Century-Old Mystery of *Puccinia striiformis* Life History Solved with the Identification of *Berberis* as an Alternate Host

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ABSTRACT

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The life history of *Puccinia striiformis* remains a mystery because the alternate host has never been identified. Inoculation of grasses using aeciospores from naturally infected *Berberis chinensis* and *B. koreana* resulted in infection on *Poa pratensis*, producing uredinia typical of stripe rust caused by *P. striiformis*. Analyses using real-time polymerase chain reaction and DNA sequence confirmed the rust fungus as *P. striiformis*. Pycnia and aecia were produced on *B. chinensis*, *B. holstii*, *B. koreana*, and *B. vulgaris* after inoculation using germinating telia of *P. striiformis* f. sp. *tritici*. Wheat inoculated with aeciospores from *B. chinensis* resulted in uredinia, which demonstrated that *Berberis* spp. also serve as alternate hosts for the wheat stripe rust pathogen. The elucidation of the complete life history for *P. striiformis* f. sp. *tritici* will provide a powerful tool to rapidly advance our knowledge of the genetics of this rust fungus, and will lead to the development of improved strategies for a better control of stripe rust.

Additional keywords: aecial host, life cycle.

Life histories (or life cycles) of most rust fungi attacking cereal crops and grasses have been well understood for more than a century. The life history of *Puccinia striiformis*, the causal organism of stripe (or yellow) rust of important cereal crops and grasses, remains a mystery because the alternate (or aecial) host for *P. striiformis* has never been identified although it has been assumed that *P. striiformis* is a macrocyclic, heteroecious fungus, based on similarities with other cereal rust fungi. Attempts to identify the alternate host by infecting various suspected plant species with germinating teliospores of *P. striiformis* have failed (4,11,15). Mains (10) suspected *Berberis* and *Mahonia* could be alternate hosts of *P. striiformis* because of its relatedness to *P. koeleriae*, *P. arrhenatheri*, and *P. montanensis* that are rusts of *Berberis*. Hart and Becker (5) followed this lead but failed to produce infection on *Berberis* or *Mahonia* with teliospores of *P. striiformis*. Due to these failures and a fact that the fungus reproduces asexually through urediniospores that can sustain itself clonally by re-infecting the telial hosts, the existence of an alternate host for *P. striiformis* in nature has been doubted (6).

In June 2009, we observed severe aecial infection on *B. chinensis*, and light infections on *B. koreana* and on 'Emerald Carousel', an interspecific hybrid between *B. koreana* and *B. thunbergii*. Preliminary inoculation on grasses using aeciospores resulted in infection only on *Poa pratensis*, producing uredinia typical of stripe rust caused by *P. striiformis*, suggesting that *Berberis* spp. might be an aecial host of this rust fungus. We report the identification of aecial hosts of *P. striiformis* f. sp. *poae*, and the elucidation of the complete life history of *P. striiformis* f. sp. *tritici.*

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MATERIALS AND METHODS

Inoculation on grasses using aeciospores. Leaves of *B. chinensis*, *B. koreana* and 'Emerald Carousel', an interspecific hybrid between *B. koreana* and *B. thunbergii*, with natural aecial infections were collected from specimen plants grown in the University of Minnesota arboretum in Chaska (MN) and ornamental plantings of Emerald Carousel in St. Paul (MN) in June 2009. Aecia-bearing leaves were suspended over seedling plants of wheat (cv. Chinese Spring and Line E), barley (cv. Hiproly and Hypana), oat (cv. Marvelous), rye (cv. Prolific), and Kentucky blue grass (*Poa pratensis*). Plants were incubated in an inoculation chamber for 16 h at 18 to 20°C in the dark. The chamber was intermittently misted to ensure high humidity (≈100% relative humidity) and maintain free moisture on leaf surfaces. After inoculation, plants were maintained in a growth chamber at 18 to 20°C with a photoperiod of 12 h.

Inoculation on *Berberis* spp. using telia of *P. striiformis* f. sp. *tritici.* Wheat straw bearing telia of *P. striiformis* f. sp. *tritici* was harvested from an experimental field at the University of California, Davis (CA). Leaf tissue was soaked in water for 24 h, rinsed thoroughly, and maintained moist by wrapping in moistened paper towel. Teliospore germination was monitored by plating teliospores onto water-agar plates and periodically observing the plates under a microscope. When teliospore germination was detected (usually 48 h after plating), straw was suspended over plants of *B. chinensis*, *B. holstii*, *B. koreana*, and *B. vulgaris*, and plants were incubated for 4 days in a mist chamber with a diurnal temperature regime (12-h night at 12°C and 12-h day at 15°C). After inoculation and incubation, plants were maintained in a growth cabinet at the same diurnal temperature/light regimes.

Inoculation on Line E wheat using resultant aeciospores produced on *B. chinensis*. Leaves of *B. chinensis* bearing aecia from telial inoculation were placed onto a piece of filter paper saturated with water in a petri plate for 6 h to promote the release of aeciospores. Water drops were placed onto aeciospore masses to make an aeciospore suspension. Seedlings of Line E wheat

were inoculated by applying spore suspension onto surfaces of primary leaves using a cotton swab. Inoculated plants were incubated for 24 h in a mist chamber with a diurnal temperature regime (12-h night at 12°C and 12-h day at 15°C). After inoculation and incubation, plants were maintained in a growth cabinet at the same diurnal temperature/light regimes. Uredinia were produced 12 days after inoculation.

DNA analysis. DNA was extracted from dried host tissue (20 to 30 mg) containing aecial, telial, or uredinial pustules. Infected leaf tissue was pulverized as described by Anikster et al. (1). DNA was isolated from pulverized tissue using an OmniPrep DNA extraction kit (GenoTech, St. Louis). Real-time polymerase chain reaction (PCR) assays specific for identification of *P. graminis* and *P. striiformis* were performed as described by Barnes and Szabo (2) using a LightCycler 480 (Roche, Indianapolis, IN). The nuclear ribosomal internal transcribed spacer (ITS) region and the 5′-end of the large subunit were amplified using PCR primers ITS1F and RUST1 as described (1). Amplification products were purified, cloned, and sequenced (1). DNA sequence alignment and phylogenetic analysis using parsimony were preformed as described (14). Nucleotide sequence data have been submitted as GenBank accession numbers GQ457304 to GQ457307 and GU382671 to GU382673.

RESULTS

Inoculations of wheat, barley, rye, oat, and *Poa pratensis* using aeciospores from *B. chinensis* (Fig. 1A) resulted in infection only on *Poa pratensis*, producing uredinia typical of stripe rust caused by *P. striiformis* (Fig. 1B). Inoculations on *Poa pratensis* using aeciospores from *B. koreana* and Emerald Carousel also produced uredinia typical of stripe rust. The rust fungus causing the aecial infections on *Berberis* spp. and uredinial infections on *Poa pratensis* was identified as *P. striiformis* by real-time PCR (Table

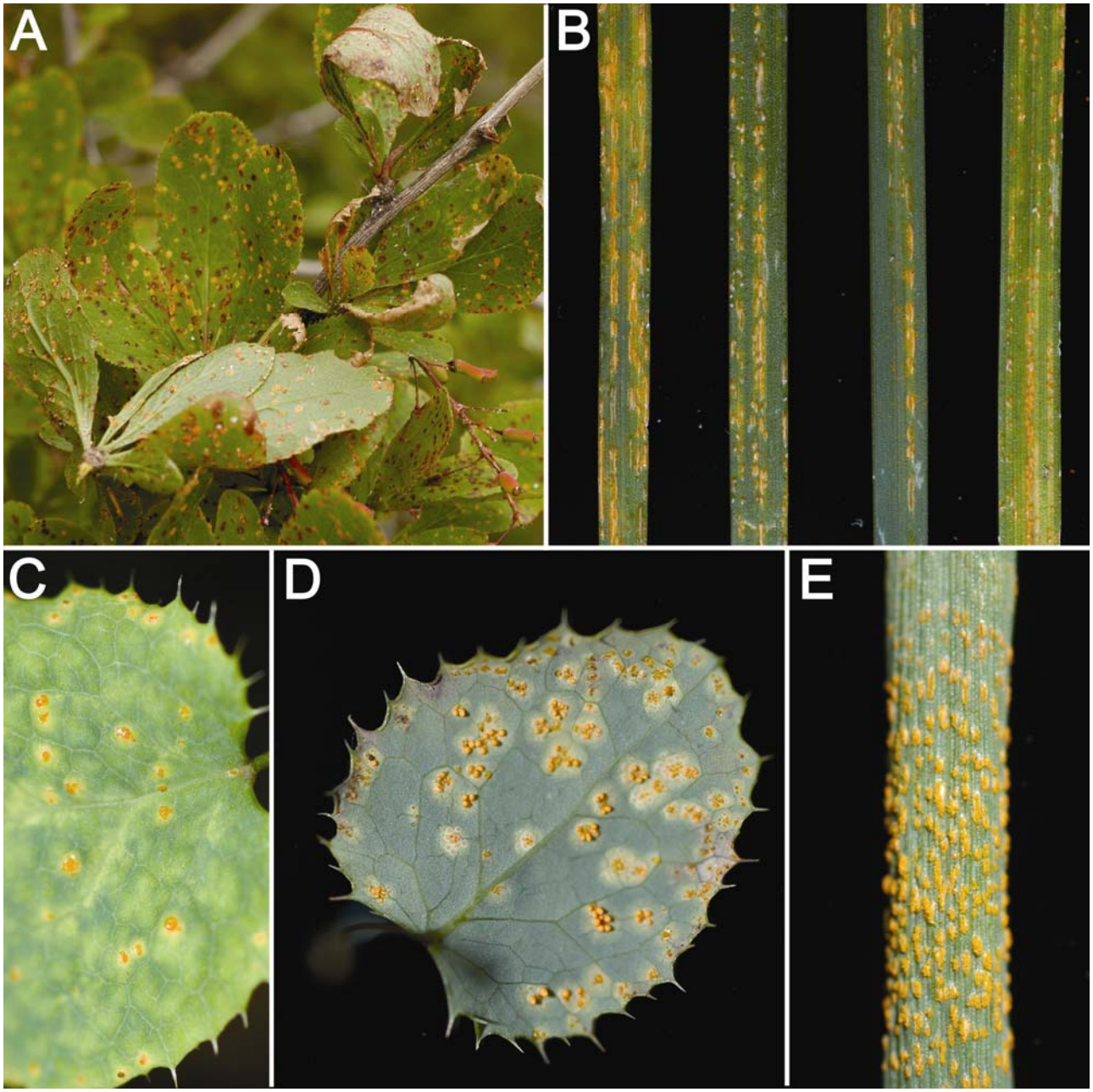

Fig. 1. A, Aecial infection on *Berberis chinensis* plants in the field; **B,** uredinia produced on *Poa pratensis* through inoculation with aeciospores from *B. chinensis*; **C,** pycnia and **D,** aecia produced on seedling plants of *B. chinensis* through inoculation with germinating teliospores of *Puccinia striiformis* f. sp. *tritici*; and **E,** uredinia produced on Line E wheat after inoculation with aeciospores from *B. chinensis* shown in **D**.

TABLE 1. Real-time polymerase chain reaction (PCR) assay for identification of *Puccinia striiformis*

Sample[a]	Host	Life-cycle stage	Location	Real-time PCR[b]	
				P. graminis	*P. striiformis*
HSZ1828	Berberis × Emerald Carousel	Aecia	St. Paul, MN	–	+
HSZ1829	Berberis × Emerald Carousel	Aecia	St. Paul, MN	–	+
HSZ1831	Berberis × Emerald Carousel	Aecia	Chaska, MN	–	+
HSZ1832	*Berberis chinensis*	Aecia	Chaska, MN	–	+
HSZ1833	*B. koreana*	Aecia	Chaska, MN	–	+
HSZ1834	*B. koreana*	Aecia	Chaska, MN	–	+
HSZ1836[c]	*Poa pratensis*	Uredinia	–	–	+
HSZ1837[c]	*Poa pratensis*	Uredinia	–	–	+
HSZ1838[c]	*Poa pratensis*	Uredinia	–	–	+
HSZ1847	*Triticum aestivum*	Telia	Davis, CA	–	+
HSZ1849[d]	*B. chinensis*	Aecia	–	–	+
HSZ1872[e]	*T. aestivum*	Uredinia	–	–	+
P. graminis	*T. aestivum*	Uredinia	–	+	–
P. striiformis	*T. aestivum*	Uredinia	–	–	+

[a] Samples were stored in rust collection at the U.S. Department of Agriculture, Agricultural Research Service, Cereal Disease Laboratory, St. Paul, MN.
[b] Positive (+) assay with crossing point value of <30; negative (–).
[c] Uredinia formed by inoculating *Poa pratensis* with aeciospores from *B. chinensis* (HSZ1832).
[d] Aecia formed by inoculating *B. chinensis* with germinating teliospores of *P. striiformis* f. sp. *tritici* (HSZ1847).
[e] Uredinia formed by inoculating *T. aestivum* (Line E) with aeciospores from *B. chinensis* (HSZ1849).

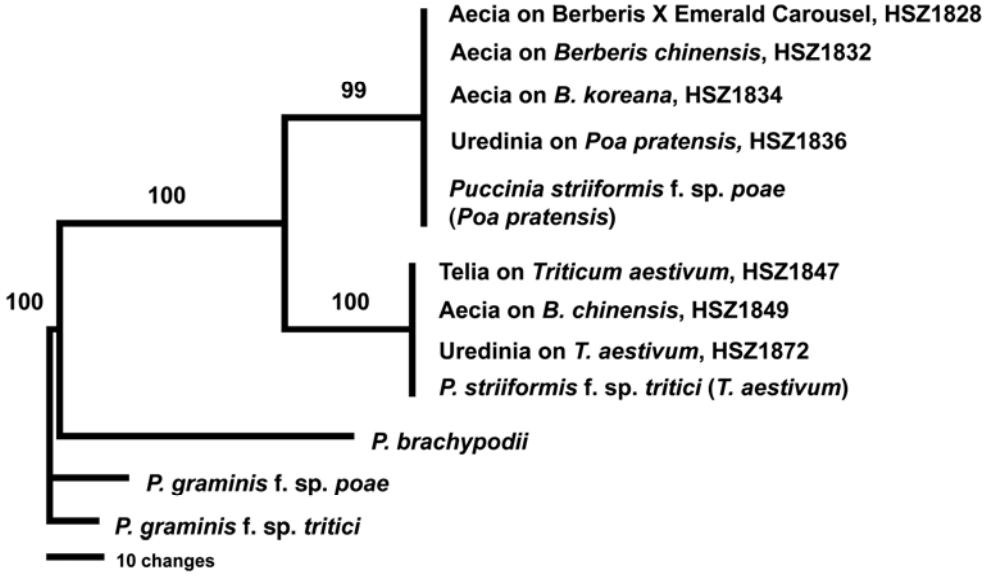

Fig. 2. Phylogenetic tree of *Puccinia striiformis* samples based on nuclear ribosomal internal transcribed spacer sequence data. Parsimony analysis resulted in an optimal tree with a length of 115 steps. Numbers above branches indicate percentage of congruent clusters in 5,000 bootstrap trials. Reference sequences used in the analysis included: *P. brachypodii* (GQ457303); *P. graminis* f. sp. *poae* (DQ417389); *P. graminis* f. sp. *tritici* (DQ417374); *P. striiformis* f. sp. *poae* (DQ417407); and *P. striiformis* f. sp. *tritici* (DQ417394).

1). DNA sequence analysis of the nuclear ribosomal internal transcribed region confirmed the identification and determined that it was *P. striiformis* f. sp. *poae* (Fig. 2).

Among several cereal rust fungi, including *P. graminis* and *P. coronata*, of which their host specificities and complete life cycles are well-known, different formae speciales of the same species share a common aecial host (7,9). We hypothesized that *P. striiformis* f. sp. *tritici*, the forma specialis specialized on wheat, also uses *Berberis* spp. as an alternate host. To test this hypothesis, freshly harvested wheat straw bearing telia of *P. striiformis* f. sp. *tritici* was used to inoculate several members of *Berberis*: *B. chinensis*, *B. holstii*, *B. koreana*, and *B. vulgaris*. Pycnia were produced at 8 days (Fig. 1C) and aecia were produced 14 days postinoculation on *B. chinensis* (Fig. 1D), *B. holstii*, and *B. vulgaris.* Aecia production on *B. koreana* appeared to be limited. Aeciospores from *B. chinensis* were used to inoculate wheat Line E, producing uredinia typical of stripe rust (Fig. 1E) 12 days after inoculation. Real-time PCR and DNA sequence analyses of the ITS region confirmed that telia on wheat straw, aecia produced on *B. chinensis*, and uredinia produced on Line E were that of *P. striiformis* f. sp. *tritici* (Table 1; Fig. 2).

DISCUSSION

Aecial infections on *Berberis* spp. (*B. chinensis*, *B. koreana*, and Emerald Carousel) were observed in June of 2009. This was unusual, in that these *Berberis* spp. are thought to be resistant to *P. graminis*, the most common rust pathogen of *Berberis* in North America. Inoculation studies and DNA analysis determined that the aecial infections were caused by *P. striiformis* f. sp. *poae*. This finding represents the first identification of an alternate host for any type of the stripe rust pathogen. In order to prove that *Berberis* spp. is an alternative host for *P. striiformis* f. sp. *tritici*, teliospores from stripe rust-infected wheat were used to inoculate *Berberis* spp., and the resultant aeciospores were used to inoculate wheat. These inoculation experiments elucidated the complete life cycle of *P. striiformis* f. sp. *tritici* (Fig. 3) and unequivocally proved that *Berberis* spp. are alternate hosts of *P.*

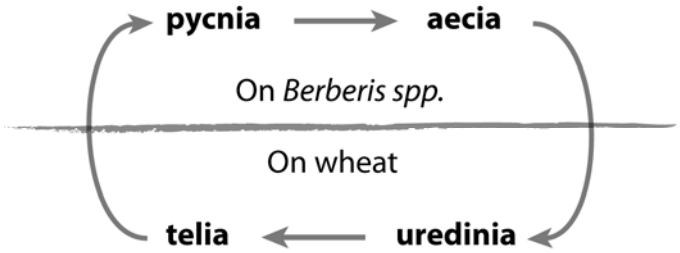

Fig. 3. Life cycle of *Puccinia striiformis* f. sp. *tritici*.

striiformis f. sp. *tritici*. DNA analysis was used to confirm the identity of the rust pathogen at each step in the life cycle. This discovery will likely provide a powerful tool to rapidly advance our knowledge on the genetics of this rust fungus and lead to the development of improved strategies for better control of stripe rust.

P. striiformis f. sp. *tritici* is known to be one of the most variable cereal rust pathogens with regard to virulence (8). Many new races are regularly identified in wheat producing areas where stripe rust is a major disease. This variability in virulence has been attributed to mutations and somatic hybridization (12) since the sexual stage was presumed to be absent. Our discovery of an alternate host has led us to hypothesize that in areas where wheat and susceptible barberry species coexist, sexual recombination has likely played an active role in contributing to pathogen variability. This hypothesis needs to be tested by isolating new races of *P. striiformis* f. sp. *tritici* from *Berberis* spp. The high degree of virulence diversity found in western China (3), the Caucasus and Central Asia (13) where *B. vulgaris* and *B. chinensis* grow naturally, and a highly aggressive race originating from eastern Africa where *B. holstii* is present, serve as indirect evidence to support this hypothesis. Wide use of ornamental *Berberis* spp. identified to be susceptible in this study, and the likely discovery of other susceptible species and hybrids to *P. striiformis* f. sp. *tritici*, highlights the concern that ornamental *Berberis* spp. has the potential for generating novel virulence combinations.

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