Factors Influencing Epidemiology and Management of Blackberry Rust in Cultivated *Rubus laciniatus*

K. B. Johnson, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331; and W. F. Mahaffee, United States Department of Agriculture–Agricultural Research Service Horticultural Crops Research Laboratory, Corvallis, OR

ABSTRACT

Johnson, K. B., and Mahaffee, W. F. 2010. Factors influencing epidemiology and management of blackberry rust in cultivated *Rubus laciniatus*. Plant Dis. 94:581–588.

The blackberry rust pathogen *Phragmidium violaceum* was first observed in Oregon in spring 2005 on both commercially cultivated *Rubus laciniatus* (Evergreen blackberry) and naturalized *R. armeniacus* (Himalayan blackberry). Several commercial plantings suffered severe economic losses. In 2006 to 2008, all five spore stages of this autoecious, macrocyclic rust pathogen were observed annually, and asexual perennation of the pathogen on old leaves or in leaf buds was not evident in the disease cycle. In field experiments, teliospore germination and infection by basidiospores occurred mostly during April. On potted “trap” plants exposed for periods of 1 week under dense collections of dead leaves bearing teliospores, basidiospore infection was associated with wetness durations of >16 h with mean temperatures >8°C. Trap plants placed under the bundles of collected leaves frequently developed spermatogonia, whereas only 1 of 630 trap plants placed in a production field of *R. laciniatus* became diseased, an indication that the effective dispersal distance of basidiospores may be limited. In growth chambers programmed for constant temperatures of 5, 10, 15, 20, 25, and 30°C, a minimum of six continuous hours of leaf wetness was required for infection by urediniospores, with >9 h required for moderate infection (>4 pustules/cm²) at 15 and 20°C. With diurnal temperature regimes averaging 5, 10, 15, 20, or 25°C, urediniospore germination and infection was highest in the range of 5 to 15°C; similarly, in the diurnal environment, >9 h of leaf wetness was required to attain moderate infection. In the field, lime sulfur applied as a delayed dormant treatment significantly suppressed teliospore germination and basidiospore infection. Over two seasons, one application of myclobutanil, a demethylation-inhibitor fungicide, applied in early May near the time of spermatogonial appearance provided effective suppression of the summer epidemic.

The rust fungus *Phragmidium violaceum* was first observed in spring 2005 on naturalized Himalayan blackberry plants (*Rubus armeniacus* Focke) along Oregon’s southern coast (17) and, within a few months, was found in many counties in western Oregon, western Washington, and northern California, including those in the Oregon’s Willamette Valley, where commercial blackberry production is concentrated (1,17; W. F. Mahaffee, *unpublished data*). In addition to infecting unwanted, naturalized blackberry, nearly all

fields of the commercial *R. laciniatus* Willd. cvs. Thornless Evergreen and Everthornless became infested with the pathogen, with several fields incurring severe economic losses. The impacted fields showed reduced fruit yield and quality, and plant vigor in the following season (K. B. Johnson, *personal observation*).

Incursion of blackberry rust into the United States represents a new production constraint for the Pacific Northwest’s \$36 million blackberry industry. Although the autecology of *P. violaceum* and epidemiology of blackberry rust has been investigated with regard to its use as a biological control agent of naturalized blackberry in Australia (4,6–8,10,18), little is known about the management of this disease in susceptible blackberry plantings (21). *P. violaceum* is an autoecious, macrocyclic rust fungus, which causes a defoliating disease on leaves and flower buds (15,22). Each spring, *P. violaceum* reportedly undergoes an obligate sexual cycle (6,10,15), with asexual perennation of this pathogen in buds or on overwintering foliage considered insignificant in the disease cycle (22). Urediniospores infect through stomata on undersides of leaves and produce a new generation of spores every 8 to 12 days (4,6). Leaf age greatly influences

susceptibility to infection, with the most susceptible foliage being the youngest, fully expanded leaves near the tips of canes (6). In late summer and through fall, uredinia differentiate to telia that produce overwintering teliospores. Physiologic races of *P. violaceum* have been reported in regions where it has been established for longer periods of time (7). In detached leaf assays, this pathogen has been shown to be sensitive to the fungicides that are commonly used to control rust diseases in other crops (21); however, there is little information of fungicide performance in the field.

The purpose of this study was to study infection and suppression of *P. violaceum* on *R. laciniatus* under western Oregon conditions. We examined the phenology of the disease cycle, identified environmental conditions that promote infection, and developed an effective chemical suppression strategy to prevent epidemic development.

MATERIALS AND METHODS

Teliospore germination. Germination of teliospores of *P. violaceum* was studied during 2006 and 2008 at two locations in western Oregon: Oregon State University’s North Willamette Research and Extension Center (NWREC), located near Aurora, and the Department of Botany and Plant Pathology Field Laboratory, located near Corvallis. A 0.12-ha planting of *R. laciniatus* cv. Thornless Evergreen, located at NWREC, was used as the source of all teliospores and for fungicide trials described below. Teliospores either overwintered in the planting or, for Corvallis experiments, leaves bearing telia were collected in fall and stored dry in an unheated screenhouse before being placed outdoors in early March. At this time, leaves bearing telia were spread uniformly between 1-by-1-m sheets of plastic, poultry-mesh fencing (18-mm grid; Tenax Corp., Baltimore, MD) that was suspended on a horizontal plane 1 m above the ground in an open area. Each suspended mesh platform held 400 to 600 leaves and was replicated three times.

Teliospores, collected weekly during spring from Aurora and Corvallis, were subjected to a germination test in the laboratory (6). The spores were scraped from leaves into a drop of distilled water and drops were spread onto microscope slides covered with a film of water agar (15

Corresponding author: K. B. Johnson
E-mail: johnsonk@science.oregonstate.edu

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

Accepted for publication 18 January 2010.

doi:10.1094/PDIS-94-5-0581

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2010.

g/liter). Slides with teliospores were incubated in the dark at 20°C for 48 h inside of closed plastic boxes lined on the bottom with moist paper towels. Percent germination (teliospores with an emerging promycelium) was determined for three sets of 100 teliospores sampled from each location. As the season progressed, the percentage of previously germinated teliospores (empty teliospore shells) also was recorded. The phenology of spore germination in Corvallis in western Oregon was compared with that observed in Montpellier in southern France (6). For this comparison, an “expected” period of teliospore germination for the cooler, western Oregon site was developed by shifting the spring germination period observed in southern France (6) by the amount that a 13-year record of mean daily temperature differed between the two locations (data for Montpellier and Corvallis were obtained from Weather Underground Inc., Ann Arbor, MI).

In 2008, the effect of lime sulfur on teliospore germination also was evaluated. In Corvallis, three subsamples of detached leaves were sprayed to near runoff with 10% (vol/vol) calcium polysulfide (Rex Lime Sulfur Solution 28%; OR-CAL Inc., Junction City, OR) mixed in water; then, after drying, they were placed in a 0.15-by-0.15-m plastic mesh bag that was positioned at the edge of the same suspended platforms that held the nontreated leaves. At Aurora, leaves bearing teliospores were sampled from plots that had received delayed dormant sprays of lime sulfur on 26 March (10%) and 16 April (2.5%) (this treatment is described below under “Chemical control”).

Basidiospore infection. A rotational “trap” plant study was conducted to identify environmental conditions that favored basidiospore infection. Plants were obtained by rooting two-node, greenwood cuttings of cv. Thornless Evergreen on a mist bench. Cuttings were then planted in 3.8-liter pots filled with Sunshine mix no. 1 (SunGro Horticulture, Bellevue, WA) and grown in a greenhouse (15 to 26°C, 16-h photoperiod). Plants were fertilized as needed (Champion 17-17-17 with micronutrients’ McConkey’s, Portland, OR) at a nitrogen concentration of 200 ppm. Prior to use, plants were trimmed to three to four actively growing canes, with individual canes ranging from 20 to 80 cm in length.

Beginning in mid-March, sets of the potted blackberry plants were exposed in the field for 7 days, after which a new set of plants was rotated into the field and the previously exposed plants incubated in a greenhouse (at approximately 18°C) for 21 days. As a control, on the day of each rotation, five symptomless plants were transferred from the propagation greenhouse to incubate with the plants that had returned from the field. Plants in the greenhouse were watered such that leaf wetness did

not occur. Potted-plant rotations were repeated through May at both Aurora (2006 and 2007) and Corvallis (2006 to 2008). At Aurora, groups of 10 plants were placed at three areas within the blackberry planting and, in Corvallis, 10 plants were placed under each of the three platforms on which the teliospore-bearing leaves had been suspended. After incubation, exposed and control plants were assessed for spermatogonia by recording the leaf node (counted down from tip) and total number of spermatogonia on all diseased leaves. After disease assessment, canes were cut back to the soil line and plants regrown for use in subsequent seasons.

Weather data were monitored at the Corvallis site with an Adcon A730 weather station equipped with standard temperature, rainfall, and leaf wetness sensors (Adcon International Inc., Davis, CA.). At Aurora, temperature, rainfall, and leaf wetness data were obtained from the Agrimet Weather Network (U.S. Bureau of Reclamation, Pacific Northwest Region, Boise, ID). As suggested previously (2,13; J. Pscheidt *personal communication*), a continuous period of leaf wetness began with rain and ended after 8 h of “dry” was recorded by the leaf wetness sensor. Consequently, adjacent wetness periods with breaks of <8 h were joined into a single event. Hourly records of temperature were used to obtain a mean temperature for each wet period. When a dry break occurred during a wetness event, only hourly temperatures that coincided with wetness were included in the mean. For 10 weekly exposure periods with multiple wetness events but no infection, all wetness periods were considered in data interpretation. For three weekly exposure periods with infection and more than one wetness event, only the most favorable period (longest and warmest) was considered in data interpretation.

Conditions for germination and infection by urediniospores. Environmental conditions giving rise to germination and infection from urediniospores were quantified in growth chambers (I-30VL; Percival Scientific Inc., Perry, IA). Plants of cv. Thornless Evergreen were produced as described above and grown in a greenhouse in 250-ml pots. Urediniospores of two local isolates of *P. violaceum* (HIM1 and ET1) were produced on blackberry plants maintained in separate, isolated growth chambers (18°C with a 16-h photoperiod). Urediniospores were collected from blackberry leaves using a cyclone spore sampler (19).

The effect of temperature on urediniospore germination was evaluated by releasing a 1:1 mixture of isolates HIM1 and ET1 inside a settling tower at a height of 30 cm above 20 petri dishes that contained water agar. Approximately 0.002 g of spores was released by blowing air at 68 kPa through a cyclone sampler for 10 s. Initially, a shield was positioned in the

tower at an intermediate height to capture spore aggregates for the first 20 s after release. After shield removal, spores were allowed to settle onto the water agar plates for 90 s. The dishes were then divided randomly and placed in growth chambers programmed with diurnal temperature regimes that averaged 5, 10, 15, 20, or 25°C (described below) over 24 h. Percent spore germination, defined as a germ tube length twice the spore diameter, was assessed by counting the number of germinated spores out of 200 spores examined per plate.

Urediniospore infection experiments were arranged in split-plot designs with temperature as the main plot and leaf wetness duration as subplots. Two types of temperature treatments were used: chambers set to a constant 5, 10, 15, 20, 25, or 30°C and chambers programmed to fluctuate diurnally with average daily temperatures of 5, 10, 15, 20, or 25°C. The diurnal temperature regimes were developed from hourly data recorded by the Agrimet station at NWREC. Leaf wetness duration treatments were created by sealing plants in humidity chambers for 0, 2, 4, 6, 8, 12, 18, and 24 h after inoculation. Three replications in time served as the blocks, with temperatures assigned to growth chambers randomly at the beginning of each replication; two plants composed an experimental unit. The experiments were repeated twice; for the diurnal temperature experiment, different sets of derived temperature regimes were used for each experiment.

For inoculation, a 1:1 mixture of urediniospores of isolates HIM1 and ET1 was suspended in a 0.05% (wt/vol) TWEEN 20 solution and adjusted to 2×10^4 urediniospores/ml with the aid of hemacytometer. The abaxial surface of the top two fully expanded leaves on each plant was misted to near runoff with a handheld atomizer (Nalgene, Rochester, NY). Immediately after inoculation, plants were sealed individually in 3-liter humidity chambers (spaghetti jars; Snapware, Mira Loma, CA) that had been misted on the inside with distilled water. The temperature and humidity within one jar in each growth chamber was recorded every 5 min by suspending a HOBO U10 Temp/RH Data Logger (Onset Computer Corp., Bourne, MA) on monofilament just below the lid. Leaves remained wet if humidity was above 95%. At the end of wetness duration periods, lids were opened to allow the leaves to dry. After opening jars from the longest wetness duration (24 h), all plants were removed from their jars and placed in a single growth chamber (18°C) for 14 days. Lesion density (pustules per square centimeter of leaf area) was assessed on the inoculated leaves. Noninoculated plants were also placed in this growth chamber to serve as a control, and all plants were bottom-watered to prevent leaf wetness during incubation. Urediniospore

infection was modeled statistically as a function of temperature and leaf wetness duration, with the actual mean temperature recorded in each chamber used as the independent variable. Temperatures in the chambers were within 0.5°C of the programmed temperature, except for the one of the 5°C diurnal temperature regimes, which averaged 7°C. Regression analysis was conducted with Proc NLIN in SAS (version 9.2; SAS Institute, Cary, NC). Response surface regression analyses were conducted with Sigma Plot 10.0 (SYSTAT Software, Inc., San Jose, CA).

Seasonal phenology of suitability of western Oregon climate for infection.

Infection responses of urediniospores to temperature in the growth chambers were used to develop an expectation of the seasonal suitability of the western Oregon climate to rapid development of blackberry rust. With growth chamber data at 12 h of wetness, lesion density was first made relative to a maximum of 1.0 and then regressed on mean incubation temperature for both the constant and diurnal temperature regimes. These equations represented optimum curves with which the relative seasonal favorability with respect to temperature could be estimated by inserting a value for mean daily temperature; data for this purpose were obtained from the National Climatic Data Center (U.S. Department of Commerce, Ashville, NC) and were composed of the 30-year average of mean daily temperature for the midpoint of each month at Salem, OR. Similarly, to estimate the seasonal favorability for leaf wetness, a surrogate variable was created from 71-year averages of days per month with >2.5 mm precipitation for Salem, OR (data from National Climatic Data Center) and these values were divided by days in a month to estimate probability of precipitation for the midpoint of each month. The product of the mid-month temperature favorability index and mid-month probability of precipitation was termed the "rust favorability index," which was plotted as a function of month of year.

Chemical control. Fungicide experiments were conducted in a 4-year-old planting of Thornless Evergreen blackberry located at NWREC near Aurora, OR. The 0.12-ha planting consisted of eight rows (43 m) with 3-by-1.8-m plant spacing. Plants were trained to a wire trellis with plot maintenance (weed control, irrigation, and fertilization) following best management practices. The planting was managed for "alternate year" fruit production where, in each season, half the rows were fruiting and the other half produced only new primocanes. In fall, rows with old fruiting canes (and primocanes associated with these plants) were cut off near ground level whereas, in the nonfruiting rows, the primocanes 2 to 3 m in length were tied to the wires to fruit the following summer.

An exception to the above cane management occurred in the first season (2005), after blackberry rust had become severe by July. As a consequence, to develop clean plants for fungicide screening, half the rows were cut off near ground level on 3 August 2005, which caused a flush of 10 to 20 new primocanes to emerge from each plant by early September. The new primocanes grew through September, achieving a length of 1 to 1.3 m before growth stopped in early October. Fungicide treatments were made on 2 and 29 September and included the following materials: myclobutanil (0.37 g a.i./liter, Rally 40 W; Dow AgroSciences, Indianapolis, IN), azoxystrobin (0.30 g a.i./liter, Abound 2SC; Syngenta, Greensboro, NC), copper hydroxide (4.8 g a.i./liter, Kocide 2000; DuPont, Wilmington, DE), and a water-treated control. Treatment suspensions were applied to near runoff (0.2 liter/plant) with a 2-liter CO₂ pressurized backpack sprayer at 275 KPa (Model GS; R & R Sprayers, Opelousas, LA). The experiment was arranged in a randomized block design with four single-plant replications. The plot area was adjacent to four rows of severely rusted blackberry, which provided a source of urediniospores. Preliminary assessment of control treatments determined that nearly all disease was located at the 9th through 13th nodal positions from the tip of the cane. Tags were affixed to leaves located at these positions on each of four randomly selected canes of each plant. Disease severity (percent leaf area with symptoms) on the upper surface of each tagged leaf was assessed on 10 and 24 October and 16 November with the aid of standard area diagrams. Rust severity assessments were summarized as area under the disease progress curve (AUDPC) using midpoint rule integration between each assessment date. AUDPC values were subjected to analysis of variance (ANOVA; SAS Proc ANOVA); Fisher's protected least significant difference test at $P = 0.05$ was used for separation of treatment means.

Spring fungicide trials conducted in 2007 and 2008 were arranged as split-plot designs with lime sulfur treatments in whole plots and timings of myclobutanil (and, in 2007, azoxystrobin followed by myclobutanil) as subplots. Whole plots consisted of a half row (11 plants) randomized in four blocks of two treatments: lime sulfur or nontreated. Subplots consisted of two adjacent plants separated by a buffer plant. Calcium polysulfide (Rex Lime Sulfur Solution 28%) mixed 10% (vol/vol) in water was applied to dormant canes on 24 March 2007 and 26 March 2008 and, because of the unusual lateness of spring 2008, 2.5% (vol/vol) calcium polysulfide (Rex Lime Sulfur Solution 28%) was applied on 16 April (a week prior to bud break). The first treatment of myclobutanil (or azoxystrobin) was timed to coincide with expected time of appearance of sper-

magonia (4 May 2008 and 30 April 2008), with the second application made just prior to bloom (4 June 2007 and 11 June 2008). Foliar fungicide subplots had a total of four treatments: myclobutanil in early May, early June, or at both timings (2008 only); azoxystrobin in early May followed by myclobutanil in early June (2007 only); and a water-treated control. All materials were applied with hand-pumped, 12-liter backpack sprayers equipped with hand held spray wands (Model 475; Solo, Newport News, VA) at a volume of 0.6 liter/plant (equivalent to 935 liters/ha).

Measurements of rust development were made on 29 April, 11 July, and 8 August 2007 and on 27 May, 3 and 17 June, and 3 July 2008. The first assessment in each season assessed density of spermatogonia, whereas the densities of uredinia were assessed on the later dates. Rust was measured by laying a 1-m² frame onto the canopy and examining the leaves within the frame for incidence of disease and number of pustules per leaf. For the first assessment, the leaves in the 5th through 10th nodal positions from the tip of the cane were examined whereas, in the later assessments, the leaves in the 1st to 5th nodes below the fruit cluster were examined. Assessments were made on both sides of the canopy with 350 to 450 leaves examined in each plot (mean of 382 leaves per 2 m²). Because disease severity remained at low to moderate levels, and because pustules were aggregated, incidence of diseased leaves was the most useful variable for evaluating development of the epidemics and effects of fungicides. For assessments of spermatogonia (29 April 2007 and 25 May 2008), the whole-plot data were analyzed with a paired t test. For the July assessment dates, the effects of subplot treatments on incidence of leaves with rust was analyzed by ANOVA; Fisher's protected least significant difference test at $P = 0.05$ was used for separation of treatment means.

RESULTS

Observations on early blackberry rust development in western Oregon. In 2006 to 2008, blackberry rust was observed each year on both naturalized *R. armeniacus* and cultivated *R. laciniatus* growing in western Oregon. Spermatogonia were observed first on *R. armeniacus*, perhaps because this host initiated leaf growth 2 weeks earlier than *R. laciniatus*. In 2008, April was abnormally cool and bud break in *R. laciniatus* occurred during the last week of the month; correspondingly, in this season, spermatogonia were not observed until near the middle of May, which was approximately 3 weeks later than in 2006 and 2007. Over 3 years of observation, symptoms or signs that would have been consistent with asexual perennation (diseased buds or development of uredinia prior to spermatogonia) were not observed.

Teliospore germination. Mean seasonal temperatures for Corvallis in early spring lagged behind those of Montpellier, France by approximately 4 weeks; consequently, based on the period of teliospore germination measured in Montpellier (6), we expected that teliospore germination in western Oregon would occur during a 9-week period extending from late March to early June, with a peak of germination occurring in early May. For the observed data, the periods of teliospore germination ranged from 8 to 10 weeks (Fig. 1; only 2008 data is shown); however, the periods of high spore germination began in early April and remained consistently high into early May, and then declined steadily during the latter half of May. By early June, most collected teliospores (>90%) were shells that had germinated previously.

In 2008, regardless of type of lime sulfur treatment (sprayed in field or onto

Fig. 1. Germination of teliospores of *Phragmidium violaceum* sampled from leaves of *Rubus laciniatus* cv. Thornless Evergreen from a field planting located near Aurora, OR (solid circle) and from bundles of detached blackberry leaves suspended on elevated platforms near Corvallis, OR (solid square) in 2008. Leaves at Corvallis were produced at the Aurora location. On 26 March 2008 (solid arrow), at Corvallis, a sub-sample of leaves was sprayed to runoff with 10% lime sulfur (open square); at Aurora, half of the plot area was sprayed with 10% lime sulfur (open circle). The Aurora field plots received an additional spray of lime sulfur (2.5%) on 17 April 2008 (dashed arrow). Each point represents a sample of 300 teliospores.

leaves inside of mesh bags), teliospores that received this treatment had greatly reduced germination relative to those that did not (Fig. 1). Over five sampling dates in April, nontreated spores averaged $66.2 \pm 4.6\%$ (standard deviation [s.d.]) and $58.9 \pm 18.9\%$ germination at Aurora and Corvallis, respectively, whereas teliospores treated with lime sulfur averaged 11.5 ± 19.4 and $4.7 \pm 5.3\%$ germination at these respective locations.

Infection by basidiospores. At Aurora, where potted plants of *R. laciniatus* were exposed in a blackberry planting for two spring seasons, only 1 of 21 weekly plant exposures resulted in an infection event. For that event, four spermatogonia were observed on one leaf of one plant (Table 1). In contrast, at Corvallis, where plants were exposed under platforms holding telia-bearing leaves, 12 of 34 weekly exposures over 3 years resulted in basidiospore infection. For weeks with infection, the incidence of diseased plants averaged $49 \pm 34\%$ (s.d.) with a mean of 2.5 ± 1.4 leaves with spermatogonia per symptomatic plant, and an average of 6.7 ± 7.0 spermatogonia per diseased leaf (Table 1). Of the 12 Corvallis exposures with basidiospore infection, 9 occurred during April, whereas 2 coincided with exposure in May and 1 from exposure in June; no symptoms developed on plants exposed in March.

For Corvallis, in total, 43 wetness periods initiated by rain were observed over the 3 years of weekly plant exposures; these wetness periods were confined to 23 of the 34 exposure weeks. A wetness period was required for infection to occur but, because of high variability in the number of infections and in wetness duration per event, the fitting of a quantitative response surface model to the data was not successful (*data not shown*). Nonetheless, categorizing the 36 wetness events by a duration of <16 or ≥ 16 h and by a mean temperature during the wetness period of $<8^\circ\text{C}$ or $\geq 8^\circ\text{C}$ placed 8 of 12 weeks with infection events into the category that experienced a wetness period ≥ 16 h with a mean temperature $\geq 8^\circ\text{C}$ (Table 2). Infection events outside of this category either were very light or had long wetness-

duration periods (53 and 77 h) with an overall mean temperature $<8^\circ\text{C}$; however, within the period was a subset period with a mean temperature $\geq 8^\circ\text{C}$ for more than 16 h (Table 2).

Conditions for germination and infection by urediniospores. Infection occurred at all constant temperatures regimes except 30°C , with the most infection occurring at either 15 or 20°C depending on wetness duration (Fig. 2A, C, and E). With diurnal temperature regimes, the greatest infection occurred at 10 to 20°C depending on wetness duration (Fig. 2B, D, and F). Under the diurnal regime, the amount of infection at 5°C after 24 h of wetness was more than twice the amount of the constant 5°C regime whereas, at 20°C , the amount of infection under the diurnal regime was only about a fifth of that observed under the constant regime. Under both regimes, a minimum of six continuous hours of leaf wetness was required for infection, with >9 h of wetness required for moderate infection (>0.4 pustules/cm 2) at 15 or 20°C under the constant regime and at 10 or 15°C under the diurnal regime.

Under the diurnal temperature regime, a significant curvilinear relationship was observed ($P = 0.02$) between the percentage of spores germinated (g) and average temperature (T_d): $g = -0.158 \times T_d^2 + 2.821 \times T_d + 39.03$ ($R^2 = 0.94$). As with the infection response, the optimal temperature for germination was 10°C , with $51 \pm 13\%$ (s.d.) germination observed, followed closely by 5 and 15°C , which averaged $48 \pm 9\%$ germination. Only 26 ± 19 and $12 \pm 5\%$ germination were observed at average daily temperatures of 20 and 25°C , respectively.

Seasonal suitability of western Oregon climate for infection. Estimated seasonal favorability for rapid development of blackberry rust based on temperature and probability of precipitation yielded a bimodal response (Fig. 3). The two periods of highest favorability coincided with mid-to late spring and early fall. Winter was predicted to be unfavorable owing to cold temperatures; July and August also were predicted to be unfavorable owing to a combination of higher than optimal daily

Table 1. Summary of weekly exposures of potted *Rubus laciniatus* cv. Thornless Evergreen to teliospore inoculum of *Phragmidium violaceum* in western Oregon during spring 2006 to spring 2008

Year	Location ^b	Overall exposure period	No. of weeks of exposure	Total no. of plants exposed	No. of weeks infection was observed	Within weeks with infection ^a			
						Incidence of diseased plants (%)	Mean no. of diseased leaves per plant	Mean spermatogonia per leaf	Mean nodal position of diseased leaves
2006	Corvallis	15 March to 6 June	12	360	5	63	2.8	10.6	5.1
	Aurora	...	12	360	1	3	1.0	4.0	7.0
2007	Corvallis	20 March to 23 May	9	270	2	38	1.8	3.3	6.7
	Aurora	...	9	270	0	0	—	—	—
2008	Corvallis	19 March to 3 June	13	390	5	39	1.8	3.9	7.0

^a After exposure, the potted plants were incubated in a greenhouse for 3 weeks after which time disease was assessed; — indicates no infection occurred at this location in this season.

^b At each location, 30 greenhouse-grown plants were exposed each week to teliospores of *P. violaceum*. Near Corvallis, plants were exposed under elevated platforms on which detached telia-bearing leaves were suspended. At Aurora, potted plants were placed in an experimental plot of *R. laciniatus*.

mean temperature and a low probability of rainfall.

Disease dynamics. At Aurora, measurable epidemics of blackberry rust were observed in the planting of *R. laciniatus* in 2005, 2007, and 2008. In 2005, on primocanes that emerged in August after the plants had been cut, no rust was observed on 9 September but older leaves began to show symptoms by 20 September. On 29 September, for water-treated plants, leaves in positions 9 to 13 from the tip of the cane (and near the base) averaged 62 to 120 rust pustules per leaf but infections were not observed at younger positions. By the final assessment in November, rust severity averaged 30% on leaves in positions 9 to 13 (Fig. 4); only an occasional pustule was observed at younger leaf positions.

Spring epidemics observed in 2007 and 2008 began with spermatogonia, which then transitioned to the aecial and uredinal phases of the disease. Spermatogonia were first detected on 29 April 2007 and 20 May 2008. In both years, disease increased through June such that, by early July, inci-

dences of diseased leaves ranged from 17% (2007) to 30% (2008) of leaves in the first five nodal positions below the fruit cluster (Fig. 5). Disease severity, however, remained relatively low, with symptomatic leaves averaging 4.2 and 4.9 pustules per leaf on 11 July 2007 and 3 July 2008, respectively. In 2007, an assessment on 14 August showed little change in the incidence of diseased leaves relative to the July assessment; pustules per leaf averaged 6.6 on this assessment date. Interestingly, in 2006, a spring rust epidemic did not develop in the plot area in spite of a large number of teliospores carrying over from the epidemic in the previous season.

Chemical control. In 2005, the effects of fungicide treatments on rust development on primocanes became apparent in

early October, with plants treated with myclobutanil showing <2% rust severity, whereas the average severity on all other treatments was >7% (Fig. 4). Disease severity increased through mid-November, with the difference between myclobutanil and other treatments maintained over the period of observation. Based on ANOVA of AUDPC, rust suppression with myclobutanil was significantly ($P \leq 0.05$) greater than with the other fungicides; azoxystrobin provided an intermediate degree of protection that was significantly ($P \leq 0.05$) better than copper hydroxide or the water-treated control.

Lime sulfur applied in early spring of 2007 and 2008 significantly (for both years; $P \leq 0.1$ or 0.05 for two- and one-tailed paired *t* test, respectively) sup-

Table 2. Frequency of defined leaf wetness and temperature events and their association with spermatogonia of *Phragmidium violaceum* on plants of *Rubus laciniatus* cv. Thornless Evergreen exposed weekly to teliospores of this pathogen near Corvallis, OR during spring 2006 to spring 2008^a

Mean temperature ^b	Length of wetness period	
	<16 h	≥16 h
<8°C		
No. of events	6	10
Events with infection	1 ^c	2 ^d
≥8°C		
No. of events	11	10
Events with infection	1 ^c	8 ^f

^a Leaf wetness events required initiation by rainfall. Adjacent wetness periods with breaks of <8 h were joined into a single event (2,13). If a dry break occurred, only hourly temperatures that coincided with wetness were included in the mean. Each week, thirty 1-year-old greenhouse-grown plants were exposed under elevated platforms on which detached blackberry leaves bearing telia of *P. violaceum* were suspended.

^b Mean temperature during period of leaf wetness.

^c In total, 11 spermatogonia were observed from this event.

^d These events had wetness periods of 53 and 77 h where, within each period, occurred a 16-h period with a mean temperature ≥8°C. Totals of 23 and 37 spermatogonia coincided with these two events.

^e In total, eight spermatogonia were observed from this event.

^f Total numbers of spermatogonia observed for these eight events were 27, 41, 225, 265, 305, 440, 935, and 2,840. For three of these events, an additional six leaf wetness periods were recorded during the weekly exposure period. Four of these wetness periods were in the category of <8°C with wetness <16 h and two were in the category of ≥8°C with wetness <16 h.

Fig. 2. Density of *Phragmidium violaceum* pustules on *Rubus laciniatus* cv. Thornless Evergreen as influenced by post-inoculation leaf wetness duration and A, C, and E, constant or B, D, and F, diurnal temperature regimes. The best fit regression models were based on the A and B, logistic or C and D, Gaussian functions (equations not shown). E and F, A two-factor Gaussian model was suitable for describing the relationship among the pustule density (Pd), leaf wetness (LW), and constant (T_c) or diurnal (T_d) temperature regimes: $Pd = 1.78 \times \exp(-0.5 \times \{[(T_c - 15.43)/5.60]^2 + [(LW - 19.97)/6.14]^2\})$ (adjusted $R^2 = 0.87$) and $Pd = -1.11 \times \exp(-0.5 \times \{[(T_d - 10.54)/-6.13]^2 + [(LW - 20.39)/6.97]^2\})$ (adjusted $R^2 = 0.85$). The models and all parameters were significant at $P < 0.0001$.

pressed the development of spermatogonia by 57 to 78% (Fig. 6). For myclobutanil, the degree of suppression of the uredinal phase depended on the time of application. Based on disease assessments made in early July, one application of myclobutanil in late April (2008) or early May (2007) provided significant ($P \leq 0.05$) control, whereas a single application of this material made in early June did not (Fig. 7). The highest suppression of rust was obtained by two treatments with myclobutanil (30 April and 11 June 2008); in contrast, in 2007, an early-May application of azoxystrobin followed by myclobutanil in early June provided an intermediate level of disease control. The interaction between delayed dormant lime sulfur (whole plot) and in-season foliar fungicides (subplots) was not significant ($P > 0.10$).

DISCUSSION

Since its first report in 2005, *P. violaceum* has caused economic damage to cultivated *R. laciniatus* cvs. Thornless Evergreen and Everthornless and, to a lesser extent, the other common species of European origin, naturalized Himalayan blackberry (*R. armeniacus*). Cultivars of *R. laciniatus* represent approximately 10% of Oregon's commercial blackberry acreage and are characterized as high yielding and very late to mature but with only intermediate fruit quality. Consequently, their

importance to the caneberry industry is partly as "insurance" to the widely grown and high quality cv. Marion, which is prone to winter injury but not susceptible to *P. violaceum* (21). Thus, if *R. laciniatus* is to continue as a cultivated blackberry, growers need to view management of blackberry rust as economical within the context of a crop with its value partially dependent on the yield performance of another cultivar.

Our results show a high likelihood for management of blackberry rust in *R. laciniatus* at low cost. The primary strategy for management is chemically achieved sanitation that targets the initiation of the disease cycle which, from a theoretical perspective, is unconventional for a foliar, polycyclic pathogen (20). Because asexual perennation of *P. violaceum* appears absent or rare (22) in the disease cycle, this pathogen undergoes an apparent obligate sexual cycle each spring, which is unlike other *Phragmidium* spp. that cause rusts on rose (11). We observed that initiation of the disease cycle from overwintering teliospores appears to require long wetness periods (≥ 16 h) at mean temperatures that are relatively warm ($>8^{\circ}\text{C}$) for western Oregon during April (Table 2). As a consequence of these constraints, we observed only two to five periods of infection by basidiospores each spring (Table 1). Importantly, in both the teliospore ger-

mination experiment (Fig. 1) and the fungicide field experiment (Fig. 6), treatment with lime sulfur suppressed the measured dependent variable, either teliospore germination or spermatogonial formation. Growers traditionally apply lime sulfur annually to dormant floricanes to suppress fungal leaf and cane spot pathogens. Our results indicate that timing of the 10% lime sulfur treatment should be made 2 to 3 weeks before budbreak, when teliospores are ready to germinate. In addition, in 2008, we applied a 2.5% lime sulfur treatment just before budbreak, which did not induce a phytotoxic response; teliospore germination data before and after this treatment (Fig. 1) suggest that this second application was potentially efficacious. Similarly, myclobutanil treatments were

Fig. 3. A, Daily average temperature (solid square), probability of precipitation (open diamond), and rainfall (open square) for Salem, OR. B, Expectation of seasonal favorability for rapid increase in blackberry rust in western Oregon based on the product of relative favorability of temperature multiplied by the probability of precipitation at the mid-point of each month. Relative favorability of temperature was based the data shown in Figure 2: constant temperature regime (solid triangle) and diurnal temperature regime (open triangle).

Fig. 4. Severity of blackberry rust on leaves of *Rubus laciniatus* cv. Thornless Evergreen in fall 2005 as influenced by treatment with various fungicides: myclobutanil (open triangle), azoxystrobin (solid diamond), copper hydroxide (open circle), and water control (solid square). Data points ($\pm$ standard error) are the average rating from four plots in an experimental planting grown near Aurora, OR. Fungicide treatments were made on 9 and 29 September.

Fig. 5. Incidence of blackberry rust on leaves of *Rubus laciniatus* cv. Thornless Evergreen from late spring to early summer 2007 (diamond) and 2008 (square). Data points ($\pm$ standard error) are the average rating from eight plots in an experimental planting grown near Aurora, OR. Data shown are the means from plots treated with water and with lime sulfur only.

more effective in late April or early May than in early June (Fig. 7) which, in both seasons, was before the appearance of the uredinial phase. Although we lack data to explain why an early myclobutanil treatment was a superior treatment, we speculate that this locally systemic material suppressed maturation of or spore formation from either the spermatogonial or aecial stages of the pathogen (14) and, thereby, also reduced initial inoculum.

In addition to the obligate telial phase, there are several reasons why targeting initial inoculum is apparently an effective strategy for management of blackberry rust. First, in blackberry, ontogenic resistance (9) of leaf tissue to the pathogen develops quickly: individual leaves at 2 weeks of age are a quarter to a third as susceptible as new, fully expanded leaves and, by 3 weeks, leaves are difficult to infect (6). For blackberry growing in Oregon, nearly all new leaf growth occurs from late April to early June, with a secondary flush of growth in September, after the fruit matures. Floral bracts are also highly susceptible but are only produced during a short period in spring. Consequently, the spring epidemic is confined to a relatively short 6- to 8-week period in the life of the crop. Second, the effective dispersal distance of basidiospores apparently is limited. This was evidenced by our observation of the extreme rarity of spermatogonia on trap plants placed in the experimental planting of *R. laciniatus* compared with infection of trap plants exposed under a dense bundle of telia-bearing leaves collected from the same planting (Table 1).

Fig. 6. Incidence of *Rubus laciniatus* cv. Thornless Evergreen leaves with symptoms of blackberry rust on A, 29 April 2007 and B, 25 May 2008 as affected by delayed dormant treatment with lime sulfur. In 2007, 10% lime sulfur was applied on 28 March; in 2008, 10% lime sulfur was applied on 26 March and, because of the lateness of this season, an additional application of 2.5% lime sulfur was made on 17 April. Bars are the mean ($\pm$ standard error) of four plots in an experimental planting located near Aurora, OR.

Once the disease cycle is initiated, dispersal of urediniospores from other plantings or nearby naturalized Himalayan blackberry likely occurs; however, given the short period of leaf and inflorescence production, epidemics initiated by urediniospores produced exogenous to the planting may not have sufficient time to complete several cycles of infection and, therefore, attain an economically damaging level of disease. Third, in addition to when leaf tissue is susceptible to infection, our analysis of the seasonal favorability of the western Oregon climate based on growth chamber response curves for infection by *P. violaceum* produced a bimodal curve with late spring to early summer and late summer to early fall being the periods most conducive to rust development. Although this analysis was simplistic, these periods coincided with increases in blackberry rust observed in the field (Figs. 4 and 5). Because of higher-than-optimum temperatures and infrequent precipitation, the analysis also predicted that the rate of increase of blackberry rust will be slow during summer, which is what we observed in 2007 (Fig. 5) and in 2008 (detailed data were not taken). Little increase of disease in summer also is likely affected by a lack of susceptible tissue. Overhead irrigation, which is common in blackberry production, partially negates the benefits of infrequent rainfall; however, the timing of irrigation can be managed to limit the length of the wetness period (12).

The severity of the blackberry rust epidemic in Oregon in 2005 drew attention to this pathogen both in terms of its potential as a constraint to the blackberry industry and as a potential biocontrol agent of naturalized Himalayan blackberry, an important noxious weed in the Pacific Northwest (5). Based on the results of the rotational trap plant study and growth chamber experiments, we have attempted to dissect why the 2005 epidemic was more severe than in subsequent seasons. Budbreak of blackberry (and many other crops) in 2005 was several weeks earlier than typical (W. F. Mahaffee, *personal observation*). Beginning in mid-March, weather records from the Aurora Agrimet station showed nine potential basidiospore infection events through mid-May (i.e., periods with >16 h of leaf wetness and mean temperature >8°C) compared with an average of 3.8 periods for 2000 to 2008 exclusive of 2005. Similarly, for May and June 2005, 15 potential periods of moderate urediniospore infection were observed (Fig. 2, defined as the conditions giving rise to >0.4 pustules/cm²) compared with an average of 6 periods in 2000 to 2008 exclusive of 2005. Thus, it appears that an early budbreak combined with weather favorable to infection were two factors that facilitated the severe disease epidemic in 2005.

Nonetheless, there may be other reasons for lack of a significant rust epidemic since 2005. For the cultivars of *R. laciniatus*, we suspect that the improved timing of the

Fig. 7. Incidence of *Rubus laciniatus* cv. Thornless Evergreen leaves with symptoms of blackberry rust on A, 11 July 2007 and B, 3 July 2008 as affected by delayed dormant treatment with lime sulfur and in-season foliar fungicide treatments. Dates of application of foliar treatments are listed under the bars; dates of application for lime sulfur were 28 March 2007 and 26 March and 17 April 2008. Bars are the mean ($\pm$ standard error) of four plots in an experimental planting located near Aurora, OR. Asterisks indicate treatment was significantly different ($P \leq 0.05$) from the water-treated control.

lime sulfur treatment, which was adopted by growers immediately, has been important in lessening the impact of local (in-field) inoculum when spring conditions have been favorable for teliospore germination and infection by basidiospores. Additionally, a 24C registration for Rally WP was obtained that doubled the rate of myclobutanil from what had been allowed on blackberry previously, and most growers make at least one treatment at the higher rate. For the naturalized populations, it was apparent in 2005 that a minor biotype of Himalayan blackberry, a facultative apomict with a population comprising a limited number of biotypes (3,5,16), was highly susceptible to *P. violaceum*. Severe rust developed on the minor biotype but only low levels of infection were observed on the more predominant biotypes (*unpublished data*). Subsequently, observations of severe rust in naturalized blackberry have become infrequent, perhaps due to pathogen-mediated selection for resistant biotypes. A reduced incidence of rust in naturalized blackberry also has likely reduced regional levels of inoculum.

In conclusion, although *P. violaceum* is now endemic in western Oregon, management of this pathogen in cultivated *R. laciniatus* should be achievable at an acceptable cost. Growers are advised to apply lime sulfur (10%) in late March or early April and to consider a second treatment (2.5%) prior to bud break (mid-April). Near the end of April or beginning of May, they should scout for the spermatogonial stage and apply myclobutanil at first detection. After the first spray, the weather should be monitored for frequency of additional wetness periods, and fields should be scouted for continued increase in disease. A second application of myclobutanil just before flowering may be warranted if the rust has continued to increase in the field. Although summer is less favorable for disease increase, we recommend that summer irrigation events be managed to limit the frequency and the length of wetness periods. After harvest, the stems of old floricanes can be cut to eliminate active pustules and teliospore formation on these tissues. If, in early September, active rust pustules are present on primocanes (next year's floricanes), an application of

myclobutanil may be useful to suppress the fall epidemic. Based on observations and experiments subsequent to the 2005 epidemic, it likely that only lime sulfur followed by a myclobutanil treatment will be required for adequate control in most seasons.

ACKNOWLEDGMENTS

Funding was provided by United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Specific Cooperative Agreement 58-5358-5-793, which was awarded through the Northwest Center for Small Fruits Research, and USDA-ARS CRIS 5358-22000-034-00. We thank T. Sawyer, K. Moukalied, and T. Neill for technical assistance; G. Koskela, J. DeFrancesco, and the field staff of the North Willamette Agricultural Research and Extension Center for the use and maintenance of the experimental blackberry planting; and J. Pscheidt for providing wetness-duration data collected at the Botany and Plant Pathology Field Laboratory near Corvallis.

LITERATURE CITED

1. Aime, M. C., and Rossman, A. Y. 2007. First report of the rust *Phragmidium violaceum* on Pennsylvania blackberry in California. *Plant Dis.* 91:1517.
2. Becker, C. M., and Burr, T. J. 1994. Discontinuous wetting and survival of conidia of *Venturia inaequalis* on apple leaves. *Phytopathology* 84:372-378.
3. Bijlsma, R.-J., and Haveman, R. 2007. *Rubus canduliger*, a new regional species from the Netherlands with notes on the range structure and the dynamics of brambles (*Rubus*, *Rosaceae*). *Folia Geobot.* 42:315-329.
4. Bruzzese, E., and Hasan, S. 1986. Host specificity of the rust *Phragmidium violaceum*, a potential biological control agent of European blackberry. *Ann. Appl. Biol.* 108:585-596.
5. Caplan, J. S., and Yeakley, A. J. 2006. *Rubus armeniacus* (Himalayan blackberry): occurrence and growth in relation to soil and light conditions in western Oregon. *Northwest Sci.* 80:9-17.
6. Evans, K. J., and Bruzzese, E. 2003. Life history of *Phragmidium violaceum* in relation to its effectiveness as a biological control agent of European blackberry. *Australas. Plant Pathol.* 32:231-239.
7. Evans, K. J., and Gomez, D. R. 2004. Genetic markers in rust fungi and their application to weed biocontrol. Pages 73-96 in: *Genetics, Evolution and Biological Control*. L. E. Ehler, R. Sforza, and T. Mateille, eds. CAB International, London.
8. Evans, K. J., Jones, M. K., and Roush, R. T. 2005. Susceptibility of invasive taxa of European blackberry to rust disease caused by the uredinal stage of *Phragmidium violaceum* under field conditions in Australia. *Plant Pathol.* 54:275-286.
9. Ficke, A., Gadoury, D. M., and Seem, R. C.

2002. Ontogenic resistance and plant disease management: a case study of grape powdery mildew. *Phytopathology* 92:671-675.
10. Gomez, D. R., Evans, K. J., Baker, J., Harvey, P. R., and Scott, E. S. 2008. Dynamics of introduced populations of *Phragmidium violaceum* and implications for biological control of European blackberry in Australia. *Appl. Environ. Microbiol.* 74:5504-5510.
11. Horst, R. K. 1983. Rust. Pages 11-12 in: *Compendium of Rose Diseases*. American Phytopathological Society Press, St. Paul, MN.
12. Huber, L., and Gillespie, T. J. 1992. Modeling leaf wetness in relation to plant disease epidemiology. *Annu. Rev. Phytopathol.* 30:553-577.
13. Jones, A. L., and Sundin, G. W. 2006. Apple scab: role of environment in pathogen and epidemic development. Pages 473-489 in: *The Epidemiology of Plant Diseases*. B. M. Cooke, B. Gareth Jones, and B. Kaye, eds. Springer, Houten, The Netherlands.
14. Kemmitt, G. M., DeBoer, G., Ouimette, D., and Imauti, M. 2008. Systemic properties of myclobutanil in soybean plants, affecting control of Asian soybean rust (*Phakopsora pachyrhizi*). *Pest Manage. Sci.* 64:1285-1293.
15. Laundon, G. F., and Rainbow, A. F. 1969. *Phragmidium violaceum* (Schultz) Wint. In: *Descriptions of Pathogenic Fungi and Bacteria*. Commonw. Mycol. Inst. Publ. No. 209. Kew, Surrey, England.
16. Nybom, H. 1988. Apomixis versus sexuality in blackberries (*Rubus* subgen. *Rubus*, *Rosaceae*). *Plant Syst. Evol.* 160:207-218.
17. Osterbauer, N., Trippe, A., French, K., McKemy, J., Bruckart, W. L., Peerbolt, T., Kaufman, D., and Aime, M. C. 2005. First report of *Phragmidium violaceum* infecting Himalaya and evergreen blackberries in North America. *Plant Health Prog.* doi:10.1094/PHP-2005-0923-01-BR.
18. Pigott, J. P., Weiss, J., Evans, K. J., and Mahr, F. 2003. Predicting the effect of the rust disease (*Phragmidium violaceum* (Schultz) Wint.) on blackberry (*Rubus fruticosus* L. agg.) in Victoria (Australia). Pages 373-381 in: *Plant Invasions: Species Ecology and Ecosystem Management*. G. Brundu, J. Brock, I. Camarda, L. Child, and M. Wade, eds. Backhuys Publishers, Leiden, The Netherlands.
19. Tervet, I. W., Rawson, A. J., Cherry, E., and Saxon, R. B. 1951. A method for the collection of microscopic particles. *Phytopathology* 41:282-285.
20. Van der Plank, J. E. 1963. *Plant Diseases: Epidemics and Control*. Academic Press, NY.
21. Washington, W. S. 1987. Susceptibility of *Rubus* species and cultivars to blackberry rust (*Phragmidium violaceum*) and its control by fungicides. *J. Phytopathol.* 118:265-275.
22. Washington, W. S. 1991. Blackberry rust. Pages 32-33 in: *Compendium of Raspberry and Blackberry Diseases and Insects*. M. A. Ellis, R. H. Converse, R. N. Williams, and B. Williamson, eds. American Phytopathological Society Press, St. Paul, MN.