Assessing host specialization among aecial and telial hosts of the white pine blister rust fungus, *Cronartium ribicola*

Bryce A. Richardson, Paul J. Zambino, Ned B. Klopfenstein, Geral I. McDonald, and Lori M. Carris

Abstract: The white-pine blister rust fungus, *Cronartium ribicola* Fisch. in Rabenh., continues to spread in North America, utilizing various aecial (primary) and telial (alternate) hosts, some of which have only recently been discovered. This introduced pathogen has been characterized as having low genetic diversity in North America, yet it has demonstrated a capacity to invade diverse environments. The recent discovery of this rust fungus on the telial host *Pedicularis racemosa* Dougl. ex Benth., raises questions of whether this host association represents a recent acquisition by *C. ribicola* or a long-standing host association that was overlooked. Here we explore two questions: (*i*) is host specialization detectable at a local scale and (*ii*) is the capacity to infect *Pedicularis racemosa* local or widespread? Genetic analysis of *C. ribicola* isolates from different aecial and telial hosts provided no evidence for genetic differentiation and showed similar levels of expected heterozygosity within a geographic population. An inoculation test showed that diverse *C. ribicola* sources from across North America had the capacity to infect *Pedicularis racemosa*. These results support a hypothesis that ability to infect *Pedicularis racemosa* is common in *C. ribicola* from North America. Utilization of *Pedicularis racemosa* by *C. ribicola* may be dependent on the co-occurrence of this host, inoculum, and favorable environments.

Key words: rust fungus, AFLP, invasive species, plant pathogen.

Résumé : Le champignon de la rouille vésiculeuse du pin blanc, *Cronartium ribicola* Fisch. in Rabenh., continue de s'étendre en Amérique du Nord, en utilisant divers hôtes aéciens (primaire) et téliens (hôtes) dont certains n'ont été que récemment découverts. On a caractérisé ce champignon pathogène introduit, comme ayant une faible diversité génétique en Amérique du Nord, mais il a cependant démontré sa capacité à envahir divers environnements. La récente découverte de cette rouille pathogène sur l'hôte télien *Pedicularis racemosa* Dougl. ex Benth., soulève la question à savoir s'il s'agit d'une acquisition récente du *C. ribicola* ou s'il s'agit d'un hôte associé méconnu, présent depuis longtemps. Les auteurs explorent deux questions : (*i*) peut-on détecter une spécialisation de l'hôte à l'échelle locale; (*ii*) la capacité d'infection sur le *Pedicularis racemosa* est-elle locale ou étendue. L'analyse génétique d'isolats du *C. ribicola* provenant de divers hôtes aéciens et téliens, n'apporte aucune preuve de différenciation génétique, et montre les degrés similaires d'hétérozygosité attendus pour une population géographique. Un essai d'inoculation montre que diverses sources du *C. ribicola* provenant de l'ensemble de l'Amérique du Nord sont capables d'infecter le *Pedicularis racemosa*. Ces résultats supportent l'hypothèse que la capacité d'infecter le *Pedicularis racemosa* est courante chez le *C. ribicola,* en Amérique du Nord. L'utilisation du *Pedicularis racemosa* par le *C. ribicola* pourrait dépendre de la co-occurrence de cet hôte, d'inoculum, et d'un environnement favorable.

Mots-clés : champignon de la rouille, AFLP, espèces envahissantes, pathogène végétal.

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Introduction

Cronartium ribicola Fisch. in Rabenh., the white-pine blister rust fungus, has caused significant mortality of five-needled, haploxylon pines in North America and widespread disruption of ecosystems where these pines were keystone species (Maloy 1997). The rust pathogen is believed to have been introduced into Europe from Asia during the mid-1800s, spreading mainly in plantations of eastern white pine (*Pinus strobus* L.) exotic to Eurasia (Spaulding 1922). The pathogen was subsequently introduced separately into eastern and western North America. In western North America, *C. ribicola* was imported on a small shipment of eastern white pine from a French nursery (ca. 100 infected trees) in 1910 to Vancouver, British Columbia. No other introductions of infected plants into western North America have been documented (reviewed in McDonald and Hoff 2001), although Hunt (2003) suggested the potential for introductions on shipments to other locations. Population genetic

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B.A. Richardson.[1] USDA Forest Service, Rocky Mountain Research Station, 1221 S. Main St, Moscow, ID 83843, USA; Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA.
P.J. Zambino, N.B. Klopfenstein, and G.I. McDonald. USDA Forest Service, Rocky Mountain Research Station, 1221 S. Main St, Moscow, ID 83843, USA.
L.M. Carris. Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA.

[1]Corresponding author (e-mail: brichardson02@fs.fed.us).

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studies support a western introduction of limited genotypes (White et al. 1996; Kinloch et al. 1998; Hamelin et al. 2000; Richardson 2006) based on limited genetic diversity of *C. ribicola* in western North America. However, at a continental scale, *C. ribicola* populations are distinct between eastern–central versus western North America, with relatively greater diversity in the east. A much larger introduction of infected trees in eastern North America versus a small introduction in the west and physical and biological barriers to gene flow are believed to be causes of this genetic differentiation (Hamelin et al. 2000).

To complete its life cycle, *C. ribicola* produces five spore stages and requires distinct aecial (primary) plant hosts (i.e., five-needled, haploxylon pines) and telial (alternate) plant hosts. Until recently, *C. ribicola* was assumed to utilize only one telial host genus, *Ribes*, in North America. Presently, telial hosts in North America are known also to include three species of the Orobanchaceae: *Pedicularis racemosa* Dougl. ex Benth., *Pedicularis bracteosa* Benth., and *Castilleja miniata* Dougl. ex Hook. (McDonald et al. 2006; Zambino et al. 2007). Despite earlier indications that *C. ribicola* is able to infect *Castilleja miniata* under greenhouse conditions (Hiratsuka and Maruyama 1976), previous field inoculations on several members of Orobanchaceae, including *Pedicularis racemosa* and *Castilleja miniata*, produced no signs or symptoms of infection (Hunt 1984). Other previous inoculations of greenhouse-grown *Castilleja miniata*, *Pedicularis canadensis* L. (native to eastern North America), and *Pedicularis resupinata* L. (native to eastern Asia) with inoculum from Wisconsin, USA, failed to produce sporulating infections (Patton and Spear 1989). However, *Pedicularis resupinata* is a known telial host of *C. ribicola* in Asia and exhibits a widespread geographical range. Recently, *C. ribicola* infections were found to be common on *Pedicularis racemosa* near Roman Nose Lake, Idaho, USA (Richardson 2006). These studies raise questions of how long *Pedicularis racemosa* has been used as a host by *C. ribicola* in North America and what factors contribute to its susceptibility.

In Asia, some geographically delimited races of *C. ribicola* are capable of utilizing one or more local species of *Ribes*, *Pedicularis*, and *Castilleja*. *Cronartium ribicola*'s utilization of telial hosts in Asia has been classified into "types" that may vary according to region or locale: *Ribes*–*Pedicularis*, *Ribes* only, and *Pedicularis* only (Stephan and Hyun 1983, reviewed in McDonald et al. 2005). Some studies have shown a correspondence between utilization of aecial and telial hosts. Yokota and Uozumi (1976) performed inoculation tests in Japan using aeciospore sources from the Japanese stone pine (*Pinus pumila* (Pallas) Regel) and the exotic eastern white pine. They found that at some sites, aeciospores from some eastern white pine sources only infected *Ribes* species, whereas at other sites, aeciospores from other Japanese stone pine and eastern white pine sources were able to infect both *Pedicularis* and *Ribes* species. Hyun and Koo (1981) reported that *C. ribicola* sources from South Korea were not capable of infecting *Ribes nigrum* L. or *Ribes hudsonianum* Richards. In a similar study, *R. nigrum* and *Pedicularis resupinata* were inoculated with rust sources from northern Germany and South Korea. Korean rust sources infected only *Pedicularis*, and German sources infected only *Ribes* (Stephan and Hyun 1983). Subsequently, phylogenetic analysis has shown genetic divergence among *C. ribicola* samples from the USA, Korea, and China (Hei et al. 2003). All of these studies suggest that some Asian *C. ribicola* populations may be genetically distinct and host-differentiated to allow exploitation of different local hosts and (or) environments.

Table 1. Sample size (*n*) and expected heterozygosity for *Cronartium ribicola* collected from five host species at the Roman Nose Lake site, Idaho. The heterozygosity of *C. ribicola* did not differ significantly ($p > 0.05$) according to host.

Host	*n*	Heterozygosity
Pedicularis racemosa	14	0.39
Ribes hudsonianum	23	0.39
Ribes lacustre	20	0.38
Pinus albicaulis	18	0.39
Pinus monticola	11	0.35

In North America, ability of geographic populations of *C. ribicola* to overcome major gene resistance in two five-needled pines has been well characterized (e.g., Kinloch and Comstock 1981; McDonald et al. 1984; Kinloch et al. 2004). However, few studies have investigated specialization to different telial or aecial hosts. Previously, cross-inoculation studies using geographically different inoculum and *Ribes* sources have shown significant differences in frequency and latency of infection. It is unclear if these phenotypic differences may be due to genetic adjustments or phenotypic plasticity (McDonald 2000).

Here, we explore two questions: (*i*) is host specialization detectable at a local scale and (*ii*) is the capacity to infect *Pedicularis racemosa* local or widespread? Two approaches were used to address these questions: (*i*) a local-scale, molecular genetic analysis of *C. ribicola* derived from two aecial hosts (*Pinus albicaulis* Engelm. and *Pinus monticola* Douglas ex D. Don) and three telial hosts (*Pedicularis racemosa*, *R. hudsonianum*, and *Ribes lacustre* (Pers.) Poir.), and (*ii*) an experiment in which *Pedicularis racemosa* was inoculated with genetically distinct *C. ribicola* from three regions in North America (western, midwestern, and eastern USA) (Hamelin et al. 2000; P.J. Zambino, unpublished data, 2005).

Material and methods

Molecular genetic analysis

Sample site and collections

Aeciospore and urediniospore collections (Table 1) were made at the North American location where non-*Ribes* telial hosts of *C. ribicola* were first discovered, near Roman Nose Lake (McDonald et al. 2006). Roman Nose Lake is approximately 20 km west of Bonners Ferry in the Selkirk Mountains of northern Idaho (48°38′N, 116°34′W), USA, at an elevation of 1700 m. The site is characterized by 20- to 30-year-old western white pine (*P. monticola*), whitebark pine (*P. albicaulis*), and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) that regenerated after a stand-replacing wildfire in 1967. White-pine blister rust cankers were present on a

Table 2. Summary of results from inoculations of *Ribes nigrum* and *Pedicularis racemosa* with 16 sources of *Cronartium ribicola*.

						Ribes nigrum		*Pedicularis racemosa*	
Region	Sample name	Origin	Aecial host	Spore type	Germ. (%)	Cutting	Leaf disk	Small plant	Detached leaves
West	HC13	N. California	MGR-SP	A	<0.1	N	I	N	N
	HC28	N. California	MGR-SP	U	28	I	I	I	I
	CO.07.07	Colorado	LP	U	89	I	I	I	I
	RN8	N. Idaho	WBP	A	100	I	I	I	I
	RN22	N. Idaho	WBP	U	40	N	I	N	L
	RN32	N. Idaho	WWP	U	34	I	N	N	L
	MC 10–1	N. Idaho	WWP	U	44	I	I	N	I
	SS2	E. Oregon	WBP	U	60	I	I	I	I
	SS7	E. Oregon	WBP	U	78	L	I	I	I
	NM03.01	New Mexico	SWWP	A	94	I	I	I	I
	WY18.20	Wyoming	LP	A	81	I	I	I	I
Midwest	MN13.04	Minnesota	EWP	A	88	I	I	I	I
	WI04. 01B	Wisconsin	EWP	U	77	I	I	I	I
East	ME01.01A	Maine	EWP	U	82	I	I	I	I
	VT01.02A	Vermont	EWP	U	94	I	I	I	I
	NY01.03	New York	EWP	A	16	I	I	N	N

Note: Inoculum was aeciospores (A) or urediniospores (U), and successful infection (I) was assessed as development of uredinia or telia within 21 days. MGR-SP, sugar pine (*Pinus lambertiana*) with major gene (*Cr1*) resistance to white pine blister rust; LP, limber pine (*P. flexilis*); WBP, whitebark pine (*P. albicaulis*); WWP, western white pine (*P. monticola*); SWWP, southwestern white pine (*P. strobiformis*); EWP, eastern white pine (*P. strobus*); Germ., germination; L, inoculated leaves lost to mold, mites, wilting/senescence; I, infection; N, no infection.

high proportion of the western white pine and whitebark pine. *Pedicularis*, *Castilleja*, and *Ribes* species were common in the understory vegetation. Uredinia of *C. ribicola* were sparsely scattered on leaves of the alternate hosts.

Aeciospore collections were made from single blister rust cankers on 11 western white pines and 18 whitebark pines. Aeciospores from each canker were used to inoculate leaf disks (ca. 20 mm diameter) from greenhouse-grown plants of the 'Heimberger' clone of *R. nigrum* (Patton 1972), which was previously shown to be susceptible to diverse North American sources of *C. ribicola* (P.J. Zambino, unpublished data, 2006). Thus, the 'Heimberger' clone should not exert genetic selection on *C. ribicola* genotypes. Before inoculation, leaf disks were washed with tap water and placed adaxial side down on moist filter paper in a 150 mm × 15 mm Petri plate. Spores were placed onto the abaxial surface of leaf disks with a sterile pipette tip and the disks misted with distilled water. One leaf disk per plate was used as a negative control. The Petri plates were sealed and then incubated at 18 °C for 2 weeks under fluorescent lights with a 12-h day length.

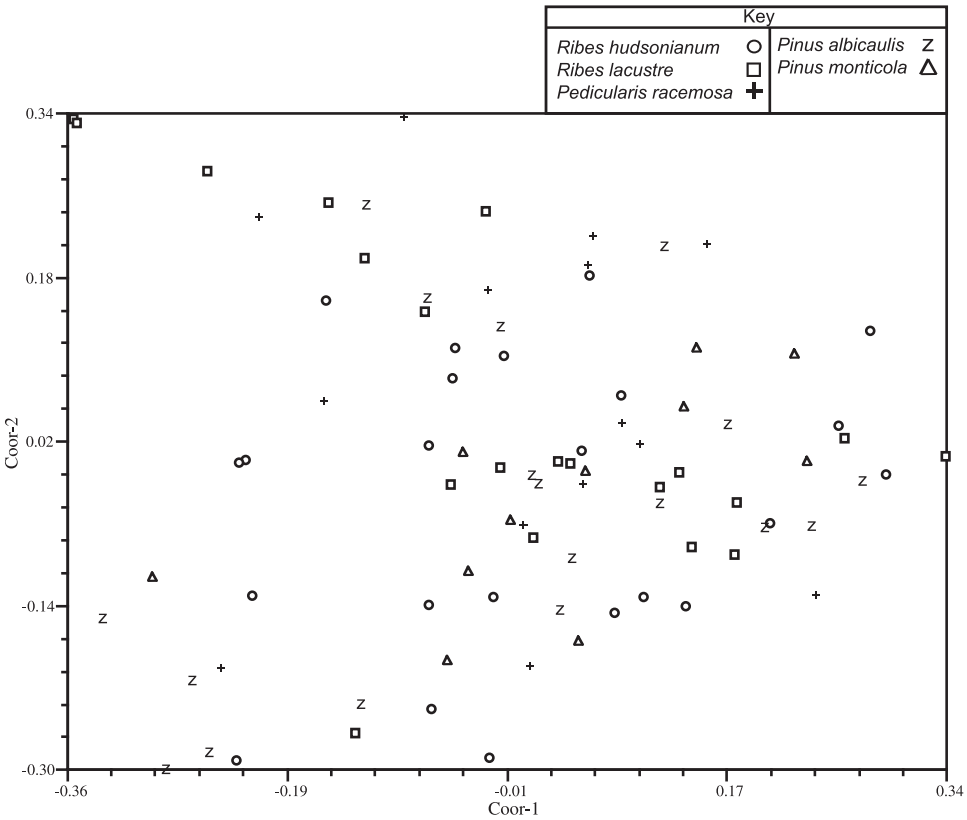
To ensure that each sample consisted of a single genotype, urediniospores were collected from a single, well-isolated uredinium on a leaf disk using a dissecting microscope and a minuten pin (0.1 mm diameter) and used to inoculate single-leaf cuttings following the methods described in McDonald et al. (2006). After uredium development, 1–2 mg of uredinospores were collected from this leaf and used to inoculate an entire, potted *R. nigrum* plant. For this series of inoculations, urediniospores were suspended in 600 μL of distilled water and the inoculum was misted onto four to five leaves using individual atomizers. Leaves were misted with water, covered with plastic bags, and incubated at 18 °C for 24 h. Plants were then moved to the greenhouse and placed into individual inoculation chambers. After 3 weeks, 50–100 mg of urediniospores were collected in gelatin capsules and dried to 40% RH before DNA extraction and storage at –20 °C. DNA was extracted from ca. 10 mg of urediniospores following a modified protocol developed by Zambino (2002).

Urediniospore collections were made from individual, naturally infected leaves of 20 *R. lacustre*, 23 *R. hudsonianum*, and 14 *Pedicularis racemosa* plants from the collection site. Uredinium-containing leaves were individually stored in a paper envelope and placed in a container with silica gel desiccant. To ensure single genotypes of *C. ribicola*, each field-collected leaf was inspected to ensure that it contained a single uredinium. Before inoculations, each leaf was sealed in a plastic envelope and placed in a 40 °C water bath for 5 min, followed by a 3– 4 h incubation at 100% RH (Zambino et al. 1997; Zambino 2000). The abaxial surface of the infected leaf was rubbed against the abaxial surface of a noninfected, *R. nigrum* single-leaf cutting. Inoculated leaves were misted with water and bagged following inoculation. Subsequently, urediniospore lines were multiplied on whole plants, as previously described.

AFLP analysis

A modified amplified fragment length polymorphism (AFLP) procedure was adapted from Vos et al. (1995). Approximately 300 ng of genomic DNA was digested for 3 h at 37 °C followed by 65 °C for 20 min, in a reaction containing 10 U of *Eco*RI and 5 U *Mse*I (New England Biolabs, Inc., Ipswich, Mass.) in a total volume of 20 μL. DNA fragments were ligated at 16 °C for 8 h using 60 U of T4 ligase (New England Biolabs, Inc.) with 50 μmol·L^{-1} *Mse*I and 5 μmol·L^{-1} *Eco*RI adapters in a final reaction volume of 20 μL. Ligated fragments were then diluted 1:10 with ster-

Fig. 1. Plot of the first two principal coordinates (Coor-1 and Coor-2) of a principal coordinates analysis for *Cronartium ribicola* isolates collected from five host sources at Roman Nose Lake, Idaho. Plot represents 14% of the total variance in a genetic distance matrix for 86 isolates obtained using 44 polymorphic AFLP loci. Symbols identify isolates by host source.



ile-distilled water. Each pre-amplification PCR reaction (30 µL total volume) contained 6 µL of template, 300 nmol·L^{-1} *Eco*RI and *Mse*I primers with no extension, 1× PCR buffer, 3 mmol·/L^{-1} $MgCl_2$, 200 µmol·L^{-1} dNTPs, and 1.5 U of Ampli*Taq* polymerase (Applied Biosystems, Inc., Foster City, Calif.). Selective amplification used eight combinations of *Mse*I/*Eco*RI primers having +2 extensions. Reactions (25 µL total volume) contained 5 µL of 1:10 diluted pre-amplification product, 1× PCR buffer, 2.4 mmol·L^{-1} $MgCl_2$, 300 µmol·L^{-1} dNTPs, 100 nmol·L^{-1} *Mse*I *Eco*RI primers, and 1.5 U of Ampli*Taq* Gold polymerase (Applied Biosystems, Inc.). The *Eco*RI primer was labeled with fluorescent dye 6-FAM (Integrated DNA Technologies, Coralville, Iowa). All reactions were conducted using an MJ PTC-200 thermocycler (Bio-Rad Laboratories, Waltham, Mass.). The thermocycling protocol has been previously described (Remington et al. 1999).

After diluting 1:4 with sterile-distilled water, selective-amplification products were separated on an ABI 3700 DNA automated sequencer (Applied Biosystems, Inc.) at the University of Wisconsin Biotechnology Center (Madison, Wisc.) using Geneflo-625 (Chimerx, Inc., Milwaukee, Wisc.) as an internal standard. Genotyper 3.7 NT (Applied Biosystems, Inc.) was used to score bands within a size range of 75 to 600 bp having a scaled peak-height threshold of 100 or greater. AFLP chromatograms of all samples were then visually checked and scored for presence (1) or absence (0) of bands. Only polymorphic AFLP bands present in at least two individuals were included in the analysis.

Samples were assigned to five groups based on host species. Because samples represented only the dikaryotic phase of the life cycle and AFLPs are dominant markers, genetic diversity and genetic structure F_{ST} values were calculated with AFLP-SURV version 1.0 (Vekemans 2002). A Bayesian approach was used to estimate the frequency of null alleles (Zhivotovsky 1999) following the Lynch and Milligan (1994) method and assuming Hardy–Weinberg equilibrium. Unbiased expected heterozygosity (H_E) within and among groups (Nei 1978) was calculated using the Lynch and Milligan (1994) method. An unpaired *t* test was used to test for significant differences in H_E among groups. To test for significant F_{ST} values between groups, a permutation test was run using AFLP-SURV version 1.0. Principal coordinates analysis was used to determine relationships among samples. NTSYS-PC ver. 2.10 (Rohlf 1998) was used to generate a Jaccard (1908) similarity matrix for isolates, transform the matrix with the *d*-center module, and plot the first two principal coordinates.

Inoculation experiments

Two inoculation experiments were carried out, using whole plants vs. detached leaves of *Pedicularis racemosa* collected from the Roman Nose Lake site. The same 16 inoculum sources from eastern, midwestern, and western USA were used for both experiments (Table 2). Previous studies have shown that these three geographic sources of inoculum are genetically distinct (Hamelin et al. 2000; P.J. Zambino, unpublished data, 2006). Two spore types (urediniospores

and aeciospores) were represented among the sources. Before inoculation, spore aliquots of each source were retrieved from –80 °C storage. Urediniospore preparation followed the protocol of Zambino et al. (1997). Germination rates were estimated for inoculum sources by dusting spores onto Petri plates containing 1.5% water agar media. Spore germination was tallied after 48-h incubation at 18 °C, 100% RH.

Whole-plant assays

Small plants were excavated with intact root systems and placed into 1 L, clear-polypropylene containers. Two plants (one 6–10 cm and one 3–7 cm) were placed in each container on a layer of moist vermiculite, and then containers were sealed with plastic lids and maintained at ≤18 °C until inoculation. One container was used per inoculum source, and one served as a negative control. Abaxial leaf surfaces of three to four fully expanded leaves per plant were dusted with spores using a small paint brush. Plants were misted with deionized water and the containers were sealed for 48 h. Then the lids were replaced with two layers of laboratory tissue. Similarly treated single-leaf cuttings of the susceptible 'Heimberger' clone of *R. nigrum* in small pots of vermiculite served as positive controls to verify that spores of each source could infect plants.

Detached-leaf assays

For each test of an inoculum source, six leaves were detached from a single, full-sized, field-grown plant. Starting with the most recent fully developed leaf and ending with the oldest healthy leaf, each leaf was rinsed with deionized water and placed in a 150 mm Petri plate lined on the bottom with moist filter paper. Leaves were placed abaxial side upward around the perimeter of the plate in order of increasing age. A leaf disk of the 'Heimberger' clone of *R. nigrum* served as a positive control. One leaf from each plate was covered with filter paper during inoculation to serve as a negative control. All leaves were misted with distilled water after inoculation, and the plate was sealed.

Whole-plant and detached-leaf assays were maintained under the same conditions after inoculation: 18 °C with a 12 h photoperiod. Leaves were examined for signs and symptoms of infection starting 11 days after inoculation and subsequently at 2- to 3-day intervals until 21 days after inoculation.

Results

AFLP analysis

Eight combinations of selective amplification primers produced 441 scoreable AFLP bands of which 44 (10%) were polymorphic among the 86 samples from the collection site. Expected heterozygosity (H_E) across *C. ribicola* isolates was 0.38. No statistically significant differences in H_E were detected among isolates grouped by host source (range in H_E = 0.35 to 0.39; Table 1). Genetic structure (F_{ST}) among host groups was 0.01, which was not statistically significant ($p > 0.05$). However, AFLP analysis distinguished all samples except for two pairs. These identical profiles were collected from two whitebark pine samples and two *R. lacustre* samples (data not shown). Pairwise F_{ST} among isolates showed no pattern with regard to host source (matrix not shown). Principal coordinates analysis utilizing two and three principal coordinates explained only 14% and 20% of the genetic variance within the distance matrix, respectively (Fig. 1 shows two coordinates, three coordinates not shown). The distribution of isolates from each host showed no discernable pattern with respect to isolates from other hosts. The AFLP analysis did not detect genetic differences among *C. ribicola* derived from different hosts.

Inoculation experiment

Spore sources from all regions were successful in infecting and producing telia and (or) uredinia on *Pedicularis racemosa* and on the positive *R. nigrum* controls (Table 2). The negative controls did not become infected for either the detached-leaf or whole-plant experiments. No readily observable difference in infection was found between inoculations utilizing urediniospores vs. aeciospores. Four inoculum sources (HC13, NY01.03, RN22, RN32) did not produce uredinia or telia on either detached leaves or whole plants of *Pedicularis racemosa*. These same inoculum sources also showed no or little infection on *R. nigrum* and had low spore germination (<40%). Infection generally appeared less abundant on *Pedicularis racemosa* than on *R. nigrum* but was not quantified. Small plants of *Pedicularis racemosa* appeared more prone to mold damage during incubation than detached leaves.

A potential relationship between *Pedicularis racemosa* leaf age and susceptibility to rust was apparent but not quantified. Young but fully expanded leaves of *Pedicularis racemosa* typically had higher numbers of lesions than older leaves, in concordance with patterns previously observed for *Ribes* alternate hosts (Mielke 1943; Zambino 2000). However, few leaves of *Pedicularis racemosa* were susceptible to infection. Even on actively growing *Pedicularis racemosa* plants, leaves with Leaf Plastochron Index (Erickson and Michelini 1957) of only two greater than the most susceptible leaves, had few or no infections and produced only telia or, occasionally, yellow–orange infections that never erupted through the epidermis. Telia and (or) uredinia often developed near the leaf margins of *Pedicularis racemosa*, particularly on older leaves, whereas infections between the mid-vein and margins were only found on the most susceptible young-mature leaves. These patterns of susceptibility with development were not quantitatively analyzed, but similar observations have been noted in field-collected *Pedicularis racemosa*.

Discussion

The AFLP analysis and inoculation experiments support a hypothesis that the ability of *C. ribicola* to utilize *Pedicularis racemosa* is widespread throughout North America and that *C. ribicola* across North America shares a common genetic background that allows successful infection of multiple telial hosts. McDonald et al. (2006) had enumerated other possible means by which *Pedicularis* and *Castilleja* might have been acquired as hosts, had the North American *C. ribicola* population initially been *Ribes*-specific. These included undetected hybridization, introductions, mutation–recombination, and phenotypic plasticity.

Naturally occurring hybrids between *C. ribicola* and a dif-

ferent North American rust, *Cronartium comandrae* Peck (Joly et al. 2004), support the potential for hybridization. It is unknown whether *C. ribicola* can hybridize with the native North American stalactiform rust fungus, *Cronartium coleosporioides* Arthur, which also utilizes some species of Orobanchaceae as telial hosts and co-occurs with *C. ribicola* at high elevation western sites. Sequencing of the rDNA ITS region has been used to detect both intraspecific and interspecific hybrids in other fungi and fungus-like organisms such as *Armillaria* and *Phytophthora* species, respectively (Hanna et al. 2007; Man in't Veld et al. 2007). The ITS of *C. ribicola* and *C. coleosporioides* differ at 16 nucleotides. However, *C. ribicola* samples from *Pedicularis racemosa* had no evidence of hybridization that could be detected in this region (McDonald et al. 2006).

If the ability to utilize non-*Ribes* hosts had been due to novel genetic changes well after introduction and spread, evidence should have been found in the geographic pattern of their occurrence. Yet *Pedicularis*-infecting strains are not locally or regionally restricted to areas where pine hosts, the two rust species, and known alternate hosts overlap. A similar argument can be used to discount new host utilization patterns from a cryptic introduction of *C. ribicola*. A small introduction might be undetectable with molecular markers; however, it is difficult to conceive a scenario where capacity to infect *Pedicularis* from a cryptic introduction could become so widespread in both eastern and western North America within a short time.

Despite the ability of *C. ribicola* from across the continent to infect *Pedicularis racemosa* in controlled tests, an unexplored question is the potential of *Pedicularis racemosa* to contribute to pine infection. The use of a single host population in the inoculation tests limits conjecture. Though it is conceivable that *Pedicularis racemosa* from the Roman Nose Lake site is more susceptible to infection than populations of this host at other locations, a field inoculation of *Pedicularis racemosa* plants at a site 170 km south of Roman Nose Lake using local aeciospores resulted in infections that produced telia (Zambino et al. 2005; Zambino et al. 2006), demonstrating natural host susceptibility to *C. ribicola* is not highly localized. In addition, natural *C. ribicola* infection of *Pedicularis racemosa* was recently found at a site in northern California (D. Vogler, personal communication, 2006). Further studies are needed to determine if other geographic sources of *Pedicularis racemosa* display variable susceptibility or resistance to *C. ribicola*.

Analysis of *C. ribicola* isolates from different telial and aecial hosts using AFLPs revealed similar levels of H_E and no detectable genetic differentiation by host (Table 1; Fig. 1). These findings corroborate the results of the inoculation experiments and those from recent greenhouse and field inoculations studies of *C. ribicola*. In a recent host-transfer experiment, aeciospores collected from whitebark pine were used to inoculate *Pedicularis racemosa* and *Castilleja miniata*. Urediniospores produced on *Pedicularis racemosa* were then transferred to *R. nigrum*. Telia produced from these infections germinated well and produced basidiospores that successfully infected western white pine seedlings (McDonald et al. 2006).

Historical reports of other *Cronartium* species occurring on North American Orobanchaceae as hosts may now need re-examination. The native *C. coleosporioides* (the stalactiform rust fungus) is reported to infect some members of *Castilleja*, *Melampyrum*, *Pedicularis*, and *Rhinanthus* species (Shaw 1973). However, infections and teliospore structures of *C. coleosporioides* are morphologically indistinguishable from those of *C. ribicola*, and artificial inoculations and genetic characterization have not been performed. At present, *C. coleosporioides* has not been reported on *Pedicularis racemosa*. Although sequence analysis of herbarium specimens represents an appealing approach to determine if *C. ribicola* utilization of *Pedicularis* and *Castilleja* species went unnoticed in North America, a large-scale survey of herbarium specimens is impractical because few specimens from these hosts are available from areas with high incidence of white-pine blister rust. To address the question of whether some infections previously identified as *C. coleosporioides* may have been *C. ribicola*, telia from two infected herbarium specimens, from 1959 and 1969, originally identified as *C. coleosporioides*, were used in PCR reactions to obtain sequences of the internal transcribed spacer of the ribosomal DNA (Richardson 2006). Sequences from both herbaria specimens matched available GenBank *C. coleosporioides* sequences (Vogler and Bruns 1998) from field-collected aeciospores.

Phylogenetic analysis of the Orobanchaceae may aid in determining clusters of species to examine for host susceptibility. Fortunately, some phylogenetic studies of this family have been conducted (Ree 2005; Wolfe et al. 2005; Bennett and Mathews 2006) and some host relationships in Asia are known. For example, in South Korea and Japan, several *Pedicularis* species are believed to be predominant hosts. In Korea, *Pedicularis resupinata* is the major host (La and Yi 1976), whereas in Japan several *Pedicularis* species have been shown to be susceptible (Yokota and Uozumi 1976). Phylogenetic relationships between Asian, European, and North American *Pedicularis* species may provide guidance for research efforts.

The ecological roles of *Pedicularis* and *Castilleja* in the life cycle of *C. ribicola* might range from critically important to insignificant, depending on ecosystem and locale, aecial and telial host phenology in relation to *C. ribicola* sporulation, and proximity of aecial and telial hosts. Because *Pedicularis* and *Castilleja* are generally more abundant in high-elevation ecosystems, it can be speculated that these telial hosts might have the greatest impact on white-pine blister rust of high-elevation, five-needled, haploxylon pines (e.g., whitebark pine and limber pine, *Pinus flexilis* James).

Results of this study should also be considered in an evolutionary and ecological context. Development of host specialization by *C. ribicola* populations is likely site- and time-dependent. This pathogen has been present in North America for a relatively short time but shows a great plasticity in host species utilization. At the Roman Nose Lake site, at least five telial hosts are present; however, non-*Ribes* telial hosts are more prevalent. Perhaps host specialization confers no major evolutionary advantage to *C. ribicola* populations in specific sites and environments where multiple hosts occur. Nevertheless, co-evolutionary conditions that favor host specialization in *C. ribicola* through genetic selection and (or) accommodation may occur over longer time

spans, especially in areas with limited co-occurrence of diverse telial or aecial hosts (West-Eberhard 2005; Suzuki and Nijhout 2006).

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References

Bennett, J.R., and Mathews, S. 2006. Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. Am. J. Bot. **93**: 1039–1051.

Erickson, R.O., and Michelini, F.J. 1957. The plastochron index. Am. J. Bot. **44**: 297–304. doi:10.2307/2438380.

Hamelin, R.C., Hunt, R.S., Geils, B.W., Jensen, G.D., Jacobi, V., and Lecours, N. 2000. Barrier to gene flow between eastern and western populations of *Cronartium ribicola* in North America. Phytopathology, **90**: 1073–1078.

Hanna, J.W., Klopfenstein, N.B., Kim, M.-S., McDonald, G.I., and Moore, J.A. 2007. Phylogeographic patterns of *Armillaria ostoyae* in western United States. For. Pathol. **37**. In press.

Hei, W., Hou, L.-B., Liu, X.-Y., and Yang, Z.-Z. 2003. The ITS region sequence analysis of Chinese pine stem rusts. *In* Proceedings Second International Union of Forest Research Organizations (IUFRO) Rusts of Forest Trees WP conference, 19–23 August 2002, Yangling, China. *Edited by* X. Mei-Qing, J.A. Walla, and W-X. Zhao. Chinese Academy of Forestry, Yangling, China. pp. 146–152.

Hiratsuka, Y., and Maruyama, P.J. 1976. *Castilleja miniata*: a new alternate host of *Cronartium ribicola.* Plant Dis. Rep. **60**: 241.

Hunt, R.S. 1984. Inoculations of Scrophulariaceae with *Cronartium ribicola.* Can. J. Bot. **62**: 2523–2524.

Hunt, R.S. 2003. White pine blister rust. Recent Res. Dev. Mycol. **1**: 73–85.

Hyun, S.K., and Koo, Y.B. 1981. Possibility of breeding blister rust resistant synthetic clones of Korean pine (*Pinus koraiensis* S. et Z.). *In* Proceedings 17th IUFRO World Congress, Division II, 6–17 September 1981, Kyoto, Japan. pp. 239–245.

Jaccard, P. 1908. Nouvelles rescherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. **44**: 223–270.

Joly, D.L., Langor, D.W., Weber, J.D., and Hamelin, R.C. 2004. *Cronartium* ×*flexili notho* sp. nov., a white pine blister rust hybrid between *Cronartium comandrae* and *Cronartium ribicola* on limber pine in Alberta. Can. J. Plant Pathol. **26**: 415 [abstr.].

Kinloch, B.B., Jr., and Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. Plant Dis. **65**: 604–605.

Kinloch, B.B., Jr., Westfall, R.D., White, E.E., Gitzendanner, M.A., Dupper, G.E., Foord, B.M., and Hodgskiss, P.D. 1998. Genetics of *Cronartium ribicola*. IV. Population structure in western North America. Can. J. Bot. **76**: 91–98. doi:10.1139/cjb-76-1-91.

Kinloch, B.B., Jr., Sniezko, R.A., and Dupper, G.E. 2004. Virulence gene distribution and dynamics of the white pine blister rust pathogen in western North America. Phytopathology, **94**: 751–758.

La, Y.J., and Yi, C.K. 1976. New developments in the white pine blister rust of Korea. *In* Proceedings 16th IUFRO World Congress, Division II, 20 June – 2 July 1976, Oslo, Norway. Norwegian Forest Research Institute, Ås, Norway. pp. 344–353.

Lynch, M., and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. **3**: 91–99. PMID:8019690.

Maloy, O.C. 1997. White pine blister rust control in North America: a case history. Annu. Rev. Phytopathol. **35**: 87–109. doi:10.1146/annurev.phyto.35.1.87. PMID:15012516.

Man in't Veld, A.M., de Cock, W.A., and Summerbell, R.C. 2007. Natural hybrids of resident and introduced *Phytophthora* species proliferating on multiple new hosts. Eur. J. Plant Pathol. **117**: 25–33.

McDonald, G.I. 2000. Geographic variation of white pine blister rust aeciospore infection efficiency and incubation period. Hortechnology, **10**: 533–536.

McDonald, G.I., and Hoff, R.J. 2001. Blister rust: an introduced plague. *In* Whitebark pine communities: ecology and restoration. *Edited by* D.F. Tomback, S.F. Arno, and R.E. Keane. Island Press, Washington, D.C. pp. 193–200.

McDonald, G.I., Hansen, E.M., Osterhaus, C.A., and Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Dis. **68**: 800–804.

McDonald, G.I., Zambino, P.J., and Klopfenstein, N.B. 2005. Naturalization of host-dependent microbes after introduction into terrestrial ecosystems. *In* Forest pathology: from genes to landscapes. *Edited by* J.E. Lundquist and R.C. Hamelin. APS Press, St. Paul, Minn. pp. 41–58.

McDonald, G.I., Richardson, B.A., Zambino, P.J., Klopfenstein, N.B., and Kim, M.-S. 2006. *Pedicularis* and *Castilleja* are natural hosts of *Cronartium ribicola* in North America: a first report. For. Pathol. **36**: 73–82.

Mielke, M. 1943. White pine blister rust in western North America. Bull. 52, Yale University, School of Forestry, New Haven, Conn.

Nei, M. 1978. Estimation of average heterozygosities and genetic distance from a small number of individuals. Genetics, **89**: 583–590. PMID:17248844.

Patton, R.F. 1972. Inoculation methods and problems in testing eastern white pine for resistance to *Cronartium ribicola. In* Biology of Rust Resistance in Forest Trees. NATO–IUFRO Advanced Study Institute: Proceedings, 17–24 August 1969, Moscow, Idaho. USDA Misc. Pub. No. 1221. pp. 373–385

Patton, R.F., and Spear, R.N. 1989. Histopathology of colonization in leaf tissue of *Castilleja, Pedicularis, Phaseolus*, and *Ribes* species by *Cronartium ribicola.* Phytopathology, **79**: 539–547.

Ree, R.H. 2005. Phylogeny and the evolution of floral diversity in *Pedicularis* (Orobanchaceae). Int. J. Plant Sci. **166**: 595–613. doi:10.1086/430191.

Remington, D.L., Whetten, R.W., Liu, B.-H., and O'Malley, D.M. 1999. Construction of an AFLP genetic map with nearly complete genome coverage in *Pinus taeda.* Theor. Appl. Genet. **98**: 1279–1292. doi:10.1007/s001220051194. PMID:12238515.

Richardson, B.A. 2006. Population genetics of *Cronartium ribicola* in western North America: assessing ongoing genetic change due to diverse hosts and environments. Ph.D. thesis, Department of Plant Pathology, Washington State University, Pullman, Wash. pp. 71–101.

Rohlf, F.J. 1998. NTSYS-pc. Numerical taxonomy and multivariate analysis system, ver. 2.00. Exeter Software, Setauket, N.Y.

Shaw, C.G. 1973. Host fungus index for the Pacific Northwest—II. Fungi. Washington Agriculture Experimental Station, Bulletin No. 766, Washington State University, Pullman, Wash.

Spaulding, P.C. 1922. Investigations of the white pine blister rust. USDA Bulletin No. 957, Washington, D.C.

Stephan, B.R., and Hyun, S.K. 1983. Studies on the specialization of *Cronartium ribicola* and its differentiation on the alternate hosts *Ribes* and *Pedicularis*. Z. Pflanzenkr. Pflanzenschutz, **90**: 670–678.

Suzuki, Y., and Nijhout, H.F. 2006. Evolution of a polyphenism by genetic accommodation. Science (Washington, D.C., **311**: 650–652. doi:10.1126/science.1118888. PMID:16456077.

West-Eberhard, M.J. 2005. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. U.S.A. **102**: 6543–6549. doi:10.1073/pnas.0501844102. PMID:15851679.

White, E.E., Foord, B.M., and Kinloch, B.B., Jr. 1996. Genetics of *Cronartium ribicola*. II. Variation in the ribosomal gene cluster. Can. J. Bot. **74**: 461–468.

Wolfe, A.D., Randle, C.P., Liu, L., and Steiner, K.E. 2005. Phylogeny and biogeography of Orobanchaceae. Folia Geobot. **40**: 115–134.

Vekemans, X. 2002. AFLP-SURV version 1.0. *Distributed by* X. Vekemans, Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Vogler, D.R., and Bruns, T.D. 1998. Phylogenetic relationships among the pine stem rust fungi (*Cronartium* and *Peridermium* spp.). Mycologia, **90**: 244–257. doi:10.2307/3761300.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414. doi:10.1093/nar/23.21.4407. PMID:7501463.

Yokota, S.-I., and Uozumi, T. 1976. New developments in white pine blister rusts in Japan. *In* Proceedings 16th IUFRO World Congress, Division II, 20 June – 2 July 1976, Oslo, Norway. Norway Forest Research Institute, ?s, Norway. pp. 330–342.

Zambino, P.J. 2000. Evaluating white pine blister rust resistance in *Ribes* after artificial inoculation. Horttechnology, **10**: 544–545.

Zambino, P.J. 2002. Dry grinding at near-ambient temperatures for extracting DNA from rust and other fungal spores. Biotechniques, **33**: 48–51. PMID:12139255.

Zambino, P.J., Echt, C., Pijut, P., and Michler, C. 1997. Desiccation, storage temperature, and heat shock affect germination of *Cronartium ribicola* urediniospores, aeciospores, and teliospores. Inoculum, **48**: 42.

Zambino, P.J., McDonald, G.I., Richardson, B.A., Klopfenstein, N.B., and Kim, M.-S. 2005. Natural infection of *Pedicularis* and *Castilleja* spp. by the white pine blister rust fungus *Cronartium ribicola* in North America. Phytopathology, **95**: S116.

Zambino, P.J., Richardson, B.A., McDonald, G.I., Klopfenstein, N.B., and Kim, M.-S. 2006. A paradigm shift for white pine blister rust: non-*Ribes* alternate hosts of *Cronartium ribicola* in North America. *In* Proceedings of the 53rd Western International Forest Disease Work Conference, 24–28 September 2005, Jackson Hole, Wyoming. *Compiled by* J. Guyon. pp. 161–163.

Zambino, P.J., Richardson, B.A., and McDonald, G.I. 2007. First report of the white pine blister rust fungus *Cronartium ribicola* on *Pedicularis bracteosa*. Plant Dis. **91**: 467.

Zhivotovsky, L.A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Mol. Ecol. **8**: 907–913. doi:10.1046/j.1365-294x.1999.00620.x. PMID:10434412.