Pathogenic Variation in Oregon Populations of *Sphaerotheca pannosa* var. *rosae*

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ABSTRACT

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Pathogenic variation in field and greenhouse populations of *Sphaerotheca pannosa* var. *rosae* was monitored on two differential cultivars. Two changes in the virulence of populations of *S. pannosa* var. *rosae* were detected by repeated sampling of conidia on greenhouse roses. These changes were associated with the introduction of infected plant material to the greenhouse. Only one pathotype was detected in powdery mildew populations sampled from two commercial greenhouses. Conidial populations on field-grown plants of *Rosa rugosa* and cultivar Dwarf Crimson Rambler consisted of at least two and four pathotypes, respectively. The presence of cleistothecia on these plants and the ratio in which the pathotypes occurred suggested that the virulence factors may have been recombined meiotically.

Rose powdery mildew, incited by *Sphaerotheca pannosa* (Wallr. ex Fr.) Lév. var. *rosae* Wor. (*S. p.* var. *rosae*), is the most serious disease on roses grown for the cut-flower market. The mechanisms and extent of variation in the fungus are unknown. In performance surveys published periodically in the *American Rose Annual*, the powdery mildew resistance ratings of individual cultivars vary both geographically and temporally. For example, the rose cultivar Tropicana (Super Star) becomes severely mildewed in Oregon but is highly resistant in other locations (1,7,8).

Several reports have suggested that pathogenic variation exists in *S. pannosa* (5,9,10,12); however, the first evidence of pathogenic specialization in the fungus was demonstrated by Bender and Coyier (3), who identified five races of *S. p.* var. *rosae*. That study involved the cultivation of monoconidial isolates of the fungus and their subsequent inoculation to a replicated series of differential hosts. To expedite this time-consuming process in this investigation, we adopted the virulence analysis concept of Wolfe and Schwarzbach (11), who describe methods for measuring the virulence of large populations against different sources of resistance. This approach is technically easier and provides results that are more representative of the mildew population. The purpose of this investigation was to use a modification of the virulence analysis concept of Wolfe and Schwarzbach to study pathogenic variation in field and greenhouse populations of *S. p.* var. *rosae* in Oregon.

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MATERIAL AND METHODS

S. p. var. *pannosa* was collected from the following field-grown rose cultivars in Benton County, Oregon, on the dates indicated: Burgundy (April 1981); *Rosa rugosa* Thunb. and Dwarf Crimson Rambler (October 1981); and Tropicana, Honey Favorite, and Sundowner (June 1982). The *S. pannosa* populations on Dwarf Crimson Rambler and *R. rugosa* were selected for study because both asexual (conidial) and sexual (cleistothecial) stages were present. This suggested the possibility of pathogenic variation within these populations caused by meiotic recombination of virulence genes. Infections on the other cultivars were chosen randomly to represent populations that occur on roses in garden plantings. Collections were made by removing several infected shoots from two or three plants of each cultivar. Shoots were placed in plastic bags and stored over ice until they were inoculated to detached leaflets or plants.

Greenhouse populations of rose mildew in Oregon were collected at two commercial rose-growing operations and at the Horticultural Crops Research Laboratory (HCRL) in Corvallis. One commercial operation was in Washington County and produced roses for the cut-flower market. This population was sampled by collecting mildewed shoots from cultivars Forever Yours, Volare, and Misty Pink in four greenhouses in March 1982. The second commercial range was in Clackamas County and was sampled in May 1982 by collecting three or four infected shoots of miniature rose cultivars Marilyn, Sarajean, and Butterscotch and two cultivars (Apricot Beauty and Ava) that are not officially recorded with the American Rose Society. Populations at the HCRL were sampled on cultivars Mary Devor and Samantha in June 1980 and April and October 1981 and on SR 70002/2 in April and October 1981.

Five races of *S. p.* var. *pannosa* were previously identified on four differential rose hosts (3). Our investigation was concerned with virulence on two of the differential hosts, a seedling rose (SR 70002/2) and cultivar Pink Parfait, and the identification of races 1, 2, and 3. In a preliminary survey, hybrid tea selections were found susceptible to one of these three races, whereas races 4 and 5 were avirulent on floricultural roses (C. L. Bender, *unpublished*). SR 70002/2 and Pink Parfait were previously assigned sequential numbers (1 and 2, respectively). Virulence formulas for races of *S. p.* var. *pannosa* were derived by listing the sequential numbers of cultivars resistant to the race on the left of the virgule (/) and susceptible cultivars on the right. Race abbreviations were assigned to each formula. Race 1 was avirulent on Pink Parfait but virulent on SR 70002/2 (2/1), race 2 was virulent on both cultivars (/2,1), race 3 was avirulent on SR 70002/2 and virulent on Pink Parfait (1/2), and races 4 and 5 were avirulent on both SR 70002/2 and Pink Parfait (1,2/). The last two races were identified by their virulence on the other two differential hosts (cultivar Dr. Huey and *R. multiflora* Thunb.). Race 4 is virulent on Dr. Huey only, and race 5 is virulent on both Dr. Huey and *R. multiflora*.

The virulence patterns in populations from Mary Devor, Samantha, SR 70002/2, Dwarf Crimson Rambler, and *R. rugosa* were analyzed on detached leaves of SR 70002/2 and Pink Parfait as described previously (3). Half-leaflet pairs were used to reduce variation in susceptibility among detached rose leaves. Pairs of half leaflets were prepared by excising equal amounts of tissue on opposite sides of the midrib. About 100–125 conidia per square centimeter were deposited on leaflets by moving a soft camel's-hair brush over the surface of mildewed tissue, then gently stroking it across the adaxial surface of the leaflets.

The deposition of conidia was observed with a dissecting microscope. The susceptibility of host tissue to a mildew population was assessed by inoculating one member of a pair with an isolate of unknown compatibility and the other member with an isolate of known compatibility. This method made possible the comparison of a virulent reaction with a reaction of unknown compatibility and improved the interpretation of test results. Each test involved 12–18 leaf tissue pairs, depending on the availability of leaf tissue.

Pathogenic variation within *S. pannosa* populations on *R. rugosa* and Dwarf Crimson Rambler was investigated by studying the virulence patterns of individual colonies initiated from these populations. One infected shoot from a plant hosting the desired population was shaken inside an isolation chamber (6) containing the original rose host. When colonies became visible 5–7 days later, conidia from individual colonies were transferred to detached leaves of SR 70002/2, Pink Parfait, and the original host. Detached leaves were rated for infection type 10 days later. The. virulence characteristics of 60 colonies from Dwarf Crimson Rambler and 30 colonies from *R. rugosa* were investigated.

In planta rather than detached leaf analysis was used to determine the virulence characteristics of *S. pannosa* populations from cultivars Burgundy, Tropicana, Honey Favorite, and Sundowner collected from two commercial rose-growing greenhouses. Shoots infected with these populations were collected and inoculated to SR 70002/2 and Pink Parfait in isolation chambers as described previously (3). Dwarf Crimson Rambler functioned as a susceptible control because all known races of the fungus are virulent on this cultivar. Seven days after the introduction of inoculum, the isolation chambers were opened and cultivars scored for the presence or absence of host necrosis and fungal sporulation.

Inoculated plants and detached leaf tissue were incubated in a growth room at 21 ± 3 C and illuminated 12 hr daily with fluorescent and incandescent lamps at an intensity of about 100 μE/m^2/sec at plant level. The relative humidity within the room was 45–55% as measured by a portable psychrometer.

RESULTS

The virulence characteristics of populations at the HCRL are presented in Table 1. The June 1980 populations on Mary Devor and Samantha were avirulent on SR 70002/2 and virulent on Pink Parfait; these characteristics indicated the presence of race 3. Plants of SR 70002/2 were not infected with *S. p.* var. *rosae* in June 1980. In April 1981, SR 70002/2 was infected with rose powdery mildew, but this population was avirulent on Pink Parfait; therefore, this population showed characteristics of race 1 pathogenicity. Populations on Mary Devor and Samantha remained avirulent on SR 70002/2 and virulent on Pink Parfait (race 3). October 1981 populations from SR 70002/2, Mary Devor, and Samantha were uniformly virulent on both test roses (race 2).

The virulence characteristics of populations from *R. rugosa* and Dwarf Crimson Rambler were more difficult to interpret. The *R. rugosa* population was avirulent on Pink Parfait but produced a heterogenous infection type on SR 70002/2. The population on Dwarf Crimson Rambler produced a heterogenous infection type on both test roses. These heterogenous reactions were characterized by macroscopically visible sporulation on some leaf disks and no sign of fungal sporulation on others. Necrotic lesions were present among sporulating colonies on Pink Parfait leaf tissue. These heterogenous reactions contrasted with the uniformly virulent or avirulent reactions observed when populations from HCRL were inoculated to the test roses (Table 1). The virulence characteristics of individual colonies originating from the Dwarf Crimson Rambler and *R. rugosa* populations are presented in Table 2. The reactions of SR 70002/2 and Pink Parfait to colonies initiated from these populations suggested that the Dwarf Crimson Rambler and *R. rugosa* populations consisted of at least four and two pathotypes, respectively. Of the 60 colonies initiated from the Dwarf Crimson Rambler population, 39 were avirulent on both test roses, 11 were virulent on SR 70002/2 only (race 1), nine were virulent on Pink Parfait only (race 3), and one had race 2 pathogenicity and infected both test roses. Twenty-seven of the *R. rugosa* colonies were avirulent on both test roses, and three were virulent on SR 70002/2 only (race 1). About 15–20 of the colonies avirulent on both differential roses were inoculated to Dr. Huey and *R. multiflora*. These colonies were virulent on *R. multiflora* but avirulent on Dr. Huey, suggesting race 5 pathogenicity. The ratio of colony types in the Dwarf Crimson Rambler population is similar to the 9:3:3:1 ratio that would be expected if avirulence were dominant and virulence for SR 70002/2 and Pink Parfait were segregating as separate genes ($0.50 > P > 0.30$). The colony type ratio in the *R. rugosa* population resembles the 3:1 ratio ($0.10 > P > 0.05$) that would be expected if avirulence were dominant and virulence on SR 70002/2 were controlled by a single recessive gene.

The results of *in planta* inoculations to the test roses are presented in Table 3. All greenhouse collections were virulent on Pink Parfait and avirulent on SR 70002/2. The virulence patterns from field collections were variable: Virulence was present for SR 70002/2 only in populations on Tropicana and Sundowner (race 1) and for both test roses on Honey Favorite (race 2); the Burgundy population was virulent only on Pink Parfait (race 3).

Table 1. Infection type and virulence characteristics of *Sphaerotheca pannosa* var. *rosae* populations at the Horticultural Crops Research Laboratory in the summer of 1980 and spring and fall of 1981 on detached leaves of a seedling rose (SR 70002/2) and cultivar Pink Parfait[a]

Population source	SR 70002/2	Pink Parfait	Virulence formula (R/S)	Indicated race
		June 1980		
Mary Devor	R	S	1/2	3
Samantha	R	S	1/2	3
		April 1981		
Mary Devor	R	S	1/2	3
Samantha	R	S	1/2	3
SR 70002/2	S	R	2/1	1
		October 1981		
Mary Devor	S	S	/2,1	2
Samantha	S	S	/2,1	2
SR 70002/2	S	S	/2,1	2

[a] 1 = SR 70002/2 and 2 = Pink Parfait. R = Resistant and S = Susceptible.

Table 2. Reactions of SR 70002/2 and cultivar Pink Parfait to individual colonies initiated from two populations of *Sphaerotheca pannosa* var. *rosae*

Population source	Virulence reaction[a]			
	Avirulent on SR and PP[b]	Virulent on		
		SR	PP	SR and PP
Dwarf Crimson Rambler	39	11	9	1
Rosa rugosa	27	3	0	0

[a] Number of 60 cultivar Dwarf Crimson Rambler colonies and 30 *R. rugosa* colonies that were avirulent on SR 70002/2 and Pink Parfait (race 4 or 5) and virulent on SR 70002/2 only (race 1), Pink Parfait only (race 3), and both SR 70002/2 and Pink Parfait (race 2).

[b] SR = SR 70002/2 and PP = Pink Parfait.

Table 3. *In planta* reactions of SR 70002/2 and cultivar Pink Parfait to greenhouse and field populations of *Sphaerotheca pannosa* var. *rosae*[a]

Collections	Virulence reaction		Virulence formula (R/S)	Indicated race
	SR 70002/2	Pink Parfait		
Greenhouse				
Washington county	R	S	1/2	3
Clackamas County	R	S	1/2	3
Field				
Tropicana	S	R	2/1	1
Honey Favorite	S	S	/2,1	2
Sundowner	S	R	2/1	1
Burgundy	R	S	1/2	3

[a] R = resistant and S = susceptible. 1 = SR 70002/2 and 2 = Pink Parfait.

DISCUSSION

Two changes were detected in the virulence characteristics of rose powdery mildew populations at the HCRL greenhouses. Both were associated with the introduction of infected plant material to the HCRL. The first change was associated with the introduction of a mildew strain on SR 70002/2, a rose selection that had been resistant for 8 yr before the 1980 growing season (3). Monoconidial isolates from this host were classified as race 1 (avirulent on Pink Parfait); this population change was detected in April 1981. The second change was associated with the introduction of mildewed Red Cascade plants in the summer of 1981. Monoconidial isolates from Red Cascade were virulent on both SR 70002/2 and Pink Parfait and were classified as race 2. Only race 2 was detected in October 1981 and in further samplings of mildew populations at HCRL greenhouses during the winter of 1981–1982 (C. L. Bender and D. L. Coyier, *unpublished*). In the present investigation, race 2 was actually more competitive than races 1 and 3 in HCRL greenhouses. The population shift to race 2 was accompanied by increased difficulty in controlling the fungus with chemicals; therefore, fungicide tolerance may be a factor in the increased competitive ability of race 2. Although race 2 is well adapted to rose ranges and presents a serious threat to growers, it was not present in populations at two commercial operations (Table 3).

In a previous report (3), monoconidial isolates cultured from field-grown *R. rugosa* plants in August 1981 were identified as race 3 (virulent on Pink Parfait only). In the present investigation, however, conidial populations on the same plants in October 1981 consisted of a mixture of two pathotypes: one avirulent on both SR 70002/2 and Pink Parfait and the other virulent on SR 70002/2 only. Therefore, during a period of 2–3 mo, there was a shift in the composition of powdery mildew races on *R. rugosa*. The virulence shifts in populations of rose powdery mildew are dynamic and can occur rapidly. In both instances where changes in pathogenicity were detected at the HCRL, they could be traced to the introduction of infected plant material into the greenhouse. Therefore, growers should quarantine any new material they receive and disinfest it thoroughly before introducing it to greenhouses.

The heterogenous responses observed on SR 70002/2 and Pink Parfait leaf tissue inoculated with populations from Dwarf Crimson Rambler and *R. rugosa* were not due to variability in tissue susceptibility, because half-leaflet pairs of the differential hosts were susceptible to isolates of known compatibility. In a previous study, avirulent isolates were unable to sporulate on SR 70002/2; isolates avirulent on Pink Parfait either failed to produce secondary conidia in macroscopically visible quantities or elicited a hypersensitive response (2). Therefore, the heterogenous reactions that occurred in response to Dwarf Crimson Rambler and *R. rugosa* inoculum were from populations of a mixture of avirulent and virulent pathotypes.

Virulence analyses of rose powdery mildew populations provided a more objective appraisal of pathogenic variability than a conventional race survey. For example, racial heterogeneity in the Dwarf Crimson Rambler and *R. rugosa* conidial populations might have been overlooked if only a few monoconidial isolates were cultured. At least four pathotypes were present in the Dwarf Crimson Rambler population, and two pathotypes were detected on *R. rugosa* when individual colonies initiated from these populations were inoculated to SR 70002/2 and Pink Parfait. The presence of cleistothecia on these plants and the ratio in which the pathotypes occurred suggests the meiotic recombination of virulence factors. In a separate study (4), monoconidial isolates from the two pathotypes on *R. rugosa* were combined on *R. rugosa* and Dwarf Crimson Rambler plants. The cleistothecia that resulted from this cross established the heterothallic nature of the fungus (4). Although the pathogenicity of ascospore infections has not been demonstrated in the laboratory, this study indicates that these spores are viable and contribute to pathogenic variation in the field.

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