Geographic Variation of White Pine Blister Rust Aeciospore Infection Efficiency and Incubation Period

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SUMMARY. Frequency of infection, main effects and interactions among four geographic sources of white pine blister rust (WPBR) (*Cronartium ribicola* J.C. Fisch.) and 10 *Ribes*-sites (*Ribes* L. species $\times$ site combinations) was investigated using leaf disk assay. Two clones, one of *R. hudsonianum* (Dougl.) Jancz. and one of *R. viscosissimum* Prush., were not infected by any sources of WPBR. One clone of *R. viscosissimum* that was not infected by two sources of WPBR was susceptible to the other sources. Highly significant WPBR source $\times$ *Ribes*-site analysis of variance interaction for incubation period and infection efficiency precluded testing main effects. Profile plots of incubation period interaction means showed orderly interaction by all WPBR sources and plots of infection efficiency showed that aggressively virulent WPBR from Oregon (Champion Mine) ranked near or at the bottom for infection efficiency for all *Ribes*-sites. Meanwhile, aggressively virulent WPBR from Idaho (Merry Creek) ranked near the bottom for infection efficiency when inoculated onto *Ribes* obtained from the Cascade Mountains but switched to the highest ranking when inoculated onto *Ribes* obtained from Idaho. Geographic interaction of white pine blister rust and *Ribes* for incubation period and infection efficiency may help to explain geographic patterns recently observed in WPBR molecular markers.

White pine blister rust is a governing force in western North American forest ecosystems supporting five-needle pines (*Pinus* L.) (Neuenschwander et al., 1999). Resistant populations of western white pine, *Pinus monticola* Dougl., selected for north Idaho remain effective (Bingham, 1983). Hazard varies across the landscape and susceptible western white pine can be grown on low-hazard sites (Hagle et al., 1989). One Oregon race of WPBR, Champion Mine, is more aggressively virulent on one resistant population of western white pine than wild-type WPBR (McDonald et al., 1984, Kinloch et al., 1999). In Idaho, the experimental Merry Creek plantation of resistant western white pine has experienced higher than expected rates of infection (McDonald and Dekker-Robertson, 1998) and may harbor another aggressively virulent race. Integrated WPBR management programs are one way to manage these complexities (Hagle et al., 1989).

An epidemiological simulator was developed to facilitate interpretation of complex site and weather interactions (McDonald et al., 1981).

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When parameterized to simulate local WPBR epidemics on various *Ribes* species and clones, WPBR sources, and weather conditions, the simulator produced large variations in WPBR behavior (McDonald, 1996). Adaptation and/or acclimation of plant pathogenic fungi can create epidemiological fitness phenotypes showing modified ability to cause disease. Such phenotypic variation is known for stem rust of wheat, *Puccinia graminis* Pers. F. sp. *tritici* Eriks. & E. Henn., (Line and Bugbee, 1964), southern leaf blight of corn, *Helminthosporium maydis* Nisikado & Miyake, (Hill and Nelson, 1976), and late blight of potato *Phytophthora infestans* (Mont.) de Bary, (Mizubuti and Fry, 1998).

Both WPBR source $\times$ *Ribes* clone interaction (McDonald and Andrews, 1981) and genetic variation of epidemiological fitness traits (McDonald and Andrews, 1982) were demonstrated for WPBR. Since *Ribes* $\times$ WPBR $\times$ site interactions must be understood to facilitate integrated management, a *Ribes* $\times$ WPBR geographic cross inoculation experiment was designed to determine influence of 1) *Ribes*-sites (*Ribes* species $\times$ site combinations) and 2) WPBR geographic source on frequency of infection, incubation period and infection frequency in the WPBR/*Ribes* pathosystem.

Materials and methods

In Fall 1981, 30 clones of *Ribes* were sampled in Oregon. *Ribes lacustre* (Pers.) Poir., *R. sanguineum* Prush, and *R. bracteosum* Dougl. supplied five clones each from two sites, Champion Mine, located about 16 miles (25.8 km) east of Cottage Grove, and Still Creek, located about 5 miles (8 km) northwest of Government Camp. In Idaho, 20 clones were sampled. *Ribes viscosissimum* and *R. lacustre* were collected at the Priest River Experimental Forest, located about 15 miles

(24.2 km) northeast of Priest River. The west fork of Merry Creek, located about 5 miles northwest of Clarkia, supplied clones of *R. hudsonianum* and *R. viscosissimum*. The 50 clones were propagated at Moscow, Idaho.

In Spring 1982, leaves were removed 21 d after emerging, from each of the 50 clones. Removed leaves were washed for 24 h in tap water. Leaf disks 1.54 cm² (0.23 inches²) in area were cut and stored at 5 °C (41 °F) for a maximum of 3 d. Six leaf disks (21 to 24 d old) from one clone and a microscope slide cover slip (spore trap) were placed in each petri dish on wet filter paper for inoculation. Disks were misted with sterile water after a set of 50 dishes was prepared. Dishes were placed at random on the floor of an inoculation chamber (2 $\times$ 2 $\times$ 2 ft (60 cm)). The chamber was constructed of fiber-glassed 0.25-inch (6.35-mm) plywood, except for the lid, which was 0.5-inch (12.7-mm) plexiglas. The lid was sealed to the chamber with wing nuts and a rubber gasket.

In the spring of 1982, bulked aeciospores were collected from five trees at each of the four sites. Each group of 50 plates (five clones $\times$ 10 *Ribes*-sites) were inoculated, with each WPBR source within 3 d of collection, to give 200 clone $\times$ site $\times$ WPBR source combinations. Measured amounts of dry talc and dry spores were thoroughly mixed to ensure deposition of single spores at the targeted rate of 50 spores/cm² (323 spores/inch²) of leaf surface. Dry talc/spore mix placed in a shell vial was in turn placed inside an erlenmeyer flask. A glass tube, inserted through a rubber stopper, was placed in the mouth of the flask and inserted into the shell vial at the bottom of the flask on one end and into the bottom of chamber on the other. The chamber, flask, and spore laden shell vial were placed under a 2-inch (5-cm) vacuum. A 2-qt

(2-L) air tank was attached to the flask via tubing and a valve. This tank was pumped to 3.5 lb/inch² (24 kPa) of ambient air. Opening the valve between the air tank and the evacuated chamber accomplished the inoculation. Ambient air exploded the talc/spore mix onto the lid of the chamber and the mix was left to settle onto the leaf disks for 2 h. Each inoculation was repeated four times to yield a total experiment of 4800 inoculated leaf disks.

After inoculation, spore traps were removed, disks were again misted and plates were sealed with several wraps of parafilm. They were incubated at 21 °C (69.8 °F) under ambient light in a temperature-controlled chamber constructed of glass and plastic sheeting and located inside a green house. Plates were then inspected daily for 30 d. Spore deposition was counted in 10 microscope fields at 200 $\times$ to estimate spores cast per dish (spores/cm²). Infections were recorded as macroscopic signs appeared. These data were used to compute numbers of infections per disk. Number of days from inoculation to appearance of each lesion was recorded (incubation period). Infection efficiency (infections/spore) was computed from the spore and lesion counts.

For the analysis of variance reported here (Systat 9, SPSS Inc., Chicago, Ill.), clones within *Ribes*-sites were pooled and dish averages were computed. The statistical model assumed that WPBR sources and *Ribes*-sites were fixed because numbers of locations were deemed inadequate to attribute results to the entire population. The formula for this model was

$$X_{ijkl} = m + C_i + S_j + R_k + (CS)_{ij} + (CSR)_{ijk} + e_{ijkl} \quad [1]$$

where X = incubation period or infection efficiency on the *l*th dish from the *i*th WPBR source, *j*th *Ribes*-site; *k*th replication, m = general mean; C_i = effect of the *i*th WPBR source; S_j =

Table 1. Analysis of variance of leaf-disk assays of incubation period of four geographic sources of white pine blister rust (WPBRs) inoculated onto 10 combinations of site and *Ribes* species (RSS)

Source	Sum of squares	df	Mean square	F ratio	P
Replication (R)	13.018	3	4.339	2.635	0.055
WPBRs	30.171	3	30.171	18.319	0.0005
RSS	1271.775	9	141.308	85.797	0.0005
RSS $\times$ WPBRs	206.634	27	7.653	4.647	0.0005
R $\times$ RSS $\times$ WPBRs	133.407	81	1.647		

Table 2. Analysis of variance of leaf-disk assays of $\ln(\text{infection efficiency} \times 10000)$ of four geographic sources of white pine blister rust (WPBR) inoculated onto 10 combinations of site and *Ribes* species (RSS).

Source	Sum of squares	df	Mean square	F ratio	P
Replication (R)	11.247	3	3.749	10.635	0.0005
WPBR	41.612	3	13.871	39.069	0.0005
RSS	132.975	9	14.775	41.617	0.0005
RSS $\times$ WPBR	23.667	27	0.877	2.469	0.001
R $\times$ RSS $\times$ WPBR	28.757	81	0.355		

effect of the j th *Ribes*-site; R_k = effect of the k th replication; $(CS)_{ij}$ = effect of the interaction of the i th WPBR source and the j th *Ribes*-site; $(CSR)_{ijk}$ = the effect of the interaction of the i th WPBR source, the j th *Ribes*-site and the k th replication, and e_{ijkl} = the effect of an individual plate. Normal probability plots of plate averages for incubation period and $\ln(\text{infection efficiency} \times 10,000)$ were nearly linear, indicating normal distributions.

Results and discussion

Nearly 98% of all leaf disks from all 15 clones of *R. bracteosum*, *R. sanguineum*, and *R. lacustre* became infected. One of five *R. hudsonianum* clones was immune to all WPBR sources. One clone of Merry Creek *R. viscosissimum* developed a single pustule when inoculated with the Priest River source and remained rust-free with the other sources of WPBR. One Priest River clone of *R. viscosissimum* was rust-free in combination with Champion Mine WPBR, while developing one pustule in combination with Priest River spores. This clone showed infections on 90% of the leaf disks inoculated with Merry Creek and Still Creek aeciospores. Plate means of incubation period computed from combinations with > two disks/plate infected (Table 1) and transformed infection efficiency computed on the basis of all plates (Table 2) showed highly significant main effects and interaction when tested with the three-way interaction term. Such interaction precludes testing significance of the main effects of WPBR source and *Ribes*-sites. Profile plots of the interaction means were constructed to visualize the *Ribes*-site interactions (Ott, 1988). Profiles of incubation period and transformed infection efficiency interaction showed a tendency toward a Cascade Mountains versus northern Rocky Mountains dichotomy (dotted

versus solid lines in Fig. 1A and B). Aggressively virulent sources did not separate for incubation period (solid boxes together in Fig. 1A) across all *Ribes*-sites. However, the infection efficiency response of the aggressively virulent Merry Creek WPBR source switched from a low ranking on *Ribes* from the Cascade Mountains to a high

ranking on *Ribes* from northern Idaho while the wild type source from Priest River remained low (Fig. 1B). Some specific combinations separated. For incubation period, the Still Creek *R. sanguineum* $\times$ Priest River WPBR and Champion Mine *R. lacustre* $\times$ Champion Mine WPBR combinations ranked longer incubation periods than their

Fig. 1. Profile plots of means of incubation period (A) and infection efficiency (B) where geographic sources of blister are Champion Mine (CM), Merry Creek (MC), Priest River (PR), and Still Creek (SC) and *Ribes* species $\times$ site combinations are CM *R. bracteosum* (CMB), SC *R. bracteosum* (SCB), CM *R. sanguineum* (CMS), SC *R. sanguineum* (SCS), CM *R. lacustre* (CML), SC *R. lacustre* (SCL), PR *R. lacustre* (PRL), MC *R. hudsonianum* (MCH), MC *R. viscosissimum* (MCV), and PR *R. viscosissimum* (PRV).

companion combinations did (Fig. 1A). The Still Creek *R. lacustre* x Merry Creek WPBR combination ranked the shortest incubation periods in its group (Fig. 1A). Combinations of Still Creek WPBR with almost all *Ribes*-sites showed high infection efficiency ranking, while combinations of Champion Mine WPBR with most of the *Ribes*-sites showed the lowest infection efficiency ranking of all the combinations (Fig. 1B).

Analysis of inoculated leaf disks indicated the WPBR-*Ribes* pathosystem is geographically dynamic and demonstrated the potential for many kinds of environmental as well as host-based genetic adjustments. There are two potential downsides of leaf-disk assay. First, wounded tissue may release an oxidative burst than can interfere with normal functioning of resistance mechanisms (Dempsey et al., 1999). Second, systemically acquired resistance, that is whole-plant resistance induced by epiphytic, endophytic, or pathogenic relationships anywhere on a plant, is known to be an important source of resistance in many pathosystems (Sturz et al., 2000). These kinds of resistance are not known in the WPBR-*Ribes* pathosystem but wounding and systemically acquired resistances are potential problems for any inoculation test that relies on detached plant parts. Nevertheless, leaf disk assays conducted under temperature gradients imposed during spore germination, infection establishment, and infection development should lead to designation of putative environmental races. The experiment described in this report should also be repeated over time on a fixed set of clones to test for WPBR founder effects (see Kinloch et al., 1998 for discussion of founder effects). Such new experiments may provide an ecological basis to help explain patterns of molecular variation observed in populations of WPBR from eastern (Et-touil et al., 1999) and western North America (White et al., 1996). Since the WPBR/western white pine pathosystem is also geographically dynamic (Meagher and Hunt, 1999), geographic issues must be addressed to develop sound management strategies.

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