}((x-x_0)/b + ((c-1)/c)^{(1/c))^{c-1}) \cdot \exp(-\text{abs}((x-x_0)/b + ((c-1)/c)^{(1/c))^{c-1})$ only if $(x \leq x_0 - b \cdot ((c-1)/c)^{(1/c))$. The parameter estimates for the *Pgg* spore curve are: $a=5000$, $b=5.44$, $c=1.12$ and $x_0=1.4$. The parameter estimates for the *Pgg*-only treatment for the spore curve multiplied by spore viability are $a = 4000$, $b = 0.4$, $c = 1.1$ $x_0 = 1.02$. The parameter estimates for the *S. filum* treatment for the rust spore curve multiplied by spore viability are $a = 3200$ $b = 0.19$ $c = 1.5$ $x_0 = 1.01$. The coefficient and standard error of the 1st parameter of each curve (which set the maximum) for each treatment are: (i) non-treated *Pgg*, 4000 ± 204 , (ii) *Pgg* with 0.05M MgSO_4 , 4040 ± 263 , and (iii) *Pgg* with *S. filum*, 3200 ± 370 . The adjusted r^2 values for each treatment curve are: (i) non-treated *Pgg*, 0.74, (ii) *Pgg* with 0.05M MgSO_4 , 0.63, and (iii) *Pgg* with *S. filum*, 0.83.

Field experiment 1: controlled inoculations. Results of the controlled inoculations with *S. filum* on *Pgg*-infected perennial ryegrass plants transplanted to the field are shown in Fig. 4 (Site 1) and Fig. 5 (Site 2). The area under the curve was calculated from 1 March through June for *Pgg* pustule data, and across all observation dates in a given year for the proportion of *Pgg* pustules infected with *S. filum*. Means and the statistical conclusions from one and two-way ANOVA are summarized for Sites 1 and 2 (Table 1). Analysis was done for data within a site, but not between sites because of a significant interaction effect between sites. There were significant effects of *S. filum* infection on *Pgg* pustule severity and the proportion of *Pgg* pustules infected with *S. filum* at both sites in 2009-2010. However, in 2010-11 the *Pgg* population did not survive the winter at either site in any treatment; therefore, there was no treatment effect on spring-time *Pgg* severity.

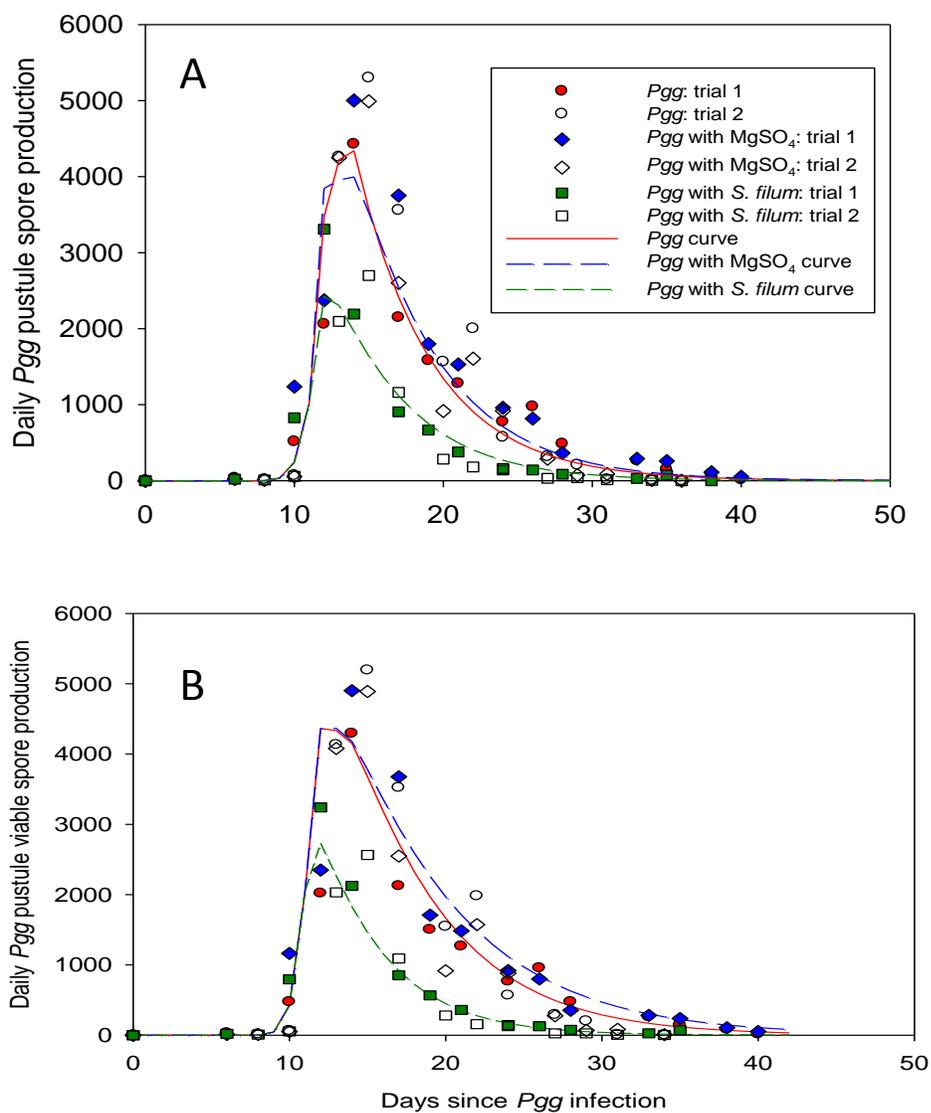


Fig. 3. *Puccinia graminis* subsp. *graminicola* (*Pgg*) daily spore production (**Fig. 3A**) and spores produced multiplied by spore viability (**Fig. 3B**). Perennial ryegrass cv. Jet plants were inoculated with a *Pgg* spore suspension and incubated for seven days (0.7 rust latent periods). Three treatments were applied (four reps of four plants per rep), and plants were subjected to misting for the first 48 h at day and night temperatures of 20 and 15°C, respectively. The three treatments applied to the *Pgg*-inoculated plants were (i) a non-treated control, (ii) 0.05 M $MgSO_4$, or (iii) *S. filum* conidial suspension. Spores were collected from individual rust pustules (4 pustules per plant) within each treatment every two or three days and counted with a hemacytometer. A sample from each replication was placed on water agar, incubated in the dark at 24°C for 24 h, and spore viability was calculated by counting the number of spores out of 20 with a germ tube longer than the spore width. A Weibull (4-parameter) curve (see text) was fit to the mean daily spore production values for each replication within each treatment. Symbols represent means for collected data from each trial; lines represent Weibull curves calculated for each treatment.

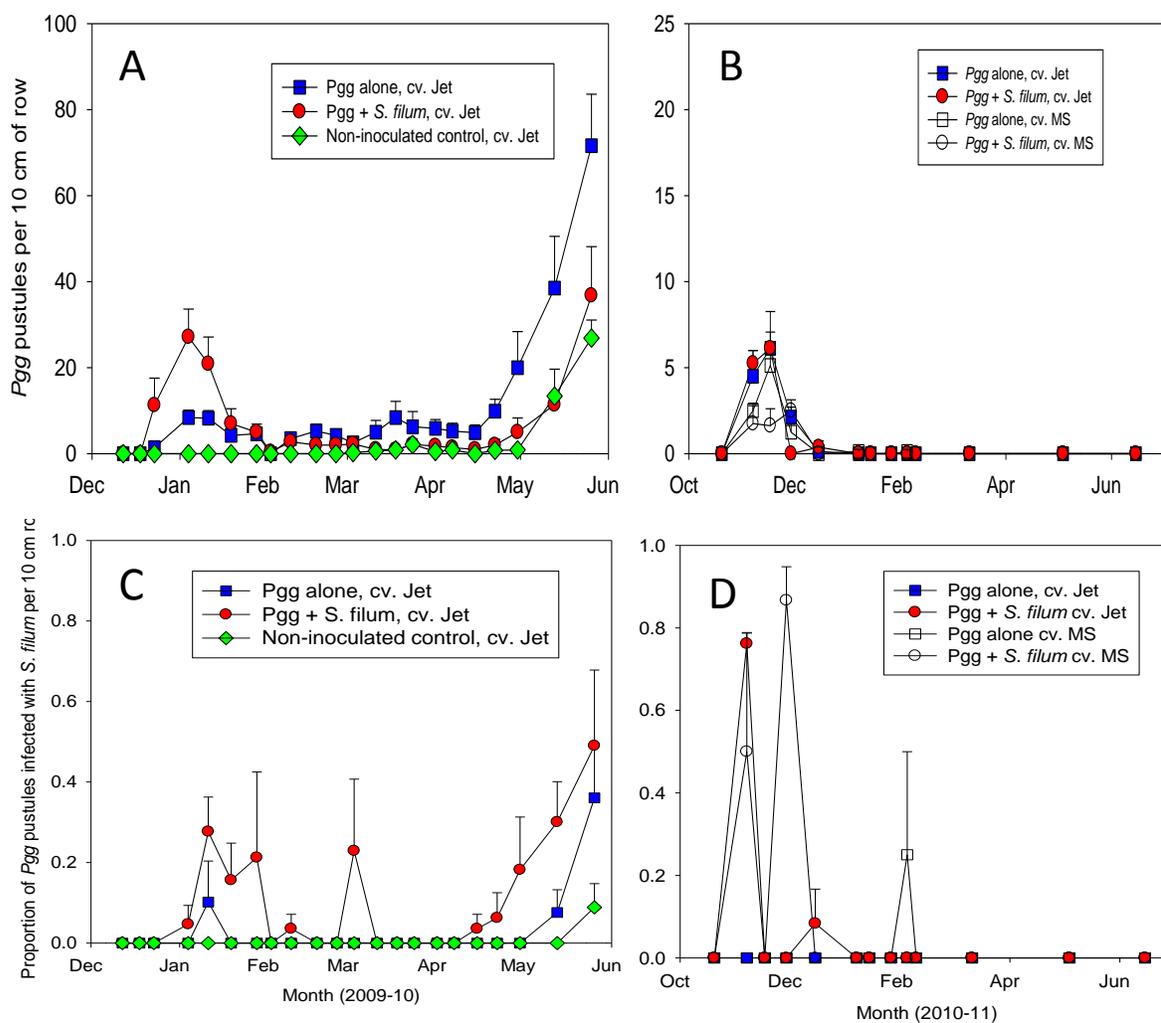


Fig. 4. Site 1 levels of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules (**Figs. 4A and 4B**) and the proportion of *Pgg* pustules infected with *S. filum* (**Figs. 4C and 4D**) plants observed repeatedly over the winter. *Pgg* and *S. filum* were inoculated under ideal conditions and out-planted to Site 1 in 2009-10 (**Figs. 4A and 4C**) and in 2010-11 (**Figs. 4B and 4D**). Data points are the means and SEM on six replications of 10 cm of row. In 2009-10 only cv. Jet was out-planted to Site 1. In 2010-11, cvs. Jet, Morningstar (MS) and Linn (not shown, results similar to Jet) were used. Non-inoculated plants did not develop *Pgg* pustules over the winter in 2010-11.

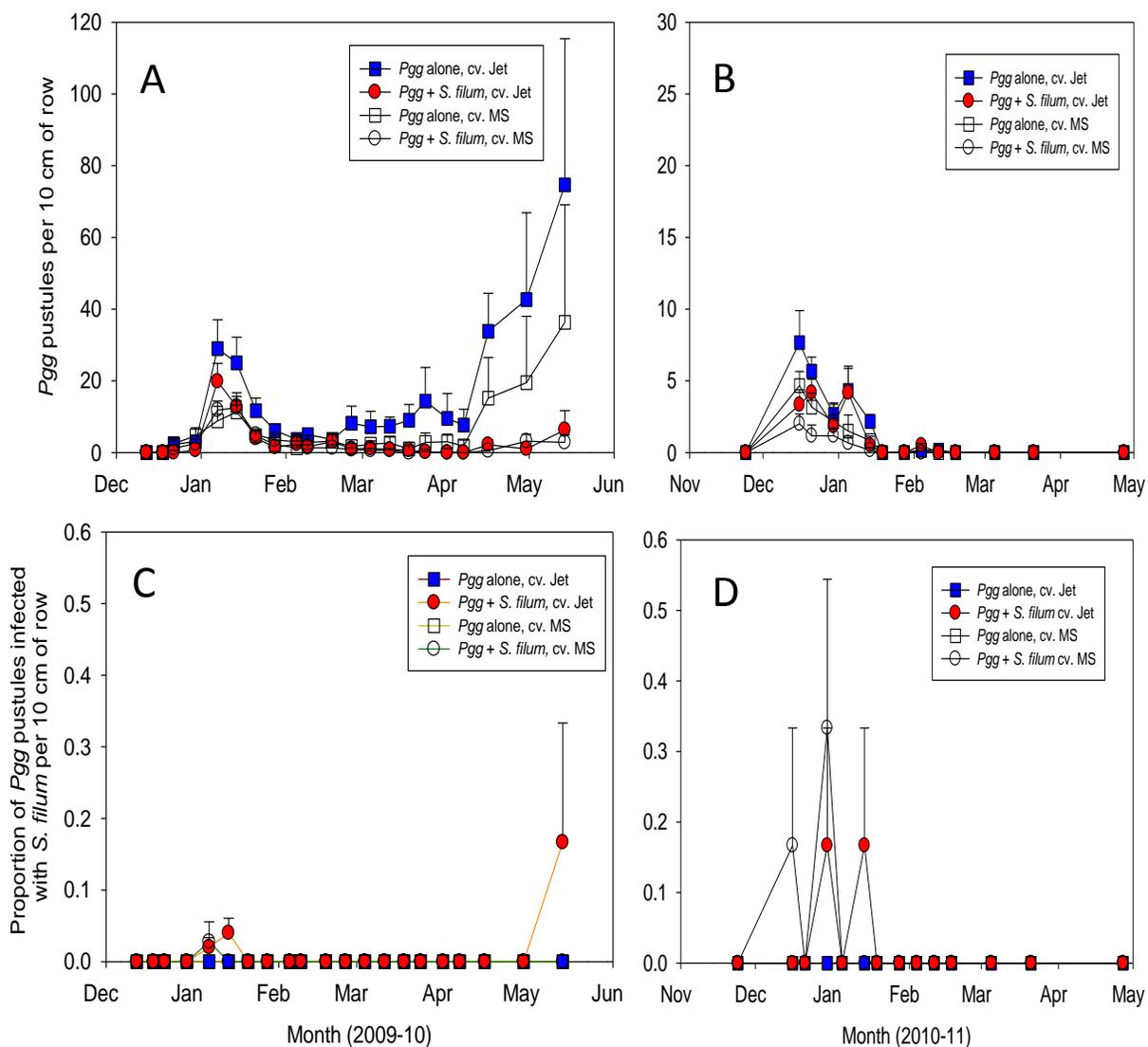


Fig. 5. Site 2 levels of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules (Figs. 5A and 5B) and the proportion of *Pgg* pustules infected with *S. filum* (Figs. 5C and 5D) plants repeatedly observed over the winter. *Pgg* and *S. filum* were inoculated under ideal conditions and out-planted to Site 1 in 2009-10 (Figs. 5A and 5C) and in 2010-11 (Figs. 5B and 5D). Data points are the means and SEM on six replications of 10 cm of row. In 2009-10 cv. Jet and Morningstar (MS) were out-planted to Site 2. In 2010-11, cvs. Jet, Morningstar and Linn (not shown, results similar to Jet) were used. Non-inoculated plants did not develop *Pgg* pustules over the winter in 2010-11.

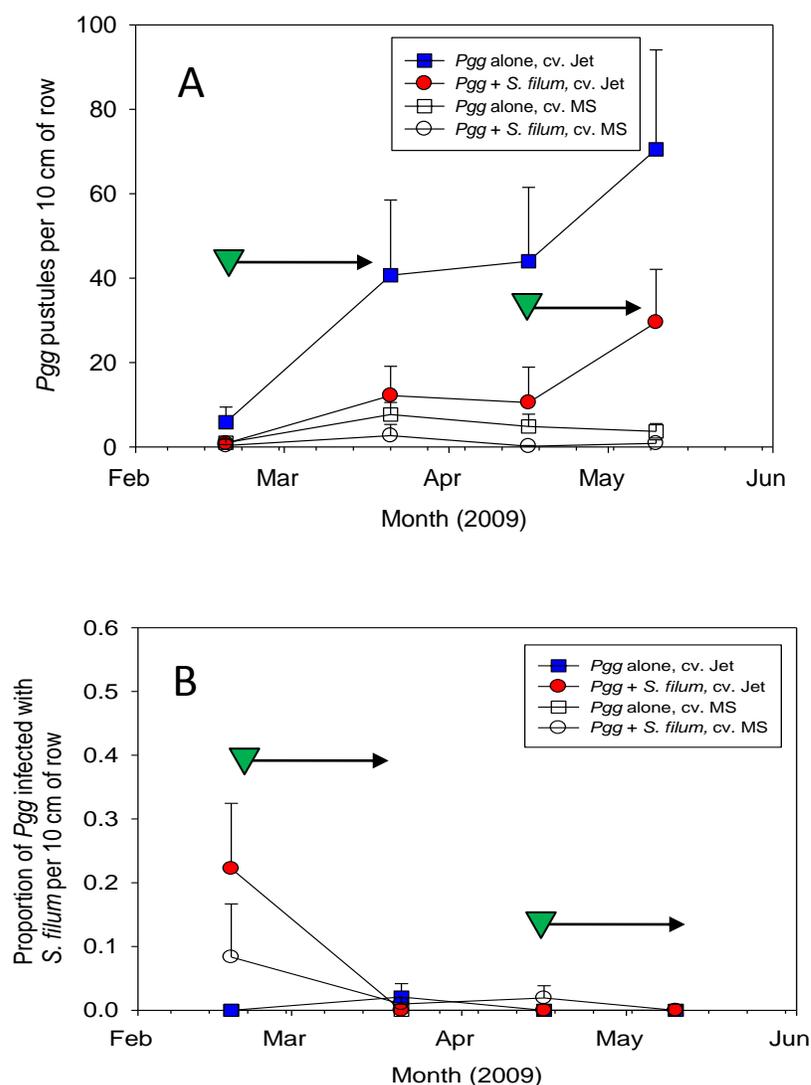


Fig. 6. *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules (**Fig. 6A**) and the proportion of *Pgg* pustules with *S. filum* (**Fig. 6B**) at Site 2. *Pgg* and *S. filum* were inoculated under controlled conditions and out-planted to Site 2 in 2009. Plants were dug for scoring immediately and again after approx. 1.5 *Pgg* latent periods (both scores are shown plotted against *Pgg* latent period). Data are the means and SEM from 10 cm of row of cvs. Jet (highly susceptible to rust) and Morningstar (MS) (moderately susceptible to rust). Non-inoculated plants did not develop *Pgg* pustules over the winter. The digging date are indicated with a small triangle, and connects with the second score (observed after 1.5 *Pgg* latent periods) with a horizontal arrow (digging dates and score dates are the same for both **Figs. 6A** and **6B**).

Table 1. Area under the disease progress curve (AUDPC) means, and statistical analysis for the number of pustules of *Puccinia graminis* subsp. *graminicola* (*Pgg*) and the proportion of *Pgg* pustules infected with *S. filum*. The two fungi were inoculated under controlled conditions and transplanted to Sites 1 and 2, in two years of experiments (2009-10 and 2010-11). Data show the mean area under the curve of *Pgg* pustules from March 1 to the end of the sampling period, and the proportion of *Pgg* pustules infected with *S. filum* over the entire sampling period on 10 cm of perennial ryegrass row. Significant differences between cv. and inoculation treatments (determined by one and two-way ANOVA, $\alpha=0.05$) are denoted by different letters. Analysis was conducted within each site, but not between sites.

Year	Cultivar	Treatment	Area under the curve			
			Site 1		Site 2	
			No. of <i>Pgg</i> pustules ^a	Proportion of <i>Pgg</i> pustules with <i>S. filum</i> (%) ^b	No. of <i>Pgg</i> pustules	Proportion of <i>Pgg</i> pustules with <i>S. filum</i> (%)
2009-10	Jet	Control	399 b	0	13 b	1 b
		<i>Pgg</i>	1510 a	0	1868 a	3 b
		<i>Pgg</i> with <i>S. filum</i>	521 b	2 a	99 b	11 a
	MS	Control	2 b	0
		<i>Pgg</i>	790 ab	0
		<i>Pgg</i> with <i>S. filum</i>	78 b	1 b
2010-11	Jet	Control	0	0	0	0
		<i>Pgg</i>	0	0	0	0
		<i>Pgg</i> with <i>S. filum</i>	0	2 a	0	12 b
	MS	Control	0	0	0	0
		<i>Pgg</i>	0	0	0	2 c
		<i>Pgg</i> with <i>S. filum</i>	0	5 a	0	19 ab
	Linn	Control	0	0	0	0
		<i>Pgg</i>	0	0	0	5 c
		<i>Pgg</i> with <i>S. filum</i>	0	1 a	0	33 a

^a Area was calculated from *Pgg* pustule severity from 1 March to 11 June in 2009-10 and to 2 June in 2010-11.

^b Area was calculated from the proportion of *Pgg* pustules infected with *S. filum* over the full duration of the experiment.

Treatment means and SEM of the observations taken from plants that were inoculated under controlled conditions, out-planted to Site 2, and then dug from the field to be scored in the glasshouse are shown for the 2009-10 field season (Fig. 6). Both the initial score and score after 1.5 latent periods was plotted against *Pgg* latent period for each of the five digging dates. Although these observations were also made in 2010-11, the levels of *Pgg* and *Pgg* infected with *S. filum* was zero on 2 February and 22 April.

In 2009-10 the *S. filum* treatment had an overall effect of three times less disease ($P = 0.013$) than the *Pgg* treatment without *S. filum*. The *S. filum* treatment had 2.5 times more *Pgg* pustules infected with *S. filum* ($P = 0.045$) than the *Pgg* treatment without *S. filum*. The Jet cultivar had seven times more disease ($P < 0.001$) than the Morningstar cultivar. Cultivar did not have a significant effect on the proportion of *Pgg* pustules infected with *S. filum*.

Field experiment 2: field inoculations. Four treatments were applied to first-year and older (2nd or 3rd year) plantings of perennial ryegrass exposed to natural inoculum of *Pgg* at Site 1 in 2009-10 and 2010-11. Treatments were: (i) non-treated control, (ii) one application of *S. filum*, (iii) monthly applications of *S. filum*, or (iv) one winter application of Quilt® fungicide. *Pgg* pustules and the proportion of *Pgg* pustules infected with *S. filum* (means and SEM) for each treatment and sampling method (cut, repeatedly sampled and dug) in each year (2009-10 and 2010-11) are plotted against time (Fig. 7 and 8, respectively). Means and the statistical conclusions drawn from a two-way ANOVA of each treatment within a year are listed (Table 2).

First-year stands of perennial ryegrass treated with one application of *S. filum* did not have significantly less *Pgg* disease than the non-treated control in either year. In 2010-11 one application of *S. filum* had a 2.56 times higher ($P = 0.04$) proportion of *Pgg* pustules infected with *S. filum* than the non-treated control when observed by the cut sampling method, but there was no significant difference found in the repeated observation or dug sampling methods.

First-year stands treated with monthly applications of *S. filum* had only 0.51 times ($P = 0.02$) the disease severity of the control treatment when observed by the repeated sampling method, and had only 0.71 times ($P = 0.03$) the disease severity of the control treatment when observed by the dug sampling method in 2009-10. There was no significant effect on *Pgg* disease severity with monthly applications of *S. filum* when the plants were observed with the cut sampling method in 2009-10. In 2010-11 first-year stands treated with monthly applications of *S. filum* had 0.64 times ($P = 0.05$) the disease severity of the control treatment when observed with the cut sampling method. There was no significant effect on *Pgg* disease severity with monthly applications of *S. filum* when disease was observed with the repeated or dug sampling methods in 2010-11.

In 2009-10 first-year stands receiving monthly applications of *S. filum* had a 10 times greater proportion ($P < 0.05$ for all sampling methods) of *Pgg* pustules infected with *S. filum* than the proportion infected on the control treatment. In 2010-11 first-year stands with monthly applications of *S. filum* had three times as many *Pgg* pustules infected with *S. filum* using the cut or dug sampling methods ($P = 0.01$ and 0.05 , respectively), and 27 times greater proportion ($P < 0.001$) of pustules infected than the control treatment when using the repeated sampling method.

Quilt® fungicide treatments on first-year stands (applied on 28 January, 2009) had a *Pgg* severity that was 0.24 times ($P < 0.001$) that of the control treatment when observed with repeated or dug sampling methods in 2009-10. The disease severity was not significantly different ($P > 0.05$) from the control treatment when observed with the cut sampling method.

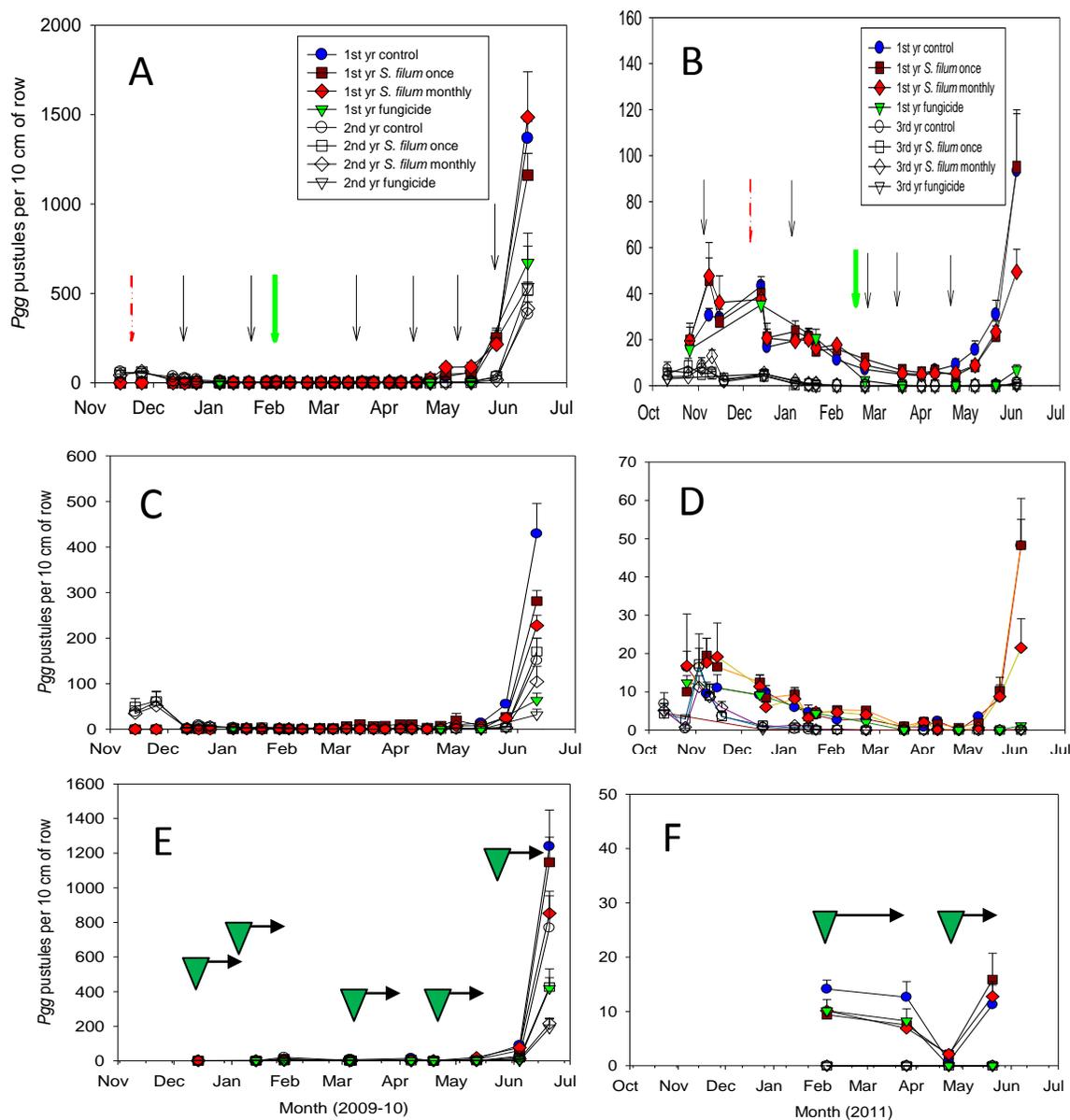


Fig. 7. *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules at Site 1 in 2009-10 (Figs. 7A, 7C, 7E) and 2010-11 (Figs. 7B, 7D, 7E). Scores were taken on 10 cm of row that was cut (Figs. 7A and 7B), repeatedly observed (Figs. 7C and 7D), or dug for scoring immediately and again after 1.5 latent periods (E and F). Treatments include (i) a control (ii) one application of *S. filum* (iii) monthly applications of *S. filum*, or (iv) Quilt® fungicide applied one time on newly planted (1st yr) or established (2nd or 3rd yr) stands of perennial ryegrass. Application dates for the different treatments are indicated by arrows, and are listed by treatment type: i) one *S. filum* application: thin dashed arrow, ii) monthly *S. filum* applications: thin solid arrows or iii) Quilt® fungicide application: thick solid arrow. The digging dates (initial score date) (indicated by the large triangles) for the dug samples (Fig. E and F), connect with the second score (observed after 1.5 *Pgg* latent periods and plotted against *Pgg* latent periods in the field) with a horizontal arrow.

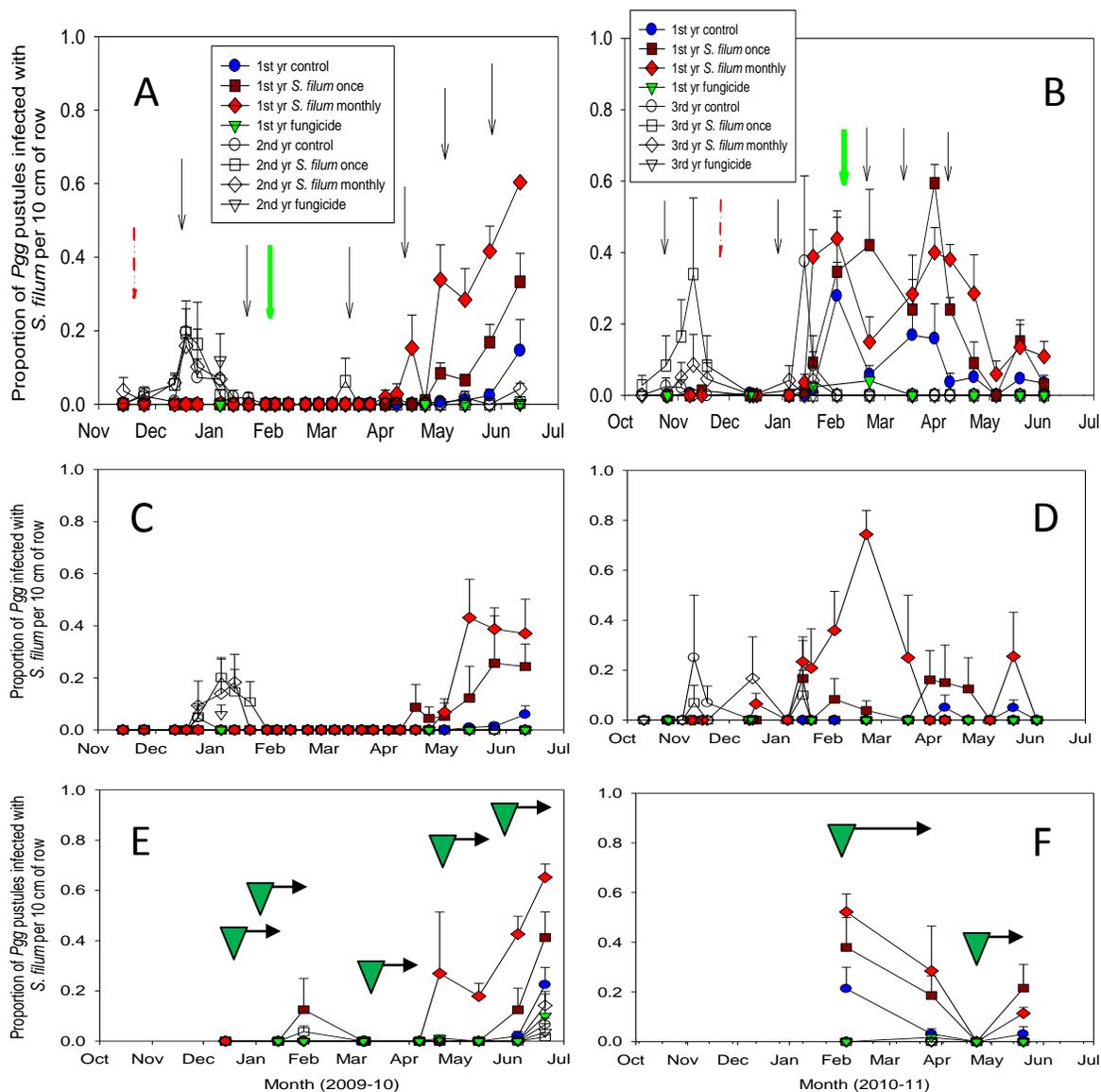


Fig. 8. Proportion of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules infected with *S. filum* at Site 1 in 2009-10 (Fig. 8A, 8C, 8E) and 2010-11 (Figs. 8B, 8D, 8F). Scores were taken on 10 cm of row that was cut (Fig. 8A and 8B), repeatedly observed (Figs. 8C and 8D), or dug for scoring immediately and again after 1.5 latent periods (Figs. 8E and 8F). Treatments include (i) control (ii) one application of *S. filum* (iii) monthly applications of *S. filum*, or (iv) Quilt® fungicide applied one time. Treatments were applied to newly planted (1st yr) or established (2nd or 3rd yr) stands of perennial ryegrass. Application dates for the different treatments are indicated by arrows, and are listed by treatment type: (i) one *S. filum* application: thin dashed arrow, (ii) monthly *S. filum* applications: thin solid arrows or (iii) Quilt® fungicide application: thick solid arrow. The digging dates (initial score date) (indicated by large triangles) for the dug samples (E and F), connect with the second score (observed after 1.5 *Pgg* latent periods and plotted against *Pgg* latent periods in the field) with a horizontal arrow.

Table 2. Field experiments with applications of *S. filum* or Quilt® fungicide at Site 1 in 2009-10 and 2010-11. Area under the curve means, and statistical analysis for *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules from 1 March to the end of the sampling period, and the proportion of *Pgg* pustules infected with *S. filum* over the entire sampling period are shown. Data are the mean area under the curve of *Pgg* pustules, and the proportion of *Pgg* pustules infected with *S. filum* along 10 cm of perennial ryegrass row across time. Three different sampling methods were used for each treatment: (i) cut and scored in the lab, (ii) repeatedly observed in the field, or (iii) dug, scored, and scored again after 1.5 *Pgg* latent periods. Significance between stand age and treatments (determined by two-way ANOVA, $\alpha=0.05$) is denoted by different letters. Analysis was conducted within each year, but not between years.

Year	Stand Age	Treatment	Area under the curve					
			<i>Pgg</i> pustules ^a			Proportion of <i>Pgg</i> pustules with <i>S. filum</i> ^b		
			Cut samples	Repeatedly observed samples	Dug samples	Cut samples	Repeatedly observed samples	Dug samples
2009-10	1	Control	15,867 a	4,630 a	12,534 a	2 b	2 b	2 b
	1	<i>S. filum</i> once	14,404 a	3,389 a	10,840 ab	7 b	9 ab	9 b
	1	<i>S. filum</i> Monthly	17,415 a	2,402 b	8,928 b	18 a	15 a	22 a
	1	Fungicide	9,799 ab	924 bc	3,672 c	1 b	0.00	1 b
	2	Control	3,758 b	1,285 bc	7,332 b	3 b	1 b	1 b
	2	<i>S. filum</i> Once	4,734 b	1,463 bc	4,231 bc	4 b	4 ab	1 b
	2	<i>S. filum</i> Monthly	3,610 b	951 bc	2,212 bc	4 b	4 ab	1 b
	2	Fungicide	5,338 b	459 c	1,607 c	2 b	3 ab	1 b
Interaction ^c (Y/N)			N	Y	Y	Y	N	Y
2010-11	1	Control	1,575 a	543 a	1,017 a	13 b	1 b	7 b
	1	<i>S. filum</i> Once	1,323 ab	564 a	800 a	33 a	9 b	20 ab
	1	<i>S. filum</i> Monthly	1,017 b	308 ab	755 a	36 a	35 a	26 a
	1	Fungicide	56 c	8 bc	571 a	13 b	0.0 b	1 b
	3	Control	7 c	1 c	0.0	4 b	2 b	0.0
	3	<i>S. filum</i> Once	5 c	0.0 c	0.0	7 b	2 b	0.0
	3	<i>S. filum</i> Monthly	5 c	1 c	0.0	1 b	5 b	0.0
	3	Fungicide	5 c	0.0 c	0.0	2 b	0.0 b	0.0
Interaction ^c (Y/N)			Y	Y	NA	Y	Y	NA

^a Area was calculated from *Pgg* pustule severity from 1 March to 11 June in 2009-10 and to 2 June in 2010-11.

^b Area was calculated from the proportion of *Pgg* pustules infected with *S. filum* over the full duration of the experiment.

^c Statistically significant interaction between treatment and stand age.

In 2010-11, first-year stands treated with fungicide (applied 4 February, 2010), had disease severity that was 0.02 ($P < 0.001$) times that of the control treatment when observed with the cut or repeated sampling methods. There was no significant difference between the fungicide treatment and the control treatment in 2010-11 when observed with the dug sampling method. The fungicide treatment did not have a significantly different proportion of *Pgg* pustules infected with *S. filum* than the non-treated control in either year.

Second-year stands of perennial ryegrass had significantly less *Pgg* disease than the first-year stands in both years. Second-year stands had 3 times less ($P < 0.001$) *Pgg* disease than first-year stands in 2009-10 and 342 times less ($P < 0.001$) disease in 2010-11 (across all treatments and sampling methods). There was a significant effect ($P = 0.002$) of fungicide in the second-year stands in 2009-10 when observed with the dug sampling method. Second-year stands did not show a significant difference from the first-year stand control treatment in the proportion of *Pgg* pustules infected with *S. filum*. There were no significant treatment effects within the second-year stands in 2010-11. There was no treatment effect from *S. filum* or Quilt fungicide applications observed in either the first or third year stand of cv. Linn using any sampling method.

Favorable field conditions for *S. filum* infection. Daily high and low temperatures and hours of leaf wetness were plotted over the winters of 2009-10 and 2010-11 at Site 1 (Fig. 9). Conditions favorable for *S. filum* infection were determined by finding days between the planting date and 15 April that had a minimum temperature above 3.5°C and 6 or more hours of leaf wetness. In 2009-10 36% of the days were favorable for infection at Site 1, and in 2010-11 40% of the days were favorable for infection.

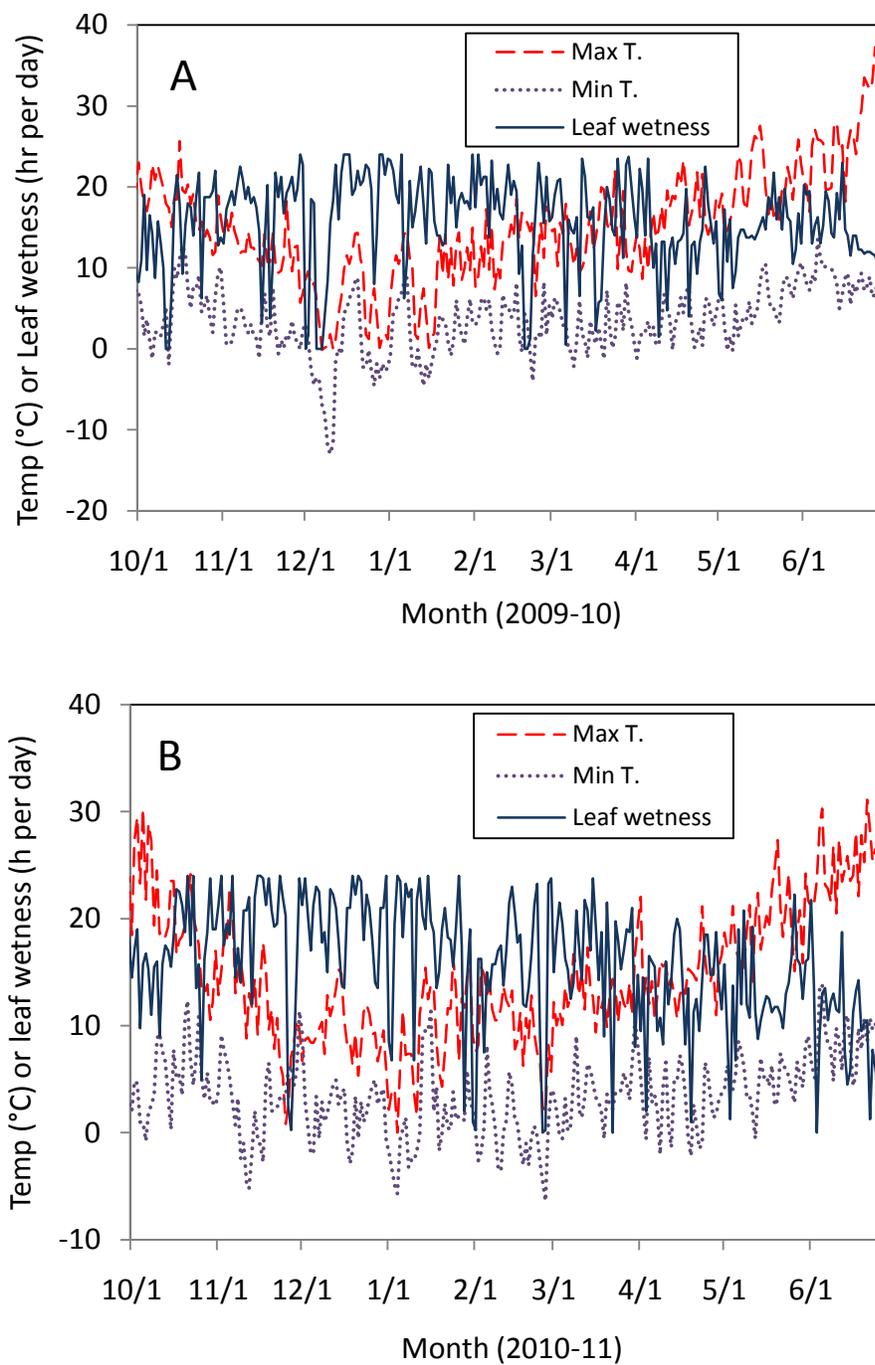


Fig. 9. Daily leaf wetness hours, plotted with high and low temperature values at Site 1 in 2009-10 (**Fig. 9A**) and 2010-11 (**Fig. 9B**). Weather data were taken every 15 min on a data logger (CR10X, Campbell Scientific) and analyzed for daily minimum (dotted line) and maximum (dashed line) air temperatures, and hours of leaf wetness (solid line).

Simulated effects of *S. filum*. *S. filum* was simulated to infect a realistic 20, 40 or 60% of *Pgg* pustules over the winter in a model (see Chapter 2) that incorporated the dynamics of plant and *Pgg* pustule growth, spore production and spore dispersal with weather conditions. *Pgg* pustules infected with *S. filum* were concluded to produce 54% fewer spores than non-infected pustules. The daily *Pgg* population output was plotted with the observed population at Site 1 over the winter of 2009-10 (Fig. 10). The model predicted that if *S. filum* infected 20, 40 or 60% of the *Pgg* pustules, *Pgg* severity (as calculated from the area under the curve from 1 March to 15 April) would be reduced by 62, 78 or 89%, respectively.

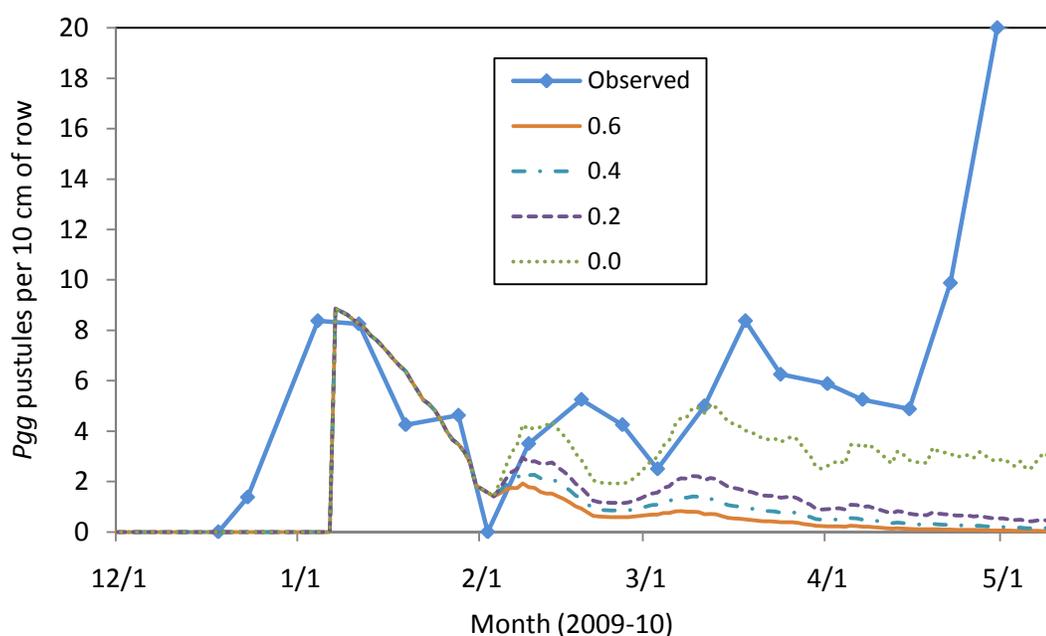


Fig. 10. Observed and simulated impacts of *S. filum* infecting 0 to 60% of *Puccinia graminis* subsp. *graminicola* (*Pgg*) pustules over the 2009-10 winter at Site 1. The model has been described in Chapter 2. The model integrates the effects of plant and *Pgg* growth dynamics and daily weather inputs on the daily number of erumpent *Pgg* pustules. Here, the model was run with a *Pgg* spore production curve set to represent the decrease in spore production observed in pustules infected by *S. filum* (Fig. 3). *S. filum* was simulated to infect 0, 20, 40 or 60% of all *Pgg* pustules. Observations are the means taken from perennial ryegrass plants inoculated with *Pgg* under ideal infection conditions and out-planted to the field (4 replications). Plants were scored for *Pgg* severity weekly.

Effects of fungicide. *S. filum* germination inhibition was measured on water agar against three fungicides along a dilution series (Fig. 11). At concentrations below 0.01% of the active ingredient (field concentrations are between 0.005 and 0.005%), chlorothalonil and azoxystrobin inhibited *S. filum* germination with a zone of inhibition between 6 and 8mm. There was no zone of inhibition around these same concentrations of propiconazole. At high concentrations (> 0.01% of the active ingredient), all three of the fungicides inhibited *S. filum* germination, but inhibition distance was 8 mm greater for axoxystrobin ($P = 0.003$), and 7 mm greater for chlorothalonil ($P = 0.012$) than for propiconazole.

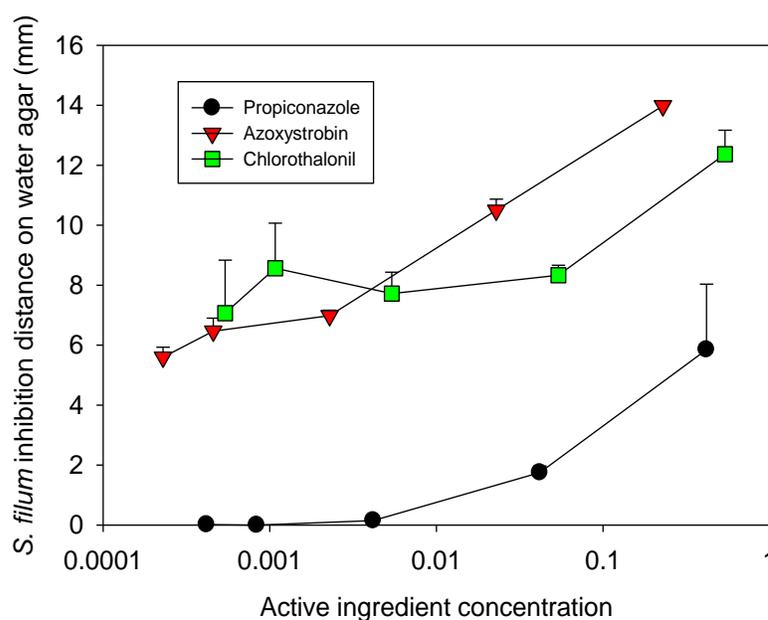


Fig. 11. *S. filum* conidiospore germination inhibition distance by three fungicides. The three fungicides and their active ingredients are: Tilt® (propiconazole 41.8%), Quadris® (azoxystrobin 22.9%) and Bravo® (chlorothalonil 54.0%). Filter discs (5.5 mm dia.) saturated in serial dilutions of each fungicide (3 replications at each concentration) were placed onto water agar plates that had been sprayed with a suspension of *S. filum* conidia. Plates were incubated with 12h dark and light periods at 23°C for five days. The distance between the edge of the filter disc and the point where there was ≥10% germination was measured. Data shown are the mean and SEM of two trials.

Discussion

Controlled inoculations. These experiments quantified conditions required for *S. filum* infection of *Pgg* pustules. Previous research by others had shown that *S. filum* does not infect *Cronartium fusiforme* pustules when both fungi are inoculated simultaneously (Kuhlman et al., 1978), but readily infects *Melampsora epitea* on willow (Pei et al., 2003), or *Puccinia recondita* on wheat (Swendsrud and Calpouzos, 1972) when conidia and urediniospores are inoculated onto leaf tissue simultaneously. Our results were closer to those reported for *S. filum* on *C. fusiforme* than for *S. filum* on *P. recondita* or *M. epitea*. *S. filum* infected 13% more 10-day-old *Pgg* pustules than 5-day-old pustules and 36% more 10-day-old pustules than 0-day-old pustules. *Pgg* infections that were zero days old at the time of *S. filum* inoculation were generally not infected by the mycoparasite.

Our experiments showed that leaf wetness duration played a significant role in the proportion of *Pgg* pustules infected with *S. filum*, as others had shown previously for *S. filum* infection on *C. fusiforme*, *P. recondita* and *M. larici epitea*. Treatments incubated with more than 6 h of mist had a 10% increase in the proportion of *Pgg* pustules infected. *Pgg* pustule treatments incubated with less than 4 h of mist were not significantly infected with *S. filum*. *S. filum* infected 42% fewer *C. fusiforme* pustules with 4 h of moisture than it did with 16 h of moist incubation (Kuhlman et al., 1978).

Incubation temperature did not have a significant effect on the number of *Pgg* pustules infected that were 10, 5 or 0 days old when inoculated with the mycoparasite. At Site 1, 30 and 40% of the days between the planting date and 15 April were favorable for *S. filum* infection (temperatures above 3.5°C and >6 h of leaf wetness).

S. filum colonizes 20.5% of *Melampsora larici-epitea* pustules on detached willow leaves and reduces spore production by 38.4% per pustule (Pei et al., 2010). In our experiments *S. filum* infected between 20 and 60% of *Pgg* pustules and reduced pustule spore production by 54%.

The reduction in *Pgg* spore production is presumed to be the mechanism by which *S. filum* reduces rust epidemics (Pei and Yuan, 2005).

A simulation model that integrated wheat leaf area, *P. recondita* and *S. filum* biology concluded that *S. filum* would reduce the severity of a leaf rust epidemic by 60-80% when 40-60% of the pustules were colonized (Hau and Kranz, 1978). An overwintering model that we developed showed *S. filum* reducing the early spring epidemic by 20-70% when 20-60% of the pustules were infected. Virulent strains of *S. filum* can infect 98% of *Melampsora epitea* pustules and reduce spore production by 98% (Yuan et al., 1999). Our model predicts that if we obtained an *S. filum* isolate that infected 98% of *Pgg* pustules and reduced spore production by 98% the rust population would approach zero by spring.

Field Experiments. Experiments with rust diseases and *S. filum* in the field have given only a qualitative understanding of how the mycoparasite lives in the field. Naturally occurring levels of *Cronartium strobilinum* uredinia infected with *S. filum* in North Florida oak stands was between 7 and 93% in 1975 (Kuhlman et al., 1978). Levels of *S. filum*-infected *Melampsora* spp. on willow plantations in Germany was between 15 and 26% in 2000-2002 (Liesebach and Zaspel, 2005). A *Melampsora* spp. epidemic was suppressed by *S. filum* without application of the mycoparasite in the UK in 1990 (Pei and Yuan, 2005).

Our controlled inoculations with *S. filum* to emerging *Pgg* uredinia on plants that were later out-planted to the field showed that *S. filum* survived the winter on *Pgg* uredinia, and reduced the *Pgg* pustule count through late spring in 2009-10. In 2010-11 *S. filum* infected *Pgg* uredinia, but the uredinia population (whether inoculated with *S. filum* or not) crashed in February, and it was therefore impossible to observe a late spring effect of *S. filum*.

S. filum applied with a backpack sprayer in the field one time showed no significant treatment difference in the severity of the *Pgg* epidemic in the spring. The one-time application

of *S. filum* did show a significant increase in the proportion of *Pgg* pustules infected with *S. filum*, compared with the non-treated control, in the cut sampling method in 2010-11. The other sampling methods did not show a significant increase in *S. filum* infected pustules in the treatment that received a one-time application of *S. filum*.

S. filum applied monthly to plants in the field showed a significant increase over the non-inoculated checks in the proportion of pustules infected in all sampling methods in both years on the first year stand. Monthly applications of *S. filum* decreased the severity of the *Pgg* epidemic when observed by several, but not all, of the sampling methods in both years. The different sampling methods may have different conclusions because the *Pgg* pustules and those infected with *S. filum* were more difficult to see in the repeatedly observed samples (*in situ*) than they were in the cut or dug samples.

One winter application of Quilt® fungicide significantly reduced the spring *Pgg* population on the first-year stands. There was not a significant effect of fungicide on the proportion of pustules infected with *S. filum*. Treatment effects were not observed on second and third year stands.

The results of this experiment provide evidence that lower *Pgg* overwintering populations give rise to less severe epidemics in the spring, supporting earlier indications of this effect (Pfender, 2004b; Shaner and Powelson, 1973). *S. filum* can infect *Pgg* pustules in first year field stands of perennial ryegrass during Oregon winter weather conditions when it is introduced in an inundative fashion on several occasions. Although Quilt® fungicide is the most effective treatment we observed at reducing winter inoculum, *S. filum* also was shown to lower the winter *Pgg* populations over the winter and into the spring.

Future research. These experiments brought up several questions that could be researched further. In the Quilt treatments, there were no recorded *Pgg* pustules from the time of the Quilt

application (February) until mid-May. It would be interesting to ascertain if the inoculum that gave rise to infections by late May came from adjacent treatment plots (which would lead us to conclude that the entire epidemic is started from a local inoculum,) or from sources kilometers away.

A second area to research further would be strategies for making *S. filum* control more effective. To do this, a greater proportion of pustules would need to be infected by the mycoparasite, and the number of urediniospores that each pustule produces would need to be reduced. Additional research focusing on finding or breeding more virulent strains of *S. filum* could be conducted to aid in this effort (Lieseback and Zaspel, 2005). Additionally, finding a way to apply the mycoparasite in a manner that is simple, inexpensive, and that insures high *S. filum* inoculum at optimal infection conditions would be necessary if this control tactic were to be used on a larger scale. A mycoparasite of powdery mildew (*Ampelomyces quisqualis*) was successfully introduced onto infected grapevines using the fungus grown on cotton wicks saturated in malt agar (Falk et al., 1995a). We observed that *S. filum* can be grown on whole grain media, and such colonized grain could be applied to a field along with fertilizer applications customarily made in early spring.

S. filum germination and growth was inhibited by azoxystrobin (Quadris®) and chlorothalanil (Bravo®) fungicides on water agar plates; however, it showed little inhibition in the presences of the propiconazole fungicide (Tilt®), especially at field rate concentrations. It would be interesting to do further work applying *S. filum* and triazole fungicides together to maximize their efficiencies.

This experiment provided further evidence that second and third year stands of perennial ryegrass cv. Jet are far more resistant to *Pgg* infection than are first year stands (Pfender, 2004b). In 2009-10 first year stands had 2.8 times greater *Pgg* severity as calculated

from the area under the curve from 1 March until 11 June than second year stands across all treatments and observation methods. In 2010-11 the first year stand had a spring *Pgg* severity (calculated from the area under the curve from 1 March to 2 June) that was 341 times greater than the third year stand. In order to better understand the mechanisms behind this resistance it would be necessary to study this phenomenon further. It would also be judicious to incorporate this information into epidemic models in order to more accurately represent this perennial ryegrass disease system.

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GENERAL CONCLUSIONS

Stem rust, caused by the fungus *Puccinia graminis* subsp. *graminicola*, causes severe yield losses on perennial ryegrass grown for seed in the Willamette Valley of Oregon. Other studies have provided evidence that the severity of stripe, and leaf rust on cereals and stem rust on grass epidemics observed during the cropping season are proportional to the amount of inoculum that overwinters. The objectives of this research were: (i) to construct and validate a mathematical model, based on empirical observations, that describes the overwintering dynamics of stem rust on perennial ryegrass (ii) simulate the effects of changing spore production and infection conditions on the stem rust overwintering survival, and (iii) quantify the effects of the mycoparasite, *S. filum*, on both individual stem rust pustules and the overwintering stem rust population.

The mathematical model that we constructed integrated processes important for the survival of stem rust over the winter including: (i) leaf and tiller growth dynamics, (ii) pustule longevity, latent period, spore production and spore dispersal, and (iii) meteorological conditions. The model was trained on six data sets collected in the field in 2002-03 and was tested with data collected at two field sites during 2009-10 and 2010-11. In both years and at both sites the model followed the general trend of the overwintering stem rust population. In 2009-10 the model predicted stem rust populations from 1 March to 15 April (calculated as area under the curve) that were not statistically different from those observed at both field sites. In 2010-11 the model predicted a population that was higher than the one observed, but this difference was not analyzed for significance because all the observed values were zero.

When the model was run with infection conditions that were set at 0, 0.125 and 0.25 times that of ideal infection conditions for two consecutive latent periods starting on 15 January, the disease severity from 1 March to 15 April was reduced by 81, 46 and 0.0% from

that observed. When the model was set at 0.5 times that of ideal infection conditions for two consecutive latent periods, the disease severity was 1.4 times greater than that observed. When the model was run with pustules set to produce 20, 40 or 60% fewer spores than observed, the disease severity from 1 March to 15 April was reduced by 74, 92 and 99% from that observed.

Sphaerellopsis filum is a mycoparasite of the rusts, and has been shown to colonize uredinia and reduce spore production. We found that *S. filum* reduces stem rust spore production over the lifetime of a pustule from 39,000 to 18,000 spores, and reduces spore viability by 4.75%. *S. filum* was able to infect significantly more stem rust uredinia with more than 6 h of continuous leaf wetness than at leaf wetness durations that were less than 6 h. *S. filum* was also able to readily infect stem rust pustules at temperatures above 5°C; we did not test *S. filum* infection favorability below 5°C. These conditions were present during Oregon winters 30 to 40% of the days between planting and 15 April in 2009-10 and 2010-11. Stem rust pustules that were 10 days old at the time of *S. filum* inoculation were more readily infected than 5-day-old pustules or 0-day-old pustules.

In controlled inoculation experiments that were out-planted to the field, the stem rust severity was up to 20 times lower and the proportion of stem rust pustules infected with *S. filum* was up to 4 times greater in plants inoculated with *S. filum* and stem rust than in plants inoculated with stem rust alone in 2009-10 at both field sites. In 2010-11 the stem rust population went to zero at both sites and treatment differences could not be observed.

In field inoculation experiments on newly planted stands of perennial ryegrass, naturally infected with *P. graminis*, stem rust severity was up to 2 times lower and the proportion of stem rust pustules infected with *S. filum* was up to 9 times greater in treatments that received monthly applications of *S. filum* than in control treatments in 2009-10. In 2010-11 stem rust severity was up to 1.5 times lower and the proportion of stem rust pustules infected with *S.*

filum was up to 35 times greater in treatments that received monthly applications of *S. filum*. One winter application of *S. filum* did not have a significant effect on the stem rust population in either year. One winter application of fungicide lowered stem rust severity by up to 5 times in 2009-10 and up to 67 times in 2010-11 from that observed in the non-treated control. One winter fungicide application did not lower the proportion of stem rust pustules infected with *S. filum* in either year, compared with the non-treated control. There were no treatment differences in either the stem rust severity or the proportion of stem rust pustules infected with *S. filum* in field inoculation experiments on established (≥ 2 years old) stands of perennial ryegrass.

S. filum was simulated to infect a realistic 20, 40 or 60% of stem rust pustules in the overwintering model. This was done by reducing the spore production and spore viability equations in the model by the amount calculated from pustules infected with *S. filum*. The model predicted that stem rust severity (calculated as area under the curve from 1 March to 15 April) would be reduced by 61, 78 or 89%, respectively.

The simulation model that was developed in this study allowed us to identify processes that limit the amount of stem rust inoculum that overwinters. Both meteorological conditions and spore production capacity were shown to affect the overwintering population dynamics of stem rust. *S. filum*, a mycoparasite of rusts, reduced stem rust spore production, and was identified as a possible overwintering biological control strategy in this study. In some controlled inoculation experiments with *S. filum* out-planted to the field, and in several monthly field inoculation experiments with *S. filum*, the overwintering stem rust population was reduced. One winter application of fungicide was also found to reduce the overwintering stem rust population. Other strategies of reducing the overwintering stem rust inoculum might include altering the host genetics so as to increase the stem rust latent period or reduce the amount of

time that individual leaves are alive. The effects of these strategies, once identified, can be simulated in the model and tested for efficacy in the field.

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APPENDIX A

Appendix Table 1

There are two interlinked models described in this table. Table 1A, the overall model, uses the leaf model outputs and meteorological conditions to determine how many infections are initiated on each day on each leaf. Table 1B, the individual leaf models, take the inputs calculated from the overall model and determine when leaf tissue grows and senesces, when individual infection cohorts erupt, and how many spores are produced in each cohort. This information is then exported to the overall model.

Table 1A overall model				
Col.	Title	Example	Formula	Description
A	Date	10/8	Input from a weather data file	Date
B	Day	281	Input from a weather data file	Day (0-365)
C	Daily Latent Period (LP)	0.1	Input from a weather data file	Daily <i>Pgg</i> latent period accumulation
D	Cumulative Latent Period today and yesterday (CLPt, CLPy)	1.1	Input from a weather data file	Accumulated <i>Pgg</i> latent periods
E	Standard Infection Value (Std InfVal)	3.0	Input from a weather data file	Relative nightly <i>Pgg</i> infection condition of maximum (range 0 to 3)
F	CLP since Planting	1.1	Input from a weather data file	Accumulated <i>Pgg</i> latent periods since planting date
G	Leaf Area Curve 1	IF(CLPt<1.047,0,IF(AND(CLPt>=1.047,CLPt<1.66),(Yesterday's leaf area+(0.6*(CLPt-CLPy))),IF(AND(CLPt>=1.66,CLPt<2.803),VLOOKUP((1.66-0.0001),return the largest number before this CLP),IF(CLPt<3.84,yesterday's leaf area+(-0.352*(CLPt-CLPy)),0))))	IF(\$F17<1.047,0,IF(AND(\$F17>=1.047,\$F17<1.66),(G16+(0.6*(\$F17-\$F16))),IF(AND(\$F17>=1.66,\$F17<2.803),VLOOKUP((1.66-0.0001),\$F\$5:\$G\$221,2,TRUE),IF(\$F17<3.84,G16+(-0.352*(\$F17-\$F16)),0))))	Equations for growth and senescence of each leaf based on heat units. The output is in cm ²
T	Total Leaf Area	0.1	SUM(G17:S17)	Leaf area on one tiller in cm ²
V	Leaf Area Proportion, Leaf 1	IF(Leaf area 1=0,0,leaf area 1/total leaf area)	IF(\$T17=0,0,G17/\$T17)	Proportion of leaf 1 tissue compared to all leaf tissue on the tiller
AI	Total Leaf Area	1.0	SUM(V17:AE17)	Sum of all of the proportions on the tiller (should be 1)
AJ	Tillers	IF(CLPt<2.67,1,(EXP((CLPt-2.67)*.2943)))	IF(F5<2.67,1,(EXP((F5-2.67)*\$AJ\$2)))	The number of tillers on an individual plant

Table 1A overall Model				
Col.	Title	Example	Formula	Description
AK	Erumpent Lesions on Leaf 1	0.0	Leaf 1! \times N18 (from leaf 1 model)	The output erumpent pustules from Leaf 1 on this day
AX	Total Erumpent Pustules	0.0	SUM(AK17:AW17) \times \$AX\$3	Sum of all of the erumpent pustules from each leaf, multiplied by the number of tillers on that plant and plants in a described length of row. (In this case 10 cm of row is 8.019 plants)
BA	Spores that land on leaf 1	Spores from leaf 1 sheet \times .53	Leaf 1! \times N248 \times BN\$2	The number of spores produced on leaf 1 multiplied by the proportion of spores expected to land on leaf 1
BN	Spores for wider dispersal	SUM((spores from leaf 1 \times .47)+(spores from leaf 2 \times .47)+(spores from leaf 3 \times .47)+(spores from leaf 4 \times .47)+(spores from leaf 5 \times .47)+(spores from leaf 6 \times .47)+(spores from leaf 7 \times .47)+(spores from leaf 8 \times .47)+(spores from leaf 9 \times .47)+(spores from leaf 10 \times .47)+(spores from leaf 11 \times .47)+(leaf 12 spores \times .47)+(leaf 13 spores \times .47))	SUM((BA17 \times BN\$3)+(BB17 \times BN\$3)+(BC17 \times BN\$3)+(BD17 \times BN\$3)+(BE17 \times BN\$3)+(BF17 \times BN\$3)+(BG17 \times BN\$3)+(BH17 \times BN\$3)+(BI17 \times BN\$3)+(BJ17 \times BN\$3)+(BK17 \times BN\$3)+(BL17 \times BN\$3)+(BM17 \times BN\$3))	The sum of the spores from each leaf multiplied by the proportion of spores that are expected to escape from the original host leaf
BP	Occupied correction factor	IF the leaf area for this leaf cohort is zero, enter zero, otherwise, calculate the Poisson distribution for the number of sites occupied on this leaf by erumpent pustules or spores from autoinfections or alloinfections divided by the total number of sites present on this leaf tissue	IF(G5=0,0,EXP(-((\$AL5 \times \$BV\$2)+(BA5 \times \$BV\$3)+(\$BN5 \times \$BV\$3 \times V5)/G5)))	Poisson correction for sites already occupied by erumpent pustules or other spores

Table 1A Overall model				
Col.	Title	Example	Formula	Description
CD	New infection factor	IF(standard infection factor=0,0,(10^standard infection factor/10^3))	IF(\$E17=0,0,(10^\$E17/10^3))	The proportion of the spores landing on susceptible tissue that can germinate, based on nightly weather conditions
CG	New Infections on Leaf 1	Number of spores retained by this leaf multiplied by the autoinfection rate. This is added to the number of spores available for wider dispersal multiplied by the alloinfection rate and the leaf tissue occupied by this leaf cohort as a proportion of leaf tissue occupied by all leaf tissue on the plant. This number of spores is then multiplied by the infection conditions today, number of tillers on the plant yesterday and the occupied site correction factor for this leaf cohort.	((BA5*\$CF\$2)+(\$BN5*\$CF\$3*V5)*\$CD6*\$AJ6*BP5)	Calculates the number of infections that will be initiated on each leaf cohort on the plant each day by multiplying leaf area, infection condition, infection rate and spores present on a daily time-step.
CT	Total new Infections Today	Adds all of the infections on this plant today	SUM(BR17:CD17)	Adds all of the infections on this plant today

Table 1B Individual Leaf				
Col.	Title	Example	Formula	Description
A	Date	10/8	Input from model overall page	Date
B	Day	281	Input from model overall page	Day (0-365)
C	Latent Period (LP)	0.1	Input from model overall page	Daily <i>Pgg</i> latent period accumulation
D	Adjusted CLP since planting CLPt (today's CLP) and CLPy (yesterday's CLP)	1.1	Input from model overall page	Accumulated <i>Pgg</i> latent periods since planting
F	Cohort Number	IF(OR(leaf area today>0,cumulative dead leaf area>0),1,0)	IF(OR(J29>0,L29>0),1,0)	Used to determine pustule and leaf cohort ID
G	Cohort ID	IF(cohort number>0,1+yesterday's cohort number,0)	IF(F29>0,F29+G28,0)	Leaf and pustule cohorts are assigned a number corresponding to the day they were initiated
H	CLP Pustule (CLPp)	IF(leaf area<=0,0,((CLPt-CLPy)+CLPp yesterday))	IF(J29<=0,0,((D29-D28)+H28))	Sums up the latent periods on this leaf when leaf area>0
I	CLPL	CLPt	D29	References the latent periods since planting
J	Leaf Area Curve 1	IF(CLPL<1.047,0,IF(AND(CLPL>=1.047,CLPL<1.66),(yesterday's leaf tissue+(0.6*(CLPL-CLPLy))),IF(AND(CLPL>=1.66,CLPL<2.803),VLOOKUP((1.66-0.0001),return the greatest leaf area before this CLPL),IF(CLPL<3.84,J28+(-0.352*(CLPL-CLPLy)),0))))	IF(\$I29<1.047,0,IF(AND(\$I29>=1.047,\$I29<1.66),(J28+(0.6*(\$I29-\$I28))),IF(AND(\$I29>=1.66,\$I29<2.803),VLOOKUP(1.66-0.0001,\$I\$7:\$J\$222,2,TRUE),IF(\$I29<3.84,J28+(-0.352*(\$I29-\$I28)),0))))	A formula specific to each leaf that gives a growth and senescence equation based on heat units. The output is in cm ²
K	LA Dies cm2	IF((today's leaf area-yesterday's leaf area)<=0,0,(today's leaf area-yesterday's leaf area))	IF((J28-J29)<=0,0,(J28-J29))	Calculates the amount of leaf tissue that died on this leaf today

Table 1B Individual Leaf				
Col.	Title	Example	Formula	Description
L	Cumulative LA death cm2	today's dead leaf area+yesterday's cumulative dead leaf area	K29+L28	Sums up the amount of leaf tissue that has died on this leaf.
M	New infections Today	Model (overall) new infections on leaf 1	Model (overall)!BR28	The number of infections that occurred today on this leaf cohort. Imported from the overall model page.
N	Erumpent Lesions Today	SUM(all of the erumpent pustules that emerged today)	SUM(O29:P29)	Sums up all the erumpent lesions from all of the pustule cohorts on this leaf.
O row 1	Cohort 1 ID	1	1	The leaf and pustule cohort for a given day.
O row 2	Number of infections initiated the day this pustule cohort arose	VLOOKUP(cohort ID,return the amount of infections initiated today)	VLOOKUP(O\$3,\$G:\$M,7,FALSE)	Looks up the the number of infections initiated on the day this cohort became established
O row 3	Leaf area available the day pustule cohort 1 was initiated	IF(VLOOKUP(pustule cohort, return the amount of leaf tissue available that day)<=0,10,(VLOOKUP(pustule cohort,return the amount of leaf tissue available that day)))	IF(VLOOKUP(O\$3,\$G:\$J,4,FALSE)<=0,10,(VLOOKUP(O\$3,\$G:\$J,4,FALSE)))	This equation looks up the amount of leaf tissue available for infection for this cohort.

Table 1B Individual Leaf				
Col.	Title	Example	Formula	Description
O row 4	Pustule cohort 1 erumpent pustules	IF(AND((CLPp<VLOOKUP(pustule cohort,return the CLPp of this pustule cohort)+pustule lifespan),(CLPp>VLOOKUP(pustule cohort, return the CLPp of the cohort)+pustule latent period),IF(IF(VLOOKUP(pustule cohort, return the CLPp of the cohort)<1.93,((initial leaf area for this cohort-cumulative leaf dead area)/initial leaf area for this cohort)*initial number of pustules present for this cohort,(leaf area today/initial leaf area for this cohort)*initial number of infections on this day)<0,0,(IF(VLOOKUP(pustule cohort, return the CLPp of the cohort)<1.93,((initial leaf area for this pustule-cumulative dead leaf area)/initial leaf area)*initial number of infections for this cohort,(leaf area today/initial leaf area for this cohort)*initial number of infections on this cohort))),0)	IF(AND((\$H6<VLOOKUP(O\$3,\$G:\$H,2,FALSE)+'Model (overall)!\$BX\$2),(\$H6>VLOOKUP(O\$3,\$G:\$H,2,FALSE)+'Model (overall)!\$BY\$2)),IF(IF(VLOOKUP(O\$3,\$G:\$H,2,FALSE)<1.93,((O\$5-\$L6)/O\$5)*O\$4,(\$J6/O\$5)*O\$4)<0,0,(IF(VLOOKUP(O\$3,\$G:\$H,2,FALSE)<1.93,((O\$5-\$L6)/O\$5)*O\$4,(\$J6/O\$5)*O\$4))),0)	Calculates the number of erumpent pustules in this pustule cohort on this day. If the infections initiated on this day have gone through at least one Pgg latent period, but haven't reached the end of their lives based on heat units, calculate Term 1 (explained later), otherwise enter 0. Term 1 splits the pustule cohorts into two groups, Group 1 and Group 2. Group 1 cohorts were initiated before the leaf started dying. The equation subtracts the cumulative dead leaf tissue from the initial leaf tissue on this leaf cohort and divides that number by the original leaf tissue. This remaining leaf tissue proportion is multiplied by the number of original pustules on this cohort. Group 2 cohorts were initiated on the leaf after the leaf started dying. For these cohorts today's leaf tissue is divided by the initial leaf tissue for this cohort and multiplied by the original number of Pgg infections initiated on this cohort. A value of zero is entered if the final pustule number is less than zero.

Table 1B Individual Leaf				
Col.	Title	Example	Formula	Description
O row 252	Daily spore production	(4000*((1.1-1)/1.1)^(1-1.1)/1.1)*(ABS((CLPp-(VLOOKUP(pustule cohort, return the starting CLPp value))-1))/0.4+((1.1-1)/1.1)^(1/1.1))^(1.1-1)*EXP(-ABS(CLp-(VLOOKUP(pustule cohort, return the starting CLPp value))-1)/0.4+((1.1-1)/1.1)^(1/1.1))^1.1+(1.1-1)/1.1)*number of erumpent pustules produced today	('Model (overall)!\$CA\$2*('Model (overall)!\$CC\$2-1)/'Model (overall)!\$CC\$2)^((1-'Model (overall)!\$CC\$2)/'Model (overall)!\$CC\$2)*(ABS((\$M226-(VLOOKUP(O\$225,\$L\$226:\$M\$430,2,FALSE))-'Model (overall)!\$CD\$2))/'Model (overall)!\$CB\$2+('Model (overall)!\$CC\$2-1)/'Model (overall)!\$CC\$2)^(1/'Model (overall)!\$CC\$2))^('Model (overall)!\$CC\$2-1)*EXP(-ABS(\$M226-(VLOOKUP(O\$225,\$L\$226:\$M\$430,2,FALSE))-'Model (overall)!\$CD\$2))/'Model (overall)!\$CB\$2+('Model (overall)!\$CC\$2-1)/'Model (overall)!\$CC\$2)^(1/'Model (overall)!\$CC\$2)^('Model (overall)!\$CC\$2+('Model (overall)!\$CC\$2-1)/'Model (overall)!\$CC\$2)*O6	The daily number of spores produced by a pustule is multiplied by the number of pustules in that cohort. The number of spores produced is determined through a Weibull (4-parameter) curve equation that was fitted to spore production data across <i>Pgg</i> latent periods.
N row 252	Total spores produced	SUM(all the spores produced today)	SUM(O252:S252)	Sum of all of the spores produced from each cohort on this leaf. This is exported to the overall model page.