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Thanks everyone for your help, from your Region Four Host Team: Left to right, John Guyon, Laura Dunning, Valerie DeBlander and Phil Mocettini. Photo by Schwandt

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A Paradigm Shift for White Pine Blister Rust: Non-*Ribes* Alternate Hosts of *Cronartium ribicola* in North America

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Abstract—Naturally occurring *Cronartium ribicola* infections were discovered in August and September, 2004 on *Pedicularis racemosa* and *Castilleja miniata* in a mixed stand of white pine blister rust-infected whitebark pine (*Pinus albicaulis*) and western white pine (*P. monticola*) in northern Idaho, at Roman Nose Lakes, ca 30 km west of Bonners Ferry. Infections were confirmed by ITS rDNA sequencing of teliospores. The ability of these species to act as host was confirmed by laboratory inoculations using aeciospores from whitebark pine. Isolates recovered from *P. racemosa* after artificial inoculations were able to infect *Ribes nigrum* and were thus not specific to an alternate host genus, and also infected western white pine seedlings. *Cronartium ribicola* was detected on additional *P. racemosa* and *C. miniata* at the site in 2005. Infections were confirmed by ITS sequencing and transfer to *R. nigrum*. In 2005, exposure of local plants to local inoculum at a second site, ca 200 km to the south, caused infections on *P. racemosa*, and laboratory inoculations implicated *C. rhexifolia* from this site as a third non-*Ribes* alternate host. Identification of these alternate hosts may significantly alter our concepts of blister-rust hazard and epidemiology, particularly for those upper montane to subalpine stands where the newly identified hosts are abundant. The use of these hosts by the introduced pathogen also raises the possibility that fungal adaptation may be one factor allowing change in this dynamic and evolving pathosystem.

Introduction

Until 2004, *Ribes* spp. (Grossulariaceae) were the only known natural alternate hosts of *Cronartium ribicola* in North America (McDonald and others In Press). Other plant species (*Pedicularis* and *Castilleja*; Orobanchaceae) are alternate hosts for some strains or species of fungi causing blister rust of five-needled pines in Asia but not in Europe (Vogler

and Bruns 1998; review in McDonald and others 2005), which is the presumed source for North American blister rust (Leppik 1970; review in Hunt 2003). Hiratsuka and Maruyama (1976) infected and obtained teliospores from *C. miniata* artificially inoculated with aeciospores from British Columbia, Canada. In contrast, other field and greenhouse inoculations using several species of Orobanchaceae and different sources of aeciospores did not infect non-*Ribes* hosts (Hunt 1984) or did not cause sporulation (Patton and Spear 1989). Natural infections of non-*Ribes* alternate hosts by *C. ribicola* had never been found in North America prior to 2004 (McDonald and others In Press).

This paper reports on studies that were conducted in 2004 (McDonald and others In Press) and 2005 on potential non-*Ribes* alternate hosts, following the discovery in late August, 2004 of telia typical of the genus *Cronartium* on four species of Orobanchaceae (*Pedicularis racemosa*, *P. bracteosa*, *Castilleja miniata*, and *C. rhexifolia*). These infections were in a mixed stand of white pine blister rust-infected whitebark pine (*Pinus albicaulis*) and western white pine (*P. monticola*) in northern Idaho (48.634109°N, 116.570817°W, elevation 1800 m), near the Roman Nose Lakes Campground, ca 30 km west of Bonners Ferry. *Cronartium ribicola* infections were confirmed for some non-*Ribes* species raising issues of the role of these alternate hosts to white-pine-blister-rust pathosystems of North America.

Materials and Methods

2004 Studies

1. To identify whether infections of *Ribes hudsonianum* var. *petiolare*, *P. racemosa*, *P. bracteosa*, *C. miniata*, and *C. rhexifolia* at the Roman Nose site were caused by *C. ribicola*, *C. coleosporioides*, or a hybrid species. *Cronartium* teliospores from natural infections were tested by direct PCR / DNA sequencing of ITS rDNA using ITS1+ITS4 primers (McDonald and others In Press).

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- Plants of suspected alternate hosts and control *R. nigrum* cuttings (Heimburger clone) were artificially inoculated (conditions for infection: 100 RH at 19 C for 48 hr) (McDonald and others In Press). *Pedicularis racemosa* and *P. bracteosa* were inoculated with three sources of aeciospores from on-site whitebark pine; *C. miniata* (two plants, from a location near Coeur d'Alene, ID) were inoculated with one of the sources.
- To test if pathogenicity is retained to *Ribes* and five-needled pines, urediniospores from lab-inoculated *P. racemosa* were used to inoculate *R. nigrum* cuttings (conditions for infection: 100 RH at 20° C for 24 hr), and teliospores from those cuttings were used to infect seedlings of western white pine in the cotyledon stage (conditions for infection: 100 RH at 16° C for 48 hr after basidiospores were cast) (McDonald and others In Press).
- To test for regular meiosis, germinating teliospores were examined for evidence of four meiotic products using scanning electron microscopy (McDonald and others In Press).
- The 11 natural infections tested from *P. racemosa* were all *C. ribicola*. This plant species had frequent infections in both years, and has a morphology that closely resembles the Asian alternate host, *P. resupinata*.
- The five *P. bracteosa* infections tested were *C. coleosporioides*.
- Castilleja miniata* hosted both rusts, with two of seven infections being *C. ribicola*.
- Castilleja rhexifolia* was identified as a host by laboratory inoculation. The infected plant produced urediniospores and teliospores identified as *C. ribicola* by ITS sequencing. The one natural infection tested was of *C. coleosporioides*.
- All aeciospores sources from Roman Nose whitebark pine produced uredinia and telia on potted *P. racemosa* and *C. miniata*, and on leaf cuttings of *R. nigrum*.
- Pedicularis*-infecting isolates grown to produce uredinia and telia on *R. nigrum* caused infections on western white pine seedlings, indicating that these isolates lack specificity to alternate host genus and retain the capacity to complete their lifecycle.
- Scanning electron microscopy indicated that germinating teliospores bear four sterigmata and basidiospores, suggesting that meiosis is regular and that nuclei migrate to different basidiospores, as expected for *C. ribicola* and other macrocyclic, heterothallic rusts.
- Pedicularis racemosa* at the site near Clarkia that had been dusted with natural spores became infected with *C. ribicola*, as confirmed by ITS sequencing, so the ability of this rust fungus to use this host is not restricted to the Roman Nose site (near Bonners Ferry, ID).
- Most field-inoculated plants of species other than *P. racemosa* were lost from various causes, so ability of *C. ribicola* to infect these hosts could not be determined. Plants in laboratory inoculations also had high mortality, indicating that optimal conditions for plant maintenance and inoculation/infection had not been obtained. More studies will be needed to evaluate regional differences in blister rust infection of potential non-*Ribes* alternate hosts.

2005 Studies

- Samples of rust from additional plants of *R. hudsonianum* var. *petiolare*, *P. bracteosa*, and *C. miniata* were sequenced.
- Samples of rust from *P. racemosa* from Roman Nose were used for direct inoculation of *R. nigrum*, to recover strains for further DNA characterization.
- In June, aeciospores from western white pine from a second site (Freezeout Saddle, 47.00885°N, 11600.846°W; near Clarkia, ID) were dusted onto local plants of *P. racemosa*, *P. bracteosa*, *C. rhexifolia*, and *C. occidentalis* to allow natural infection processes.
- Plants of *P. racemosa*, *P. bracteosa*, *C. rhexifolia*, and *C. occidentalis* from the site near Clarkia were removed from the field and inoculated with *C. ribicola* under laboratory conditions.

Results

- All rust collections were either *C. ribicola* or *C. coleosporioides*, with no hybrids detected at 27 ITS sites that differ between the species.

11. For the successful *C. miniata* and *C. rhexifolia* inoculations, only one to two leaves or floral bracts of similar age out of the entire plant showed infection.

Discussion

These studies have demonstrated that non-*Ribes* species are being used as alternate hosts at an upper montane site with a short, cool growing season. The use of *Pedicularis* and *Castilleja* spp. as alternate hosts in North America could have a major impact on our understanding of disease epidemiology, site-specific risks for pine infection, and naturalization/adaptation in blister-rust pathosystems. Assessment of the importance of these alternate hosts will depend on geographic distributions of such non-*Ribes* alternate hosts, rates of infection, environmental conditions and host developmental physiology that affect windows for infection, and interactions with genetic structure of *C. ribicola*. Testing interactions with rust populations will require artificial inoculations; however, short windows of receptivity of tissues to infection and other characteristics of species in the hemiparasitic Orobanchaceae may make such studies difficult. Negative results from inoculations are not necessarily conclusive.

The lack of previous reports of natural alternate hosts in the Orobanchaceae and the mixed results of previous attempts at artificial inoculation of these species (Hiratsuka and Maruyama 1976; Hunt 1984; Patton and Spear 1989) could have been caused by differences in environmental or experimental conditions, genetic differentiation of rust and/or host populations, local adaptation via either standard genetic mechanisms or phenotypic plasticity, or even a separate, cryptic introduction. Also, prior to the advent of easy identification by molecular techniques, some *C. ribicola* infections on Orobanchaceae may have been mistakenly ascribed to *C. coleosporioides* (see Farr and others 1995 for listed hosts for this species).

ITS sequence data provided no evidence that hybridization between species was the source for *C. ribicola* that utilizes the Orobanchaceae; however, introgression with *C. ribicola* could mask past hybridization events with other *Cronartium* species. In contrast, Joly and others (2004) and Hamelin and others (2005) have demonstrated that hybrid aecia between *C. ribicola* and *C. comandrae* (the Comandra rust pathogen) occur on limber pine in British Columbia, although they have not determined

alternate hosts for these natural hybrids, nor their persistence in nature.

Our reported detection of *C. ribicola* on Orobanchaceae, the finding of natural hybrids between *C. ribicola* and a rust of hard pines (Joly and others 2004; Hamelin and others 2005), and recent expansion of blister rust into new pine hosts and environments indicate that blister-rust pathosystems in North America are dynamic. The blister-rust fungus could be changing and adapting by multiple mechanisms, even as hosts are responding to the presence of the recently introduced pathogen. We expect that the current report is only the first of many shifts in the blister-rust paradigm that will be revealed through more critical examination of rust and host populations, the responses of blister-rust fungi to different environments, and genetic and epigenetic mechanisms of adaptation in *C. ribicola* and its hosts.

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