Barberry Does Not Function as an Alternate Host for *Puccinia striiformis* f. sp. *tritici* in the U. S. Pacific Northwest Due to Teliospore Degradation and Barberry Phenology

M. N. Wang, Department of Plant Pathology, Washington State University, Pullman 99164-6430; and X. M. Chen, USDA-ARS, Wheat Genetics, Quality, Physiology, and Disease Research Unit, and Department of Plant Pathology, Washington State University, Pullman 99164-6430

Abstract

Wang, M. N., and Chen, X. M. 2015. Barberry does not function as an alternate host for *Puccinia striiformis* f. sp. *tritici* in the U. S. Pacific Northwest due to teliospore degradation and barberry phenology. Plant Dis. 99:1500-1506.

Sexual reproduction of the stem rust pathogen, *Puccinia graminis* f. sp. *tritici* (*Pgt*), on barberry (*Berberis vulgaris*) has been shown to provide initial inoculum for the development of the disease on wheat and barley and also generate diverse races of the pathogen. However, in our previous study, the stripe rust pathogen, *P. striiformis* f. sp. *tritici* (*Pst*), was not found on barberry in the U. S. Pacific Northwest. To determine why *Pgt* is able to infect the alternate host, while *Pst* cannot under the natural conditions, the viabilities of teliospores of both *Pgt* and *Pst* were investigated from 2011 to 2014 by determining the germination rates using telial samples collected periodically from wheat fields. Teliospores of *Pst* usually produced in July were physically degraded during winter, and their germination rate decreased from 50 to 90% in August to less than

1% in the following March and no germination after May. In contrast, *Pgt* teliospores usually produced in July and August remained physically intact and physiologically dormant, and could not germinate until February. Germination of *Pgt* teliospores gradually increased to 90% in May, at which time young leaves of barberry were susceptible to infection. In addition, a time-series experiment was conducted for inoculation of barberry plants with *Pst* teliospores. The results showed that *Pst* teliospores need a minimum of 32 h continual dew-forming conditions to infect barberry, and infection reaches a peak after incubation of inoculated plants for 88 h. The lack of a prolonged period of leaf wetness conditions during the season of telial maturity effectively negates *Pst* infection of barberry plants in the Pacific Northwest.

Both stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), and stem rust, caused by *P. graminis* Pers.:Pers. f. sp. *tritici* Erikss. & E. Henn. (*Pgt*), are important diseases of wheat in many regions of the world (Chen 2005; Jin 2011; Kolmer et al. 2009; Leonard and Szabo 2005; Roelfs 1985b; Wellings 2011). In the U. S. Pacific Northwest (PNW), stripe rust occurs every year and is able to cause devastating epidemics in the entire region, while epidemics of stem rust occur less frequently and are limited to eastern Washington and northern Idaho where alternate host barberry plants exist (Chen 2005, 2014; Jin 2011; Roelfs 1985a, b; Sharma-Poudyal and Chen 2011; Wang et al. 2015).

Although alternate hosts for *Pgt* has been well known since de Bary's work in the 1860s (Chabelska 1938; Eriksson and Henning 1894; Leonard and Szabo 2005), the aecial hosts for *Pst* remained unknown until recently (Chen et al. 2012; Jin et al. 2010; Wang et al. 2011; Zhao et al. 2013). As an alternate host, common barberry (*Berberis vulgaris* L.) plays an important role for stem rust epidemics in the United States, especially the PNW (Jin 2011; Peterson 2003; Roelfs 1982; Wang et al. 2011, 2015). Aecia on barberry can provide the initial inoculum of highly diverse races to cause stem rust in wheat and barley (Jin 2011; Roelfs 1982; Wang et al. 2015). Although sexual reproduction has been strongly suggested for *Pst* in some regions (Ali et al. 2014) and demonstrated under controlled

conditions (Chen et al. 2012; Jin et al. 2010; Wang and Chen 2013) and at a very low frequency under natural conditions (Zhao et al. 2013), evidence for involvement of alternate hosts in stripe rust epidemics under natural conditions is still limited.

Because both stripe rust and stem rust occur with their alternate host plants in the PNW, it is important to determine the role of the alternate host in the epidemiology of the diseases. In a previous study, the *Pst* population was found to be clonal, providing indirect evidence that the alternate host is not important for stripe rust (Cheng and Chen 2014). In a companion study to characterize aecia collected from barberry plants from 2010 to 2013 using pathogenicity tests and molecular markers, we found that barberry plants in the PNW were infected by *Pgt* and to a much lesser extent by other formae speciales of *P. graminis*, but we did not detect any *Pst* aecia (Wang et al. 2015). All these studies demonstrated that barberry is essential for stem rust, but we found no evidence for a role for barberry in stripe rust in the PNW. As teliospore viability is essential for infection of an alternate host, our hypothesis is that *Pst* and *Pgt* differ in teliospore viability under the natural conditions in the PNW. The specific objectives of the present study were to determine 1) year-round germination rates of *Pst* and *Pgt* teliospores and 2) the duration of moisture required for infection of barberry by *Pst* teliospores. The results of the present study, together with regional weather conditions and phenology of the primary and alternate hosts, enable us to answer the question of why *Pgt* is able to infect an alternate host, but *Pst* cannot in the PNW.

Corresponding author: X. M. Chen; E-mail: xianming@wsu.edu

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

Accepted for publication 8 April 2015.

<http://dx.doi.org/10.1094/PDIS-12-14-1280-RE>

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2015.

Materials and Methods

Sampling of *Pst* and *Pgt* telia. Telial samples were randomly collected from wheat leaves before harvest and from stubble after harvest once a month from July to the next May from five sites in one of our experimental fields near Pullman, Washington. The field was planted with spring wheat cultivar Lemhi, which is susceptible to both stripe rust and stem rust, without fungicide application. For germination tests, three types of telial samples were used: 1) wheat stubble laying on the ground in the field (hereafter referred as "ground" samples); 2) wheat plants standing in the field before or

after harvest (referred as “standing” samples); and 3) wheat plants kept at room temperature (referred as “room” samples). Telia of the room samples were tested from July 2011 to May 2012, and those of field ground and standing samples were tested for germination rates in three seasonal cycles, from July 2011 to May 2012, July 2012 to May 2013, and July 2013 to May 2014.

Morphological characterization. Morphological and structural changes of telia, including evidence of degradation or damage, were examined both macroscopically and microscopically. For microscopic examinations, a Zeiss Stemi 2000-C stereo microscope and a Zeiss Axioskop 40 light microscope (Diagnostic Instruments, Inc., Sterling Heights, MI) were used. Characteristics of telia and teliospores included size, color, arrangement, shape, position within or outside host epidermis, and degradation.

Determination of optimal conditions for germination of *Pst* and *Pgt*. Initially, the optimal temperature was determined for *Pst* teliospore germination using teliospores of isolate 09-134, identified as race PST-127 using the old differentials and as PSTv-11 using the new set of *Yr* single-gene differentials (Wan and Chen 2012, 2014), that had been collected from inoculated plants grown in a greenhouse and stored for a month at 4°C. Teliospores of *Pgt* from wheat plants that had been collected in August 2010 and kept outdoors through the winter to break dormancy (Anikster 1986) were used for determining the optimal temperature and time point for germination in the middle of April 2011. Five temperatures, 5, 10, 15, 20, and 22°C, were used in separate tests for teliospore germination, all at 100% humidity. The procedure of making spore suspensions and incubation were detailed as below.

Determination of viability and germination of time-series teliospore samples from fields. To determine how long *Pst* teliospores can survive in cool and wet conditions of the winter, teliospore germination was determined under various artificial conditions in the laboratory. Wheat straw bearing telia sampled from the field was cut into 5 cm fragments, placed on filter papers misted with sterile water in a petri dish, incubated at 5°C, and periodically inspected for teliospore germination. The teliospore suspension and incubation and calculation of germination rates were carried out basically following the procedure as previously described (Anikster 1986) with modifications as described below.

Wheat leaves or straws bearing telia were flushed under slowly running tap water for 5 min to remove dirt and saprophytes. The leaves or straws were cut into 5 cm fragments. The fragments were divided into two parts; one for direct observation under a microscope to check the spore germination, and the other was wrapped with wet tissue paper and kept at 4°C for 3 h to soften the tissues. For a germination test, about 100 *Pst* or 20 *Pgt* telial sori from 10 randomly selected straw fragments picked under a stereomicroscope were transferred into a 1.5-ml centrifuge tube, and mixed in 1.0 ml sterile water. The different numbers of telia were used because *Pst* telia were much smaller than those of *Pgt*. Teliospores were gently scattered with a round glass rod, and the spore suspension was vigorously stirred for 3 min to disperse spores. A 100 µl teliospore suspension was transferred onto a sterile glass slide. The slide was inverted and placed on glass rods on moist filter papers in a petri dish. The spore suspensions were incubated at 10°C (for *Pst*) or 15°C (for *Pgt*) for 48 h and spore germination was observed under a microscope. The teliospores producing a four-celled promycium were considered germinated and therefore viable. For each test, three replicated slides were used. About 100 to 300 teliospores were observed to calculate germination rate for each replication. For the low germination cases, up to 600 teliospores were observed.

Determination of the duration of moisture required for *Pst* teliospores to infect barberry. The teliospores of isolate 09-134 from wheat cultivar Nugaines grown in a greenhouse were collected, and the spore suspensions were made of about 500 telia in 5.0 ml sterile water checked under a stereomicroscope. An aliquot (50 µl) of the teliospore suspension (about 300 to 500 teliospores) were brushed on to the adaxial surface of a 10-day old barberry leaf. The inoculated plants were kept in a dew chamber with 100% humidity at 10°C in the dark for 8 to 120 h at the same condition for inoculation of wheat

plants with *Pst* urediniospores (Cheng and Chen 2014). Three plants were randomly taken from the dew chamber at 8-h intervals, and placed in a growth chamber with 8 h dark / 16 h light and a diurnal temperature cycle of 4 to 20°C as described for *Pst* tests with urediniospores (Cheng and Chen 2014). Plants were inspected daily for symptoms and signs of infection. The days after inoculation were recorded when pycnia and aecial pustules first appeared. The number of pycnia on each inoculated leaf was counted when pycnia were fully developed.

Statistical analyses. Significant differences among the germination rates in the optimal temperature tests and the tests of the monthly samples were determined by analysis of variance (ANOVA) using SAS software (SAS Institute Inc., Cary, NC). The infection efficiencies of teliospores measured as numbers of pycnia on inoculated barberry leaves were compared among different incubation durations, also using ANOVA.

Results

Formation of uredinia and telia in wheat fields. Table 1 shows the monthly mean temperatures and precipitations of Pullman, WA and the phenology of wheat crops, barberry plants, and *Pst* and *Pgt* fungi around Pullman, WA from July 2011 to May 2014. Although the weather conditions varied from year to year, the hottest weather was in July to August and the coldest weather in December to January. Monthly precipitation generally was the highest (25.1 to 120.9 mm) from late fall to middle spring (October to April), low (23.4 to 47.0 mm) in late spring and early summer (May to June), and scarce (0.0 to 14.5 mm) from middle summer to middle fall (July to September) with an exception in September 2013. With the temperature and moisture conditions, winter wheat was planted in October and harvested in August, and spring wheat was planted in April and harvested in September. Stubbles could be found after harvest and even in fields planted with the next crop. Uredinia of *Pst* were generally found on wheat plants from May until September before spring wheat harvest (Table 1), and occasionally as early as March (in 2011) and as late as October on early planted winter wheat or volunteer wheat plants (data not shown). Wheat stripe rust was extremely severe in 2010 and 2011, about normal in 2012 and 2013, and low in 2014 (data not shown). Uredinia of *Pgt* could not be found until July on winter wheat and both spring wheat and spring barley (Table 1). Fresh telia of *Pst* generally appeared on leaves and leaf sheaths of winter wheat in late June and could be found afterward until winter wheat and spring wheat harvests. Fresh telia of *Pgt* could be found on leaves and stems few days after uredinial formation, while it took several weeks to see *Pst* telia after first observation of uredinia. Stem rust in wheat and barley fields was moderate in 2010 to 2012, severe in 2013, and hard to find in 2014 (data not shown). During this study and the accompany study (Wang et al. 2015), aecia were found on barberry plants in the Palouse region surrounding Pullman in 2010 to 2013, but very few in 2014. Pycnia and aecia first appeared as early as in mid-May (2010), but usually in late May (Table 1). The peak of aecial formation was in late May to mid-June, and aecia could not be found from August to October when leaves were fallen. The critical period of barberry infection by *Puccinia* species was from May to June.

Telial structures and timing of teliospore germination. Telia of *Pst* grow subepidermally in wheat tissue (Fig. 1A) and form nearly spherical sori consisting of teliospores inside and an outside peridial cell layer. To expose telia, it is necessary to remove the epidermis. As teliospores are enclosed in telia and covered by the telial peridium layer, they cannot germinate until the epidermis and peridium break. In contrast, a *Pgt* telium forms a tough and thick pseudoparachenma on the plant surface (Fig. 1A). Teliospores are born on the pseudoparachenmata without a peridium or the peridium break open, and after overwintering they can be germinated without breaking telia.

Telia of *Pst* under the epidermis of leaf sheaths remain physically intact after harvest in late summer and early fall under the usually dry conditions in the Palouse region. In general, precipitation gradually increases from October onwards and then decreases after

March, and conditions then become dry from July through September (Table 1). During the high-precipitation season of rain and snow from October to March, leaf epidermal tissue becomes fragile, and telia can be exposed, but teliospores in sheaths are usually not exposed even in the following spring because they are covered by an epidermal layer. With the decomposition of wheat straws and leaves in the high-precipitation season, telia were gradually degraded. Most telia were discolored and the leaf sheaths covered by saprophytic fungi (*Alternaria* and *Cladosporium* spp.) during and after winter (Fig. 1B). Unlike *Pst* telia that were submerged under the epidermis layer of host, *Pgt* telia readily erupted through the epidermis and became exposed on the surface of leaves and stems (Fig. 1A). *Pgt* telia appeared to be resistant to winter weather as

color and structure of *Pgt* telia remained unchanged in the spring (Fig. 1B). When telia-bearing straw was tapped over a glass slide, *Pgt* teliospores could be collected. This method did not work for *Pst* teliospores.

Teliospores of *Pst* readily germinated when mature during the crop season, during the summer after crop harvest, and in the fall (Fig. 1C), while *Pgt* teliospores could not be germinated (Fig. 1D). After winter, *Pst* teliospores became degraded and could not be germinated, and almost no normal *Pst* teliospores were observed in April (Fig. 1E). In contrast, *Pgt* teliospores became readily germinated in April (Fig. 1F).

Optimal temperatures for teliospore germination. Figure 2 shows the germination rates of *Pst* and *Pgt* at five temperatures (5,

Table 1. Weather conditions (monthly mean temperatures and precipitations) and phenology of wheat, barberry, *Puccinia striiformis* f. sp. *tritici* (*Pst*), and *P. graminis* f. sp. *tritici* (*Pgt*); and germinations of *Pst* and *Pgt* teliospores after incubation at 10°C for 48 h using telial samples collected under different field conditions from Pullman, Washington in July 2011 to May 2014

Month of sampling	Weather ^a		In wheat fields				On barberry plants				Teliospore germination (mean ± sd, %)			
			Growth stage		Uredinia ^b		Telia ^c		Barberry		<i>Pst</i>		<i>Pgt</i>	
	Mean temp. (°C)	Precip. (mm)	Winter wheat	Spring wheat	<i>Pst</i>	<i>Pgt</i>	<i>Pst</i>	<i>Pgt</i>	Stage ^d	Presence of aecia ^e	Standing ^f	Ground ^g	Standing	Ground
2011-2012														
July	16.9	7.1	Milk-maturing	Heading-milk	+	+	OC	OC	OL+GB	+	72.5±5.1	NS ^h	NT ⁱ	NT
August	19.6	0.0	Harvest	Milk-maturing	+	-	OC	OC	OL+GB	-	91.6±1.9	NS	0	0
September	17.5	5.6	Stubble	Harvest	+	-	OC	OC	OL+RB	-	74.2±5.4	62.3±8.5	0	0
October	9.6	35.8	Planting	Stubble	+	-	OS	OS	OL+RB	-	28.3±3.2	28.9±0.5	0	0
November	2.7	52.8	Seedling	Stubble	-	-	OS	OS	No leaves	-	33.0±3.8	11.0±2.2	0	0
December	-0.2	46.0	Overwintering	Stubble	-	-	OS	OS	No leaves	-	12.3±4.5	1.3±1.2	0	0
January	0.2	53.1	Overwintering	Stubble	-	-	OS	OS	No leaves	-	2.5±1.3	0.4±0.4	0	0
February	1.9	37.1	Overwintering	Stubble	-	-	OS	OS	No leaves	-	1.0±0.2	0.3±0.0	41.3±7.4	6.0±2.3
March	3.9	120.9	Tillering	Stubble	-	-	OS	OS	No leaves	-	0.5±0.2	0	30.3±14.8	3.0±0.3
April	9.1	63.2	Tillering	Planting	+	-	OS	OS	Leaf out	-	0	0	31.8±2.0	4.1±0.8
May	11.4	37.1	Jointing	Tillering	+	-	OS	OS	YL+FL	+	0	0	45.4±5.1	4.5±0.7
2012-2013														
June	14.5	47.0	Jointing-FL	Jointing-boot	+	-	OC	OS	OL+GB	+	NT	NT	NT	NT
July	19.7	14.5	Milk-maturing	Heading-milk	+	+	OC	OC	OL+GB	-	49.8±3.7	NS	NT	NT
August	19.8	0.0	Harvest	Milk-maturing	+	-	OC	OC	OL+GB	-	56.3±2.4	NS	0	0
September	16.3	0.5	Stubble	Harvest	+	-	OC	OC	OL+RB	-	24.7±1.4	52.2±16.6	0	0
October	9.1	67.8	Planting	Stubble	+	-	OS	OS	OL+RB	-	28.3±3.7	55.6±3.8	0	0
November	5.0	82.8	Seedling	Stubble	-	-	OS	OS	No leaves	-	30.9±2.6	26.2±6.2	0	0
December	0.6	76.2	Overwintering	Stubble	-	-	OS	OS	No leaves	-	16.2±4.0	7.0±1.0	0	0
January	-2.7	40.9	Overwintering	Stubble	-	-	OS	OS	No leaves	-	5.1±1.6	2.7±0.8	4.5±6.6	0
February	2.5	30.5	Overwintering	Stubble	-	-	OS	OS	No leaves	-	2.5±1.9	0.2±2.9	2.2±0.6	7.1±3.7
March	5.3	25.1	Tillering	Stubble	-	-	OS	OS	No leaves	-	0.2±0.1	0.1±0.1	20.4±7.8	5.3±0.8
April	7.5	62.5	Tillering	Panting	-	-	OS	OS	Leaf out	-	0	0	26.9±6.0	17.6±0.7
May	12.8	26.2	Jointing	Tillering	+	-	OS	OS	YL+FL	+	0	0	37.3±9.6	11.3±2.0
2013-2014														
June	15.3	43.4	Jointing-FL	Jointing-boot	+	-	OC	OS	OL+GB	+	NT	NT	NT	NT
July	20.4	5.1	Milk-maturing	Heading-milk	+	+	OC	OC	OL+GB	-	64.4±3.3	NS	NT	NT
August	20.7	11.9	Harvest	Milk-maturing	+	-	OC	OC	OL+GB	-	83.2±7.6	NS	0	0
September	16.8	59.2	Stubble	Harvest	+	-	OC	OC	OL+RB	-	72.3±2.9	71.2±9.3	0	0
October	7.7	11.7	Planting	Stubble	+	-	OS	OS	OL+RB	-	87.8±8.5	64.4±8.8	0	0
November	2.7	60.2	Seedling	Stubble	-	-	OS	OS	No leaves	-	32.6±9.2	89.1±3.9	0	0
December	-2.6	43.9	Overwintering	Stubble	-	-	OS	OS	No leaves	-	7.8±2.5	10.6±3.3	0	0
January	0.3	56.1	Overwintering	Stubble	-	-	OS	OS	No leaves	-	3.1±0.9	2.0±0.4	0	0
February	-2.2	77.5	Overwintering	Stubble	-	-	OS	OS	No leaves	-	1.0±0.2	1.0±0.0	1.5±0.4	1.6±0.1
March	5.1	67.3	Tillering	Stubble	-	-	OS	OS	No leaves	-	0.3±0.1	0.2±0.1	20.0±3.2	5.9±2.0
April	7.9	40.4	Tillering	Panting	-	-	OS	OS	Leaf out	-	0	0	68.8±5.3	17.2±2.7
May	13.3	23.4	Jointing	Tillering	+	-	OS	OS	YL+FL	-	0	0	84.5±5.4	22.5±1.4

^a The weather data of Pullman were obtained from the U. S. National Climatic Data Center (<http://www.ncdc.noaa.gov>).

^b Presence (+) and absence (-) of uredinia on wheat crops.

^c Presence of telia on crop (OC) or on stubble (OS).

^d OL = old leaves that are not susceptible to *Puccinia* infection; GB = green berry (only young green berries can be infected by *Puccinia* spp. YL = young leaves that are susceptible to *Puccinia* infection; FL = flowering; and RB = red berries that cannot be infected by *Puccinia* spp.

^e Presence (+) and absence (-) of aecia. Aecia were identified as mostly *Pgt* and few other formae speciales of *P. graminis*, but no *Pst* (Wang et al. 2015).

^f Telia-bearing wheat straws were standing in the field.

^g Telia-bearing wheat straws were fallen on the ground.

^h NS indicates that no telial samples on ground were available before the wheat crop was harvested.

ⁱ NT indicates that no telia were formed at the time of sampling.

10, 15, 20, and 22°C) for both 24 and 48 h incubations. For *Pst*, germination rates were significantly different among tested temperatures ($P < 0.05$), except between 10 and 15°C ($P > 0.05$) (Fig. 2A and B). The incubations at 24 and 48 h were not significantly different in germination rates at each of the same temperature settings. At 5°C, teliospores of *Pst* germinated slowly with about 14% of teliospores germinated within 24 h. Highest teliospore germination rates, 87.1 and 86.0%, were observed with incubation at 10°C for both 24 and 48 h, respectively (Fig. 2A and B). At 10°C, septa divided the promycelium into four cells and each cell had one nucleus within 24 h, each cell of a promycelium subsequently produced one sterigmata and one basidiospore, about 10 to 20% of basidiospores formed 24 to 48 h, and most basidiospores germinated at 10°C 48 to 72 h (data not shown). At 20°C, the germination rate (55.8% for 24 h and 57.3% for 48 h) was significantly reduced compared with 10 and 15°C ($P > 0.05$), and at 22°C, the germination rate (8.0% for 24 h and 12.0% for 48 h) was further reduced (Fig. 2A and B). At 22°C, promycelial growth was very slow and only short promycelia were observed within 48 h; and promycelia gradually became degraded, and no basidiospores were observed within 72 h (data not shown). Thus, temperatures between 10 and 15°C were optimal for *Pst* teliospores to germinate and produce basidiospores.

Teliospores of *Pgt* did not germinate at 5°C (Fig. 2A and B). The germination rate was 71.9% for 24 h and 74.2% for 48 h at 10°C; significantly increased to 82.4% (for 24 h) and 82.7% (for 48 h) at 15°C and 82.7% (for 24 h) and 94.4% (for 48 h) at 20°C; and slightly decreased to 73.4% (for 24 h) and 84.4% (for 48 h) at 22°C. Thus, temperatures between 15 and 20°C were found to be optimal for *Pgt* teliospore germination with production of basidiospores. At 15 and 20°C, teliospores produced promycelium containing four nuclei and about 10% of promycelia began to produce sterigmata and basidiospores within 24 h, which was faster than the basidiospore

formation of *Pst* (data not shown). Based on these results, 10°C for *Pst* and 15°C for *Pgt* and 48 h incubation were used in the subsequent germination tests of field samples to determine teliospore viability and capability of germination.

Viability of teliospores under controlled conditions. As *Pst* teliospores are prone to germinating immediately when mature while *Pgt* teliospores have a very long period of dormancy, the teliospore viability was determined by both germination and spore integrity. Although *Pst* teliospores can readily germinate when mature, no germination was observed directly on wheat plants throughout the entire study of four years. In contrast, germinated *Pgt* teliospores were observed when examining telial samples collected in May 2012, which were produced in August 2011. Thus, the germination tests were conducted using teliospore suspensions for both *Pst* and *Pgt*.

To compare *Pst* teliospore survivals under warm and dry versus cool and wet conditions, germination rates were measured for teliospores from telial samples kept under controlled conditions after collection from the field in July 2011. When kept at room temperature (22 to 23°C) and dry conditions, the germination rate of *Pst* teliospores decreased gradually from 79.3% in July to 1% the next June (Fig. 3A). When kept wet at 5°C, the germination rate dropped quickly from 90.9% 5 days after collection to 1.1% 14 days after collection, and no germinated teliospores were observed 21 days after collection (Fig. 3B). The results indicated that high moisture has a great negative effect on viability of *Pst* teliospores.

Fig. 1. Telia and teliospores before and after winter. **A**, Telial sori (T) of *Puccinia graminis* f. sp. *tritici* (*Pgt*, upper) and *P. striiformis* f. sp. *tritici* (*Pst*, lower) on wheat sheath in fall. **B**, Telial sori (arrows) of *Pgt* (upper) and *Pst* (lower) on wheat sheath in spring. *Pst* telial sori covered by leaf epidermis. **C**, Germination of teliospores (TS) of *Pst* collected in fall tested at 10°C for 48 h showing promycelia (PM). **D**, No germination of teliospores (TS) of *Pgt* collected in fall tested at 15°C for 48 h. **E**, Degraded teliospores (TS) of *Pst* observed in spring. **F**, Germination of *Pgt* teliospores (TS) collected in field in spring tested at 15°C for 48 h, showing promycelia (PM).

Fig. 2. Germination rates of teliospores of *Puccinia striiformis* f. sp. *tritici* (*Pst*) and *P. graminis* f. sp. *tritici* (*Pgt*) incubated for **A**, 24 and **B**, 48 h at different temperatures.

Viability of teliospores under field conditions. The germination data for the standing and ground samples from the field for both *Pst* and *Pgt* in the three year cycles (July 2011 to May 2012, July 2012 to May 2013, and July 2013 to May 2014) are shown in Table 1. For *Pst* standing samples, fresh teliospores collected in July had mean germination rates of 72.5, 49.8, and 64.4% for the 2011 to 2012, 2012 to 2013, and 2013 to 2014 cycles, respectively. The germination rates were the highest in August and then gradually decreased, dropping below 1.0% by March. No viable teliospores were detected in April and May in any of the three year cycles. The ground samples had a similar trend, except that no such telial samples could be found in July and August before harvest. The germination rate dropped from 52.2 to 71.2% in September of the three years more quickly ($P < 0.05$) to below 1.0% by January or February. In spring, telia became degraded. Most teliospores were fragmented into single cells, and the cells became empty or had shrunken cytoplasm (Fig. 1E).

In contrast, *Pgt* teliospores did not germinate until January or February (Table 1). Telia and teliospores were not degraded. Teliospores from the standing samples became capable of germination in January or February, and the germination rate reached the highest in May.

Fig. 3. Germinations of *Puccinia striiformis* f. sp. *tritici* teliospores from telial samples kept under controlled conditions after collection from a field near Pullman, Washington in July 2011. Germination was measured after teliospore suspension incubated in Petri dishes at 10°C for 48 h. **A**, Telial samples stored at room temperature (22 to 23°C). **B**, Telial samples stored in moist petri dishes at 5°C.

Teliospores from the ground samples had much lower germination rates than the standing samples ($P < 0.05$), but generally had a similar increasing trend. Compared with *Pst*, a relatively low percentage of *Pgt* telia and teliospores were in a degraded state after winter.

Duration of moisture for infection of barberry with *Pst* teliospores.

When inoculation with *Pst* teliospores was successful, pycnia appeared on the upper surface of barberry leaves 12 to 16 days after inoculation and aecia formed on the lower surface of leaves 16 to 20 days after inoculation. Pycnia and aecia did not form on barberry plants incubated in the dew chamber for less than 32 h, and plants incubated at the same conditions for 32 h or longer had pycnia and aecia (Fig. 4). The infection efficiency, measured as the number of pycnia per leaf, increased as the plants were incubated longer, reaching the highest level when plants were incubated for 88 h, and then gradually dropping on plants incubated for more than 88 h.

Discussion

The telial stage is an important means of perennation for numerous rust fungi. It enables survival over unfavorable conditions (Mengden 1984). Teliospores function like rest spores in other fungi, appearing when host plants start maturing and when weather becomes too hot for production and infection by urediniospores. For a functional telial stage, teliospores need to retain viability under conditions unfavorable for the asexual stage and disease development. Compared with uredinia and urediniospores, telia and teliospores of both *Pgt* and *Pst* are dark colored and teliospores are thick walled, which should protect them from damage by UV light and other weather factors. *Pgt* and *Pst* have different mechanisms and physical structures to retain viability of teliospores. Teliospores of *Pgt* are naked in a telium, and a large portion of the telium erupts through the host plant epidermis. The exposed teliospores can easily absorb water for germination. However, very long dormancy prevents *Pgt* teliospores from germinating and thus they survive the long hot summer and cold winter. *Pgt* teliospores need to go through wet-dry cycles during the cold winter before they can germinate. This mechanism enables *Pgt* teliospores to germinate at the right time to infect susceptible young barberry leaves and fruit in the spring, as demonstrated in the present study and the accompany study (Wang et al. 2015). In contrast, *Pst* teliospores do not have dormancy, or have a very short dormancy, and can produce basidiospores immediately at maturity, as reported by Wright and Lennard (1978) and demonstrated in the present study. Moreover, the plant epidermis and the fungal peridial layer covering *Pst* teliospores prevent them from germination without crushing the telia. Since the seasonality of teliospore germination does not coincide with the short window of vulnerability of alternate hosts, Rapilly (1979) questioned the ability of *Pst* basidiospores to survive and to

Fig. 4. Effect of dew chamber incubation duration on infection of barberry leaves inoculated with telia of *Puccinia striiformis* f. sp. *tritici*.

infect an alternate host in nature. The degradation of telia and teliospores after winter explains why barberry plants cannot be infected by *Pst*, at least under the natural conditions of the Palouse region of the PNW (Wang et al. 2015).

There is some potential for teliospores to infect barberry plants just when they are produced on wheat crops in July in the Palouse region (Wang et al. 2015); however, common barberry plants leaf out in mid-April to early May and the window of susceptibility for young leaves is usually passed when *Pst* produces mature telia in late June and July (Wang et al. 2015). Although the phenology of barberry reduces the probability of infection by fresh teliospores in the summer, we should not rule out the possibility. However, the condition of 32 h dew period required for *Pst* teliospores to infect barberry seldom occurs after June in the Palouse region. Actually, during late spring and early summer in both 2010 and 2011, weather was unusually wet and cool, causing the most severe epidemics of wheat stripe rust in the past decade (Chen 2014). Although a relatively long period of dew formation occurred almost every night in the early May to late June in both years, it was hard to have continuous dew formation for more than 32 h. We observed severe *Pgt* aecial infection, but no *Pst* aecia on barberry plants in these years (Wang et al. 2015). Furthermore, because our experiment was conducted using teliospore suspensions, it should take an even longer dew period for dry teliospores as inoculum to infect barberry. Therefore, *Pst* infection of barberry under the natural conditions in the Palouse region remains improbable.

Stripe rust and stem rust fungi may represent the extremes for germination types of rust fungal teliospores. Anikster (1986) studied conditions for teliospore germination using 27 rust fungi species representing five genera (*Puccinia*, *Uromyces*, *Tranzschelia*, *Frommea*, and *Melampsora*). After teliospores were floated on water for 24 to 144 h and incubated on agar at 16°C, germination was observed in most species tested, including several formae speciales of *P. graminis*, but the germination of *Pgt* teliospores failed. These results may indicate great differences existing among different species and formae speciales of rust fungi. Natural infection of barberry plants by the bluegrass stripe rust fungus, caused by *P. pseudostriiformis* (previously regarded as *P. striiformis* f. sp. *poae*) (Abbasi et al. 2004; Liu and Hambleton 2010), reported in Minnesota (Jin et al. 2010) may indicate conditions for the fungal teliospore germination different from those required for *Pst*. In the present study, we only researched teliospore survival in the PNW, whose winters typically have a rain and snow mixture, which is favorable for *Pgt* teliospore survival, but detrimental to *Pst* teliospores. Teliospores of the stripe rust fungi including *P. pseudostriiformis* may have better survival under dry conditions. Compared with *Pst*, uredinial sporulation of *P. pseudostriiformis* occurred much later, typically in September and October in the PNW. When telia are produced in fall, they can be highly viable in early winter. If telia are covered by snow through much of the winter, they might have good viability in the spring to germinate and infect barberry plants.

Together with the long moisture requirement for teliospore germination and basidiospore infection of barberry, the information of *Pst* teliospores preferring dry conditions for survival can be used to identify regions in the world where *Pst* could potentially infect barberry. In such regions, wheat crops should mature late in the fall and the weather should be relatively dry in late fall and throughout the winter, or covered by snow. In the spring, when barberry plants leaf out and/or during the summer when some species of barberry continually produce new leaves, continuous rain of a couple of days or more can keep leaves wet long enough for *Pst* teliospores to complete the process of germination and basidiospore infection. Such cropping and weather conditions are possible in regions like southern Gansu, China, where natural *Pst* infection of barberry was reported (2013). In that region, spring wheat is grown in high elevation areas and not harvested until August and September, and abundant telia can form as late as those months. In that region, abundant barberry plants grow wild near wheat fields and villages, as reported by Zhao et al. (2013) and observed by the second author of this paper. The winter and early spring in the region are generally dry and cold, and rainy periods are quite common in the spring. Growers keep wheat straw

in high piles near wheat fields and sometimes close to barberry plants, which creates conditions favorable for teliospore survival. Teliospores on such straws should last longer than those on straws in the fields. The region is one of the major stripe rust “hotspots” in China (Zhao et al. 2013). Although *Pst* can survive from year to year by the uredinial stage, occasional infection of barberry may generate new races. Regions with similar cropping and weather patterns may exist in other countries. Nevertheless, worldwide, alternate hosts should not be important for stripe rust.

Although barberry is not important for stripe rust, it is very important for stem rust in the PNW. Therefore, destroying common barberry plants near fields will help reduce stem rust. The barberry plants most close to fields as mentioned in the accompany study (Wang et al. 2015) were destroyed. For the management of stripe rust, the effort should be put into developing and growing cultivars with durable and high levels of resistance, and using fungicides when necessary (Chen 2014). Although it is not important for disease management, the discovery of alternate hosts for the stripe rust pathogen has provided a genetic system to study important traits of the pathogen and understand the host-pathogen interactions (Chen et al. 2012; Rodriguez-Algaba et al. 2014).

Acknowledgments

This research was supported by the U. S. Department of Agriculture, Agricultural Research Service (Project No. 5348-22000-015-00D), Washington Grain Commission (Project No. 13C-3061-5665), and Idaho Wheat Commission (Project No. 13C-3061-4232). PPNS No. 0676, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences, Agricultural Research Center, HATCH Project Number WNP00663, Washington State University, Pullman, WA 99164-6430. We thank Dr. Yue Jin for technical advice for teliospore germination. We are grateful to Drs. Frank Dugan and Weidong Chen for critical review of the manuscript.

Literature Cited

- Abbasi, M., Hedjaroude, Gh. A., Scholler, M., and Goodwin, S. B. 2004. Taxonomy of *Puccinia striiformis* s. l. in Iran. *Rostaniha* 5:71-82.
- Ali, S., Gladioux, P., Leconte, M., Gautier, A., Justesen, A. F., Hovmöller, M. S., Enjalbert, J., and de Vallavieille-Pope, C. 2014. Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici*. *PLoS Pathog* 10:1-12.
- Anikster, Y. 1986. Teliospore germination in some rust fungi. *Phytopathology* 76: 1026-1030.
- Chabelska, H. 1938. Life-cycle of the rust on *Atzelltsa strigosa* Labill. *Palest. J. Bot. Jerus. Ser.* 1:101-103.
- Chen, X. M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Can. J. Plant Pathol.* 27:314-337.
- Chen, X. M. 2014. Integration of cultivar resistance and fungicide application for control of wheat stripe rust. *Can. J. Plant Pathol.* 36:311-326.
- Chen, X. M., Wang, M. N., Wan, A. M., Cheng, P., and Cheng, J. J. 2012. Sexual or asexual reproduction, which one is more important for stripe rust? Pages 36-37 in: *Disease Risk and Food Security*, W.-Q. Chen et al., eds. *Proc. 13th Int. Cereal Rusts and Powdery Mildews Conf.*, Beijing, China, China Agricultural Science and Technology Press.
- Cheng, P., and Chen, X. M. 2014. Virulence and molecular analysis support asexual reproduction of *Puccinia striiformis* f. sp. *tritici* in the U. S. Pacific Northwest. *Phytopathology* 104:1208-1220.
- Eriksson, J., and Henning, E. 1894. Die Hauptresultate einer neuen Untersuchung über die Getreidcroste. *Z. Pflanzenkr.* 4:66-73, 140-142, 197-203, 257-262.
- Jin, Y. 2011. Role of *Berberis* spp. as alternate hosts in generating new races of *Puccinia graminis* and *P. striiformis*. *Euphytica* 179:105-108.
- Jin, Y., Szabo, L. J., and Carson, M. 2010. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host. *Phytopathology* 100:432-435.
- Kolmer, J., Chen, X. M., and Jin, Y. 2009. Diseases which challenge global wheat production – the wheat rusts. Pages 89-124 in: *Wheat: Science and Trade*. B. F. Carver, ed. Wiley-Blackwell, Ames, IA.
- Leonard, K. J., and Szabo, L. J. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. *Mol. Plant Pathol.* 6:99-111.
- Liu, M., and Hambleton, S. 2010. Taxonomic study of stripe rust, *Puccinia striiformis* sensu lato, based on molecular and morphological evidence. *Fungal Biol.* 114:881-899.
- Mengden, K. 1984. Development and physiology of teliospores. Pages 375-398 in: *The Cereal Rusts Vol. I: Origins, Specificity, Structure, and Physiology*. W. R. Bushnell and A. P. Roelfs, eds. Academic Press, New York.
- Peterson, P. D. 2003. The common barberry: The past and present situation in Minnesota and the risk of stem rust epidemics. Ph. D. Dissertation, North Carolina State University, Raleigh.
- Rapilly, F. 1979. Yellow rust epidemiology. *Annu. Rev. Phytopathol.* 17:59-73.

- Rodriguez-Algaba, J., Walter, S., Sørensen, C. K., Hovmøller, M. S., and Justesen, A. F. 2014. Sexual structures and recombination of the wheat rust fungus *Puccinia striiformis* on *Berberis vulgaris*. *Fungal Genet. Biol.* 70:77-85.
- Roelfs, A. P. 1982. Effects of barberry eradication on stem rust in the United States. *Plant Dis.* 66:177-181.
- Roelfs, A. P. 1985a. Wheat and rye stem rust. Page 3-37 in: *The Cereal Rusts Vol. II: Diseases, Distribution, Epidemiology and Control*. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, Inc. New York.
- Roelfs, A. P. 1985b. Epidemiology in North America. Pages 403-434 in: *The Cereal Rusts Vol. II: Diseases, Distribution, Epidemiology and Control*. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, Inc. New York.
- Sharma-Poudyal, D., and Chen, X. M. 2011. Models for predicting potential yield loss of wheat caused by stripe rust in the US Pacific Northwest. *Phytopathology* 101:544-554.
- Wan, A. M., and Chen, X. M. 2012. Virulence, frequency, and distribution of races of *Puccinia striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordeii* identified in the United States in 2008 and 2009. *Plant Dis.* 96:67-74.
- Wan, A. M., and Chen, X. M. 2014. Virulence characterization of *Puccinia striiformis* f. sp. *tritici* using a new set of Yr single-gene line differentials in the United States in 2010. *Plant Dis.* 98:1534-1542.
- Wang, M. N., and Chen, X. M. 2013. First Report of Oregon grape (*Mahonia aquifolium*) as an alternate host for the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) under artificial inoculation. *Plant Dis.* 97:839.
- Wang, M. N., Wan, A. M., and Chen, X. M. 2015. Barberry as an alternate host is important for *Puccinia graminis* but not for *Puccinia striiformis* in the U. S. Pacific Northwest. *Plant Dis.* 99:1507-1516.
- Wang, M. N., Wan, A. M., Chen, X. M., and Evans, C. K. 2011. Barberry is more important as an alternate host for stem rust than for stripe rust in the U. S. Pacific Northwest. Page 166 in: *Oral Presentations, Poster Abstracts, Participants and Program of BGRI Technical Workshop*, St. Paul, MN.
- Wellings, C. R. 2011. Global status of stripe rust: a review of historical and current threats. *Euphytica* 179:129-141.
- Wright, R. G., and Lennard, J. H. 1978. Mitosis in *Puccinia striiformis*. *Light microscopy*. *Trans. Br. Mycol. Soc.* 70:91-98.
- Zhao, J., Wang, L., Wang, Z. Y., Chen, X. M., Zhang, H. C., Yao, J. N., Zhan, G. M., Chen, W., Huang, L. L., and Kang, Z. S. 2013. Identification of eighteen *Berberis* species as alternate hosts of *Puccinia striiformis* f. sp. *tritici* and virulence variation in the pathogen isolates from natural infection of barberry plants in China. *Phytopathology* 103:927-934.