Field Production, Germinability, and Survival of *Puccinia striiformis* f. sp. *tritici* Teliospores in China

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Abstract

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, has been recently demonstrated to be heteroecious and macrocyclic. The pathogen depends on wheat as the primary (telial) host and barberry as the main alternative (aecial) host to complete the complete life cycle. Viable teliospores are essential for the initiation of sexual reproduction of *P. striiformis* f. sp. *tritici*. However, no exact source of viable teliospores has been discovered to produce basidiospores to initiate infection on susceptible barberry. In the present study, we investigated the telial production and teliospore germinability of *P. striiformis* f. sp. *tritici* in wheat fields in Gansu and Qinghai provinces and wheat straw stacks in Qinghai in 2018 and 2019. Field production of *P. striiformis* f. sp. *tritici* teliospores was observed commonly at all growth stages of winter and spring wheat plants. The percentage of leaves bearing viable teliospores and germination rate of *P. striiformis* f. sp. *tritici* teliospores were 78.5 and 5.1% at tillering stage, 83.2 and 9.4% at early jointing stage, and 91.8 and 4.9% after booting stage in 2018 and 2019, respectively. Among wheat straw samples bearing telia collected from Qinghai province, samples with germinable teliospores ranged from 23.9% in January to 4.4% in June in 2018 and from 10.3% in January to 6.0% in May in 2019, with an overall mean germinability of 5.9%. This study showed that teliospores are produced at all growth stages of wheat under field conditions, and teliospores harbored in wheat straw stacks after wheat harvest are able to survive through winter for potential infection of barberry to the next spring.

Keywords: *Puccinia striiformis*, stripe rust, telial production, teliospore germinability, wheat

Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss., is a destructive foliar disease of wheat, and epidemics can result in yield losses of wheat worldwide (Chen 2005, 2020; Li and Zeng 2002; Wellings 2011). The rust pathogen is heteroecious and macrocyclic, completing the whole life cycle via the development of five various spore stages on two unrelated hosts (Jin et al. 2010; Zhao et al. 2013). Urediniospores and teliospores are produced on primary hosts (cereals and grasses) and pycniospores and aeciospores on alternative hosts (*Berberis* spp. and *Mahonia* spp.) after being infected by basidiospores produced from teliospores.

Teliospores are vital to initiate the sexual cycle of the rust fungus. Teliospores germinate to produce basidiospores under favorable conditions (Hiratsuka 1973; Jin et al. 2010; Wang and Chen 2015), and basidiospores infect young tissues (leaves, leaf stalks, and berries) of barberry to commence sexual reproduction (Jin et al. 2010; Li et al. 2016). Therefore, viable teliospores are essential for initiating sexual reproduction of *P. striiformis* f. sp. *tritici* when nearby barberry plants produce young leaves or young tissues (shoot and berries) in spring.

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The natural infection of *P. striiformis* f. sp. *tritici* on susceptible barberry bushes has been identified in China (Li et al. 2016; Wang et al. 2016; Zhao et al. 2013). However, natural infection of *P. striiformis* f. sp. *tritici* on barberry has not been reported in Europe or in the United States, or in other regions of the world (Berlin et al. 2012; Wang et al. 2015). Previous research showed that the teliospores are unable to infect barberry because they are degraded under the fluctuating moisture and temperature conditions in the U.S. Pacific Northwest (Wang and Chen 2015). Teliospores lost viability within 2 weeks under moist conditions even at a low temperature. However, the teliospores were able to germinate after being stored for 10 months under dry conditions at room temperature (Wang and Chen 2015; Wang et al. 2015). Thus, climatic conditions are considered one of critical factors influencing whether *P. striiformis* f. sp. *tritici* teliospores germinate to produce basidiospores for infecting alternative hosts.

The stripe rust pathogen produces teliospores on infected wheat tissues mostly at the end of growth stage under field conditions (Kurt 1984; Wang and Chen 2015). Likewise, teliospores can be produced on the seedling and adult plant stages of wheat under controlled conditions (Ali et al. 2010; Tian et al. 2016; Wan et al. 2019; Yuan et al. 2018). Eriksson and Henning (1896) reported that the formation of teliospores and basidiospores of the stripe rust fungus was easily observed every year on wheat, and teliospores could germinate to generate basidiospores in both autumn and spring. A previous study by Raeder and Bever (1931) reported that mature teliospores of the stripe rust pathogen could germinate immediately, but teliospores of other rust fungi would not germinate until the next spring. Wang and Chen (2015) reported that, under natural conditions in spring in the U.S. Pacific Northwest, *P. graminis* f. sp. *tritici* (the wheat and barley stem rust pathogen) could infect barberry, which is essential for wheat stem rust epidemics in the region, but *P. striiformis* f. sp. *tritici* did not. Additionally, production of telia and teliospores varies at different weather conditions, plant growth stages, and *P. striiformis* f. sp. *tritici* isolates (Wan et al. 2019).

Winter wheat is grown in different regions of China, but harvesting time is quite different. With spring wheat grown in some regions, wheat growth extends almost all year long. The different crop systems result in the overlap of the different stages of wheat crops. Based on the cultivation systems of winter and spring wheat, characteristics of *P. striiformis* f. sp. *tritici* oversummering and overwintering, and frequencies of stripe rust epidemics, the wheat production regions in China are divided into 15 stripe rust epidemic zones (Zeng and Luo 2006). Thus, the formation of *P. striiformis* f. sp. *tritici* teliospores even at the late growth stage of wheat could last for several months. Before the present study it was unknown whether *P. striiformis* f. sp. *tritici* could produce teliospores at any early growth stage of wheat under field conditions. Furthermore, it was not clear where and to what extent teliospores could be viable under different regional wheat growing conditions. Especially in the wheat production areas of western China, wheat straws are commonly piled into cone-shaped stacks for various purposes, and the stacks are often in vicinity of barberry bushes and kept until the next summer. Because wheat straw stacks provide dry conditions, teliospores on straws in stacks may have advantage for survival and therefore can be an important source of viable teliospores for infection of nearby barberry plants. However, no research had been done to test the hypothesis before the present study. Therefore, the present study investigated production and germinability of *P. striiformis* f. sp. *tritici* teliospores during the wheat growing season in different regions and survival of *P. striiformis* f. sp. *tritici* teliospores in wheat straw stacks in China.

Materials and Methods

Field surveys of *P. striiformis* f. sp. *tritici* telial production. The Qinling Mountains, mainly in Shaanxi Province, are a major east–west mountain range that separates China into the climatically different northern and southern parts. Wheat growth stages vary dramatically in the northern and southern regions, but stripe rust affects both regions (Zeng and Luo 2006). Winter wheat is planted in late autumn and harvested in spring in the southern region and planted in mid- to late autumn and harvested in summer to autumn in the northern regions. Wheat continually grows in winter in the southern region because the temperatures are mostly >0°C in winter, whereas wheat plants are mostly dormant in the northern region in winter because temperatures are often below freezing (China Meteorological Administration, http://www.cma.gov.cn/en2014/). In the present study, winter wheat fields in Sichuan, Yunnan, Guizhou, and southern Shaanxi provinces in the southern winter wheat region were randomly surveyed for *P. striiformis* f. sp. *tritici* telial production because *P. striiformis* f. sp. *tritici*-infected wheat plants often produce urediniospores in these provinces in winter. Similar field surveys were also conducted after winter in the *P. striiformis* f. sp. *tritici* overwintering areas of Gansu (mainly winter wheat), Qinghai (both winter and spring wheat), and central Shaanxi province (winter wheat) in the northern regions, where wheat leaves are often dead in winter because of below-freezing temperatures, but *P. striiformis* f. sp. *tritici* may survive in living leaves and leaf sheaths of wheat through winter (Ma et al. 2017; Shi et al. 2005; Yao et al. 2014). In both southern and northern regions, field surveys were conducted in locations where barberry plants were found in previous studies (Du et al. 2019; Zhao et al. 2013 , 2016). Approximately 30 wheat fields around a single village were checked for telial formation before the early jointing stage from January to June in the 2018 and 2019 crop seasons, depending on the location. Up to three stripe rust foci were visually checked, and the numbers of wheat leaves bearing telia and those with only uredinia were counted in a 0.25-m^2 area in each focus. The number of wheat leaves bearing telia was divided by the number of leaves bearing uredinia or telia to calculate the percentage of telial formation. When wheat crop reached booting stages, counting telial leaves was stopped as the percentage reached 100%. Samples of wheat leaves bearing telia were collected from the fields once a month from January to July in both the 2018 and 2019 crop seasons to check teliospore germination.

Wheat straw stacks in Huzhu and Datong counties of Qinghai and Qinzhou and Gangu counties of Gansu were checked for the presence of telia and teliospore germinability. In these regions, growers commonly pile up wheat straw in stacks for various uses, and stacks may last up to the next summer. Five villages in each county were surveyed for telia in straw stacks. Straw samples with telia were collected once in a month from January to June for checking teliospore germinability. The samples were kept in a zippered plastic bag containing allochroic silica gel (Qingdao Haiyang Chemical Co., Ltd, Shandong, China) for drying and kept at 4°C for later use.

Tests for teliospore germination. Teliospores were tested for germination via the method as described by Wang and Chen (2015) with slight modification. Leaves or leave sheaths bearing telia were washed under tap water and then cut into approximately 5-cm-long fragments. These fragments were presoaked in deionized water in a Petri dish at 4°C for 24 h. Approximately 30 telia from each fragment were randomly picked and placed on the flat surface of a clean concave glass slide. Leaf epidermis over telia was removed with a scalpel to make a teliospore suspension in 100 µl of deionized water. The teliospore suspension was transferred onto the concave surface of a glass slide. The slide was placed in a Petri dish and supported by two parallel glass rods on water-saturated filter paper. The dish was covered and kept in an incubator for 24 to 72 h at 10°C for teliospores to germinate. After incubation, germination of teliospores was observed with a light microscope (Olympus BX53, Tokyo, Japan). In the method described by Blackman (1903), a teliospore germinated to produce promycelium with four cells was considered viable. Three fragments from different leaves or leaf sheaths were used in the germination tests, and a total of 300 teliospores were used to calculate the germination rate for each telial sample. To determine the percentage of leaves bearing viable teliospores in each stack, ≦10 leaves were tested for teliospore germination.

Statistical analysis. The percentages of telial formation and teliospore germination were calculated in Excel in Microsoft Office 2010 (Microsoft, Redmond, WA). Analysis of variance (ANOVA) was performed in the Mixed Model of SPSS version 22.0 (SPSS Inc., Chicago, IL) to determine the differences in telial production and teliospore germination between regions, wheat growth stages, and months and between the 2018 and 2019 crop seasons.

Results

Telia formation. Telial pustules of *P. striiformis* f. sp. *tritici* were observed at various locations throughout the surveyed regions (Fig. 1A to C; Supplementary Table S1). In the northern winter wheat region, telia were initially found on dead or lower yellowish-green leaves of wheat in early March 2018 and early April 2019 in Tianshui, Gansu. Likewise, early formation of telia was observed in early April in middle-western Shaanxi and early June in Qinghai in both the 2018 and 2019 crop seasons. In the southern winter wheat region, *P. striiformis* f. sp. *tritici* telia were observed on the low part of plants as early as late December in Yunnan, early January in Guizhou, mid-January in Sichuan, and early March in southern Shaanxi. These observations indicated that initial formation of *P. striiformis* f. sp. *tritici* telia in the southern winter wheat region was earlier than in the northern winter wheat region as long as wheat plants were infected.

Production of telia on wheat plants varied between crop seasons. Telia were observed in 14 (53.8%) of 26 wheat fields from eight counties of five provinces in 2018 but only 10 (27.8%) of 36 fields from 10 counties of four provinces in 2019 (Supplementary Table S1). On average, telia were found in 24 of 62 wheat fields in both years, or 38.71%. Telia were observed at various wheat growth stages from tillering and early jointing stage before winter and in early spring in all winter wheat growing regions, except for Qinghai, in which these growth stages of wheat occur in late May to early June. The percentage of telial formation ranged from 0 to 88.9% at different sampling sites and averaged 7.3% at the tillering stage and 9.3% at the early jointing stage (Table 1). The average percentage of telial formation at all surveyed sites was 8.4%. Telial formation rates did not differ significantly between areas ($P = 0.5302$), growth stages ($P = 0.2304$), months ($P = 0.1257$), and years ($P = 0.2031$) (Table 2). However, differences in percentage of telia were observed in wheat straw stacks between the two years (Fig. 2A and B; Table 3). Of 56 wheat straw stacks surveyed in each year in Qinghai and

Gangu provinces in the *P. striiformis* f. sp. *tritici* oversummering regions, 21 (37.5%) in 2018 and 12 (21.4%) in 2019 had *P. striiformis* f. sp. *tritici* telia (Table 3).

Germination of teliospores from field samples. Under a microscope, teliospores were observed in 24 of 27 samples that were collected from tillering to early jointing stage, and 39 of 40 samples from early booting to soft-dough stage were capable of germination (Fig. 1D; Supplementary Table S2). However, the germination rates were different between 2018 and 2019 ($P = 0.02$). Over the two years, 87.4% of the samples had viable teliospores, including 78.5% at tillering stage, 83.2% at early jointing stage, and 91.8% from booting to soft-dough stage (Table 1). No significant differences were found between growth stages, areas, and months. The interactions between year and month ($P = 0.7758$), year and growth stage ($P = 0.9103$), month and growth stage ($P = 0.0694$), and year, month, and growth stage ($P = 0.0988$) showed no significant differences (Table 2). However, the germination rate of teliospores varied significantly between months ($P < 0.01$) (Table 1). The highest teliospore germination rate was 28.7%, and the mean value was 5.9% across all sites in both years. The mean teliospore germination rate was 5.1% at tillering stage, 9.4% at early jointing stage before early spring, and 4.9% from booting stage to soft-dough stage across the two growth seasons. The mixed model analysis indicated that month was most significantly associated with teliospore germination ($P = 0.0022$), followed by growth stage ($P = 0.0540$), whereas regions ($P = 0.8806$) and years ($P = 0.9317$) were not significant factors for variation in teliospore germination (Table 2). The interaction between year and month ($P = 0.8170$) and between growth stage and year ($P = 0.3719$) displayed nonsignificant differences in teliospore germination, whereas those between growth stage and month were significant ($P = 0.0111$). The interaction between area, growth stage, and month showed no significant difference ($P = 0.1087$). Similarly, a significant difference was detected in the interaction between growth stage and month. Teliospores sampled from early jointing stage of wheat in February and after booting stage of wheat in July had higher teliospore germination than those from other growth stages (Fig. 3). In general, teliospores from most telial samples were capable of germinating, although the overall germination rate was low.

Germination of teliospores from straw stack samples. Germinability of teliospores from wheat straw stacks in Qinghai and Gansu provinces was checked monthly in 2018 and 2019 (Fig. 2C and D). The minimum, maximum, and mean germination rates of teliospores for each location and sampling date are provided in Supplementary Table S3. In Qinghai, 15 of the 32 stacks sampled in 2018 had telia, and teliospores from three samples had germinated teliospores, and in 2019, seven of the 43 stacks had telia and five had viable teliospores (Table 3). In Gansu, six of 24 stacks had telia, only one had viable teliospores in 2018, and none of the stacks had viable teliospores, although five of the 13 checked stacks had telia in 2019. Teliospores in samples collected up to June in 2018 and May in 2019 in Qinghai were able to germinate, whereas those sampled after March in Gansu were not. The percentage of leaves bearing viable teliospore in wheat straw stacks ranged from 83.3% in January to 45.0% in June 2018 and from 64.4% in January to 3.3% in May 2019 (Table 4), showing a gradual decrease during this period or later. The highest mean germination rate of teliospores appeared in January in both years and since then has gradually decreased. The germination rate of teliospores ranged from 8.8 to 38.0% with a mean of 23.9% in January 2018 and from 5.0 to 18.0% with a mean of 10.3% in January 2019. The germination rate ranged from 2.9 to 6.6% with a mean of 4.4% in June 2018 and from 0 to 28.7% with a mean of 6.0% in May 2019 (Table 4). The mixed model analysis showed a significant difference in teliospore germination rate between the two years ($P = 0.0423$), but not between months from January to June ($P = 0.0503$). No significant difference was found in germination in the interaction between month and year ($P = 0.6485$) (Table 2). The results indicated that wheat straw stacks harbor teliospores for survival until May or June, which matches the susceptibility of young barberry leaves.

Discussion

In the present study, telia were observed early at the seedling stage of wheat plants in fields, indicating that telial formation is common in fields at all growth stages of wheat in China. Teliospore formation is affected by many factors, mainly race type, temperature, and wheat variety (Ali et al. 2010; Pillai et al. 1978; Takahashi et al. 1965; Zimmer and Schafer 1960). Capacity for teliospore formation varies with race types in some rust fungi. A report by Pillai et al. (1978) showed that races of *Puccinia graminis* f. sp. *tritici*, the causal agent of wheat stem rust, varied in their tendency to form teliospores, whereas race 122 took more time for teliospore formation than races 14, 21, 34, and 117A. Likewise, various race types of *P. coronata* var. *avenae*, the cause of crown rust of oat, also showed remarkable differences in capacity to produce telia and teliospores under experimental conditions. Speed of telial formation of *P. coronata* var. *avenae* on oats was not correlated with range of virulence and specific virulence of the pathogen, resistance, and maturity of the host but appeared to be a function of a specific relationship between the particular host and parasite (Zimmer and Schafer 1960). Similarly, the difficulty and degree of telial formation of *P. recondite* f. sp. *tritici*, the causal pathogen of wheat leaf rust, on young wheat seedlings was not related to race; however, it varied between combinations of fungus race and wheat variety (Takahashi et al. 1965). For *P. striiformis* f. sp. *tritici*, the capacity for telial production of Asian *P. striiformis* f. sp. *tritici* isolates was higher than that of European or Mediterranean isolates (Ali et al. 2010). Teliospore formation of *P. striiformis* f. sp. *tritici* varies with changes in temperature. High temperatures ($\geq 20°C$) are beneficial for *P. striiformis* f. sp. *tritici* teliospore formation, because a shorter time is necessary at high temperatures than at low temperatures. The temperature range for *P. striiformis* f. sp. *tritici* teliospore formation is lower than that for *P. graminis* f. sp. *tritici* teliospore formation (Pillai et al. 1978). A study by Zimmer and Schafer (1960) reported that temperature directly altered the rate of telial formation of *P. coronata* var. *avenae*. Teliospore formation of *P. graminis* f. sp. *tritici* was completed in 3 to 5 days at 15°C, more rapidly than at 30°C, and the number of telia containing teliospores at 30°C was three to five times greater than at 15°C (Pillai et al. 1978). Although we did not relate telial production to temperatures in the present study, the higher telial formation in the late growth stages than in the early stages across the surveyed regions was apparently related to high temperatures.
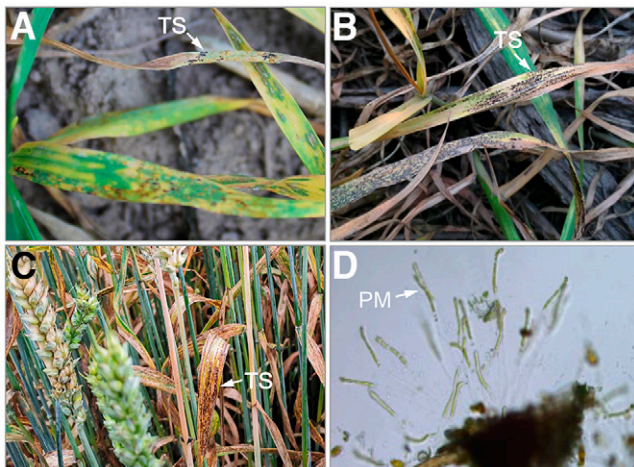

Fig. 1. Formation and germination of *Puccinia striiformis* f. sp. *tritici* telia on wheat foliage in fields. **A,** Telial sori (TS) of *P. striiformis* f. sp. *tritici* formed on leaves of wheat at tillering stage in Tianshui, Gansu of *P. striiformis* f. sp. *tritici* overwintering area in April 2019. **B,** TS of *P. striiformis* f. sp. *tritici* produced on wheat leaves at early jointing stage in Jiange, Sichuan of *P. striiformis* f. sp. *tritici* oversummering area in March 2018. **C,** TS of *P. striiformis* f. sp. *tritici* formed on leaves of wheat at soft-dough stage in Guide, Qinghai in July 2019. **D,** Germination of *P. striiformis* f. sp. *tritici* teliospores produced on wheat foliage at early jointing stage in winter in Sichuan in February 2018, tested at 10°C for 72 h, showing production of promycelia (PM).

Teliospore formation is also related to resistance and susceptibility of wheat varieties. Takahashi et al. (1965) reported that the telia of *P. recondite* f. sp. *tritici* were difficult to form on highly resistant wheat varieties but formed easily on moderately resistant varieties, especially for varieties with an X-type reaction (mixed reaction) rather than highly susceptible varieties. Likewise, teliospore formation of *P. striiformis* f. sp. *tritici* is also related to growth stage of wheat and susceptibility or resistance of wheat varieties. Under the same environmental conditions, *P. striiformis* f. sp. *tritici* forms teliospores more readily on the late growth stages of wheat than in the early stages. Teliospore production on wheat seedlings can be induced by high temperatures. The factors affecting telial formation of *P. striiformis* f. sp. *tritici* should be systemically studied in the future. In previous studies, we surveyed the distribution and growth of barberry plants (Wang et al. 2016; Zhao et al. 2013), and in the present study we focused on teliospores on wheat plants. Future studies should consider both primary and alternative hosts simultaneously.

Wheat straw stacks provide a unique environment for prolonging teliospore viability. In rural areas of China, especially the northwestern region, wheat growers commonly stack wheat straws for feeding livestock, burning for cooking or keeping warm, and many other purposes. Straw stocks often last 1 year from harvest to harvest. Under such conditions, teliospores embedded underneath wheat straw can remain viable much longer than those exposed in fields. Because straw stacks provide dry and constant temperature conditions, teliospores can survive longer. Based on our field investigations and results of the present study, teliospores inside wheat straw stacks are still viable at the time when barberry bushes near stacks and around wheat fields are growing new shoots and leaves. Thus, viable teliospores can potentially infect barberry plants, and when infected barberry plants can release aeciospores to infect wheat crops.

In the present study, we demonstrated that field production of teliospores can occur in all growth stages of wheat from January to July in different wheat growing regions of China. Almost all telial samples containing teliospores were capable of germination and germinated immediately after collection. Although in the present study teliospore germination showed remarkable differences between months, germination rates were lower than those reported in the U.S. Pacific Northwest (Wang and Chen 2015). However, germination rates gradually decreased from January to May or June, similar to those of telial samples stored at room temperatures and under dry conditions but different from those under field conditions in the

Table 1. Percentage of telial formation of *Puccinia striiformis* f. sp. *tritici*, wheat leaves bearing viable teliospores, and germination rate of teliospores from wheat plants at different growth stages and months in fields in different wheat growing regions in the 2018 and 2019 cropping seasons

Treatment	No. of sites	Percentage of telial formation (%)			No. of sites	Percentage of leaves bearing viable teliospores (%)			Germination rate of teliospores (%)		
		Minimum	Maximum	Mean ± SE[a]		Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
Growth stage	62	0	88.9	8.4 ± 2.1	67	0	100.0	87.4 ± 3.3	0	28.7	5.9 ± 0.8
Tillering stage	29	0	88.9	7.3 ± 3.4	13	0	100.0	78.5 ± 9.8	0	14.2	5.1 ± 1.3
Early jointing stage	33	0	50.0	9.3 ± 2.7	14	0	100.0	83.2 ± 9.6	0	28.6	9.4 ± 2.4
From booting to soft-dough stage	0	–[b]	–	–	40	0	100.0	91.8 ± 3.1	0.6	10.5	4.9 ± 0.8
P[c]				0.2304				0.8712			0.054
Area	62	0	88.9	8.4 ± 2.1	67	0	100.0	87.4 ± 3.3	0	28.7	5.9 ± 0.8
P. striiformis f. sp. *tritici* winter reproducing area (southwestern area)[d]	31	0	63.8	9.0 ± 2.8	21	0	100.0	85.1 ± 6.3	0	28.6	6.2 ± 1.4
P. striiformis f. sp. *tritici* overwintering area (northwestern area)[e]	31	0	88.9	7.8 ± 3.3	46	0	100.0	88.5 ± 3.9	0.4	28.7	5.8 ± 0.9
P				0.5302				0.9308			0.8806
Year	62	0	88.9	8.4 ± 2.1	67	0	100.0	87.4 ± 3.3	0	28.7	5.9 ± 0.8
2018	26	0	50.0	11.1 ± 2.7	38	0	100.0	89.9 ± 4.0	0	28.6	5.5 ± 0.9
2019	36	0	88.9	6.4 ± 3.1	29	0	100.0	84.2 ± 5.6	0	28.7	6.5 ± 1.4
P				0.2031				0.0183*			0.9317
Month	62	0	88.9	8.4 ± 2.1	67	0	100.0	87.4 ± 3.3	0	28.7	5.9 ± 0.8
Jan	15	0	12.5	2.8 ± 1.1	8	50.0	100.0	93.8 ± 6.3	1.3	11.9	5.8 ± 1.4
Feb	10	0	63.8	11.4 ± 6.6	10	50.0	100.0	88.1 ± 5.7	1.9	28.6	11.1 ± 3.0
Mar	23	0	50.0	7.0 ± 2.6	32	0	100.0	86.7 ± 5.3	0	18.2	4.0 ± 0.7
Apr	8	0	88.9	22.4 ± 11.1	4	0	100.0	46.9 ± 24.1	0	14.2	5.4 ± 3.4
May	0	–	–	–	8	88.9	100.0	95.8 ± 2.0	0.4	8.8	4.2 ± 1.0
Jun	6	0	23.5	3.9 ± 3.9	2	100	100.0	100 ± 0	1.6	9.6	5.6 ± 4.0
July	0	–	–	–	3	100	100.0	100 ± 0	7.2	28.7	14.5 ± 7.1
P[c]				0.1257				0.9961			0.0022**

[a] SE = standard error.
[b] Dashes indicate no data.
[c] P = probability value. Single asterisks and double asterisks indicate significant differences at 0.5 > P > 0.01 and 0.05 > P > 0.0001, respectively.
[d] In the *P. striiformis* f. sp. *tritici* winter reproduction area, weather is mild in winter, and aerial parts of wheat plants are still living and growing slowly. Simultaneously, *P. striiformis* f. sp. *tritici* can develop continuously to produce urediniospores after infection on autumn-sown wheat in autumn or reinfect healthy wheat plants.
[e] In the *P. striiformis* f. sp. *tritici* overwintering area, green tissues of wheat plants are dead in winter, and *P. striiformis* f. sp. *tritici* survives as mycelia inside leaf sheaths under the ground.

United States (Wang and Chen 2015). The differences could result from the different climatic conditions and whether the samples were immediately tested for germination.

Although teliospores inside wheat straw stacks until May 2019 were still able to germinate, the percentage of leaves bearing viable teliospores and the germination rates of teliospores were not high. This finding could be attributed to the fact that the telial samples were collected from the older wheat straw stacks because farmers often piled straw stacks on top of the old ones. Thus, the difference in teliospore germination could be attributed to other factors including teliospore aging, genetic variability of *P. striiformis* f. sp. *tritici*, and the possibility of fungicide application, which may also influence teliospore survival. Studies by Allen (1965) and Sussman and Halvorson (1967) demonstrated that the dormancy of teliospores is determined by the nature of the spore, not by the presence of inhibitory environmental factors. Under unfavorable environmental conditions, dormancy may be not favorable for *P. striiformis* f. sp. *tritici* (Wang and Chen 2015; Wright and Lennard 1978). We observed that most of the tested teliospores could immediately germinate once they matured. We also found that teliospores in wheat leaves dried and stored at low temperatures (4 to 5°C) could maintain germinability for >1.5 years (J. Zhao et al., *unpublished data*). Factors including teliospore viability, the tenderness of young tissue (mainly leaves) of barberry bushes, and favorable weather conditions mostly affect sexual reproduction of *P. striiformis* f. sp. *tritici* (Li et al. 2016; Wang and Chen 2015).

In China, wheat is grown in a wide range of cropping times, and thus growth stages overlap in different wheat production regions and even in some smaller areas of same regions. In the present study, teliospores produced in January and February were capable of germination to form basidiospores. Basidiospores could potentially infect leafing-out barberry plants in late March or early April in the northern regions or earlier in the southern regions. The results support the existence of *P. striiformis* f. sp. *tritici* sexual reproduction in spring under natural conditions (Zhao et al. 2013). From the Qinghai samples, we found high germination rates of teliospores formed in July. During this month, barberry plants are still producing new shoots and leaves. Thus, it will be useful to conduct rust surveys on barberry in this region to determine whether *P. striiformis* f. sp. *tritici* infects barberry plants around July.

Teliospores can survive throughout winter in some regions of China. Winter and spring wheat crops are usually harvested in autumn in high-elevation areas such as the Qinghai and Gansu regions surveyed in the present study. These regions typically have cool and dry autumn, winter, and early spring, which are favorable for

teliospore survival, especially in straw stacks. In these regions, barberry bushes start to produce new leaves and shoots from late April, and aecia appear in late May or early June (Wang et al. 2016; Zhao et al. 2013). Under favorable conditions, susceptible barberry can be infected by rust fungi from spring to early summer, producing aeciospores to infect wheat crops. Most recently, we obtained *P. striiformis* f. sp. *tritici* isolates from rusted barberry leaves close to wheat straw stacks in which we observed telia in Qinghai (W. Chen et al., *unpublished data*) and continue to study the connections between *P. striiformis* f. sp. *tritici* on barberry plants and telia in straw stacks.

In summary, *P. striiformis* f. sp. *tritici* produces teliospores at all wheat growth stages under field conditions in China. Teliospores collected from wheat fields and straw stacks showed a wide range of germination rates under controlled conditions favoring for teliospore germination. Wheat straw stacks maintain teliospore viability to the
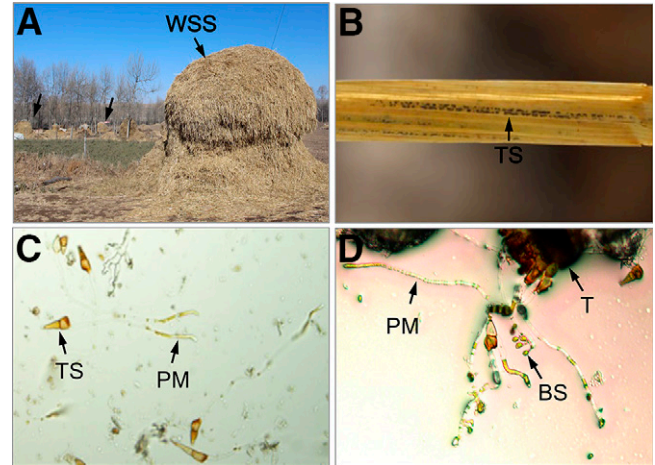

Fig. 2. Presence and germination of *Puccinia striiformis* f. sp. *tritici* teliospores collected from wheat straw stacks near wheat fields. **A,** Wheat straw stacks (WSS) in a spring wheat production area after harvest in Datong, Qinghai in 2018. **B,** Telial sori (TS) of *P. striiformis* f. sp. *tritici* on wheat leaves and leaf sheaths collected from wheat straw stacks in Qinghai in 2018. **C,** Germination of *P. striiformis* f. sp. *tritici* teliospores (TS) on wheat leaves and leaf sheaths collected from wheat straw stacks in May 2019 in Qinghai and tested at 10°C for 24 h, showing extension of promycelia (PM). **D,** Germination of *P. striiformis* f. sp. *tritici* teliospores (T) on wheat leaves and leaf sheaths collected from wheat straw stacks in May 2019 in Qinghai, showing extension of promycelia (PM) and basidiospores (BS).

Table 2. Effects of area, growth stage, year, and month on telial formation, percentage of leaves bearing viable teliospores, and teliospore germination of *Puccinia striiformis* f. sp. *tritici* in wheat fields, and germination of teliospores from straw stacks

Factor and interaction	Percentage of telial formation in field			Percentage of leaf bearing viable teliospores			Germination of teliospore in wheat			Germination of teliospore from stacks		
	df[a]	F[b]	P[c]	df	F	P	df	F	P	df	F	P
Area	1	0.3999	0.5302	1	0.0076	0.9308	1	0.0228	0.8806	–	–	–
Growth stage	1	1.4766	0.2304	2	0.1384	0.8711	2	3.1166	0.0540	–	–	–
Year	1	1.6660	0.2031	1	5.9988	0.0183*	1	0.0074	0.9317	1	4.8189	0.0423*
Month	4	1.9026	0.1257	6	0.0990	0.9961	5	4.1374	0.0022**	4	2.9597	0.0503
Area × growth stage	0	–[d]	–	0	–	–	0	–	–	–	–	–
Area × year	0	–	–	0	–	–	0	–	–	–	–	–
Area × month	0	–	–	0	–	–	0	–	–	–	–	–
Growth stage × year	0	–	–	1	0.0128	0.9103	1	0.8136	0.3719	–	–	–
Growth stage × month	1	2.5873	0.1144	2	2.8326	0.0694	2	4.9820	0.0111*	–	–	–
Year × month	3	0.0943	0.9628	1	0.0821	0.7758	1	0.0542	0.8170	3	0.5602	0.6485
Area × growth stage × year	0	–	–	0	–	–	0	–	–	–	–	–
Area × growth stage × month	0	–	–	0	–	–	1	2.6784	0.1087	–	–	–
Area × year × month	0	–	–	0	–	–	0	–	–	–	–	–
Growth stage × year × month	0	–	–	1	2.8417	0.0988	0	–	–	–	–	–
Area × growth stage × year × month	0	–	–	0	–	–	0	–	–	–	–	–

[a] *df* = degrees of freedom.
[b] *F* = F-crit.
[c] *P* = probability value. Single asterisks and double asterisks indicate significant differences at $0.5 > P > 0.01$ and at $0.05 > P > 0.0001$, respectively.
[d] Dashes indicate no data.

next spring, overlapping with regrowth of barberry in spring. Viable teliospores may produce basidiospores, infecting susceptible barberry plants to complete the sexual cycle for releasing aeciospores to infect wheat crops. Therefore, *P. striiformis* f. sp. *tritici* teliospores produced in wheat fields and harbored in wheat straw stacks are sources of inocula attacking barberry plants to initiate *P. striiformis* f. sp. *tritici* sexual reproduction in China. The results provide useful information for identifying the inoculum sources of viable teliospores that can infect barberry under natural conditions in China and may elucidate the epidemiology of stripe rust and lead to new strategies for control of the disease considering possible sexual reproduction of the pathogen on alternative hosts. More studies are needed on the natural infection of barberry plants by basidiospores produced from teliospores and the role of aeciospores as inocula for attacking wheat crops.

Table 3. Percentages of wheat straw stacks (WSS) carrying telia and germinable teliospores of *Puccinia striiformis* f. sp. *tritici* in Qinghai and Gansu in 2018 and 2019

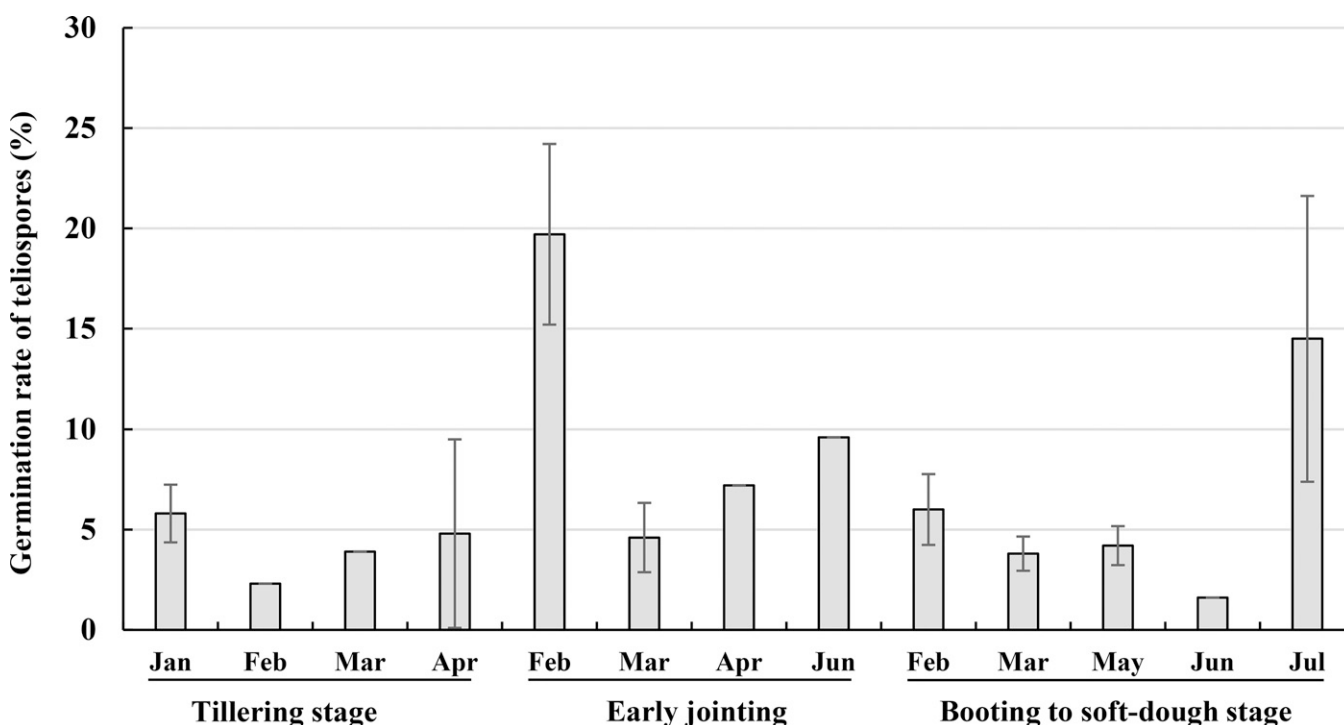| Year	Province	No. of WSS	No. of WSS with telia	Percentage (%)	No. of WSS with germinable teliospores	Percentage (%)
2018	Gansu	24	6	25.0	1	4.2
	Qinghai	32	15	46.9	3	9.4
Subtotal		56	21	37.5	4	7.1
2019	Gansu	13	5	38.5	0	0
	Qinghai	43	7	16.3	5	11.6
Subtotal		56	12	21.4	5	8.9

Fig. 3. Germination of *Puccinia striiformis* f. sp. *tritici* teliospores sampled in different months and growth stages. Each bar represents the standard error.

Table 4. Percentage of leaves bearing germinable teliospores and germination rate of teliospores from wheat straw stacks (WSS) in different months during the 2018 and 2019 cropping seasons

Sampling date	No. of WSS	Percentage of leaves bearing germinable teliospores (%)	Germination rate of teliospores from WSS (%)		
			Minimum	Maximum	Mean ± SE[a]
30 Jan 18	3	83.3	8.8	38.0	23.9 ± 6.9
11 Mar 18	3	70.0	0.0	39.0	15.5 ± 9.8
16 Apr 18	2	67.5	5.0	11.7	8.1 ± 0.4
31 May 18	2	51.4	0.0	18.0	7.4 ± 2.6
25 Jun 18	2	45.0	2.9	6.6	4.4 ± 0.8
22 Jan 19	5	64.4	5.0	18.0	10.3 ± 0.4
19 Mar 19	3	34.4	2.0	19.3	8.4 ± 2.6
18 Apr 19	3	3.3	0.0	0.4	0.04 ± 0.04
18 May 19	3	3.3	0.0	28.7	6.0 ± 6.0
24 Jun 19	ND[b]	ND	ND	ND	ND

[a] SE = standard error.
[b] ND = no data because of the removal of the straw stack.

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