Isolation and Identification of Races of *Sphaerotheca pannosa* var. *rosae*

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ABSTRACT

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Nine isolates of *Sphaerotheca pannosa* var. *rosae*, the pathogen that causes powdery mildew of roses, were obtained from infections on seven hybrid rose cultivars and two *Rosa* spp. Isolates were initiated on detached leaves, increased on host plants, and inoculated to rose cultivars to test for differential reactions. Virulence was evaluated by assessing the infection type of individual lesions and the percentage of leaf area covered with sporulating colonies. Five races of *S. pannosa* var. *rosae* were identified, and virulence formulae were developed to describe them on four differential rose cultivars. The pathogenicity markers described in this investigation will facilitate future genetic studies of *S. pannosa* var. *rosae*.

Additional key word: Erysiphaceae.

Pathogenic specialization of *Sphaerotheca pannosa* (Wallr.) Lev. has not been extensively studied. Yarwood (20) reported the occurrence of two strains of *S. pannosa* that differed in the size of lesions initiated on apricot (*Prunus armeniaca* L.) fruit. Kable et al (10) suggested the existence of a race of powdery mildew fungus pathogenic on rose and peach (*P. persica* [L.] Batsch.). Observations in both reports were based on the relationship of disease frequency in infected orchards to distance from mildewed rose bushes. Using detached shoots and leaves, Coyier (3) and Mence and Hildebrandt (12) found that conidia from *Rosa virginiana* Mill. would not produce sporulating colonies on most commonly grown rose cultivars, although they readily infected *R. virginiana* and *R. rugosa* Thunb. These authors did not use monoconidial isolates to establish pathogenic specialization or confirm their observations on host plants.

In the spring of 1980, plants propagated from an unnamed seedling rose, a descendent of the rose cultivar Tropicana, became severely infected with rose powdery mildew at the Oregon State University Botany and Plant Pathology Farm in Corvallis. This seedling, abbreviated SR 70002/2 in this paper, was developed by Walter Lammerts (Lammerts Hybridization Gardens, Freedom, CA 95019) and has been used as a source of resistance in his breeding program. During the 8 yr prior to the 1980 growing season, infection on this rose had been limited to small lesions on the receptacle. This change in host reaction suggested the introduction of a new pathogenic race of the fungus.

The purpose of this investigation was to develop methods of isolating and identifying the races of *S. pannosa* var. *rosae* Wor. that occur on roses. A preliminary report of the results has been published (2).

MATERIALS AND METHODS

Isolates. Nine monoconidial isolates of *S. pannosa* var. *rosae* were obtained from field and greenhouse plantings of the rose cultivars and species listed in Table 1. Field collections were made from naturally infected plants in Benton County, OR. Greenhouse collections were made from infected plants at the Horticultural Crops Research Laboratory in Corvallis, OR, except for the isolate from the miniature rose Red Cascade, which was cultured from plants in Clackamas County, OR.

Selection of these isolates involved consideration of the genetic diversity of their sources. Isolates on SR 70002/2 and Tropicana were of interest because these roses are genetically related, and both were noted for their mildew resistance when first introduced to most geographic locations. Isolates from cultivars Pink Parfait, Mary Devor, and Samantha were collected as representative of races that might be found on hybrid teas, grandifloras, and other modern commercial roses. The isolate on *R. rugosa* was chosen because the genes of this species have not been introduced into the lineage of modern floricultural cultivars (16). Mildew isolates from cultivar Dr. Huey and *R. multiflora* Thunb. were studied because these two roses are grown as rootstocks. The isolate on cultivar Red Cascade was selected primarily because it appeared to be extremely virulent on that cultivar.

A detached-leaflet culture technique was used to establish monoconidial isolates. Leaf material was removed from plants which were maintained free of mildew in isolation chambers (4). Leaflets were mounted on the adhesive side of a 2.5 × 7.5-cm piece of waterproof tape in which six 6-mm-diameter holes were punched. The tape was mounted on a microscope slide in a petri dish which served as a reservoir. Water was supplied to the tissue

TABLE 1. Identification and origin of nine isolates of *Sphaerotheca pannosa* var. *rosae*

Isolate	Rose host	Location of planting	
		Field	Greenhouse
1	SR 70002/2	X	
2	Red Cascade		X
3	Pink Parfait		X
4	Dr. Huey	X	
5	*Rosa multiflora*	X	
6	Mary Devor		X
7	*R. rugosa*	X	
8	Tropicana	X	
9	Samantha		X

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through a wick made of untreated cotton twine. Single conidia were transferred to detached leaf material by using the method described by Coyier (5). Colonies were usually visible in 7–10 days. Conidia were then dispersed with a camel's-hair brush to susceptible tissue of host plants maintained in isolation chambers. One isolate from each host was maintained as a stock culture on the original host cultivar.

Cultivars. In preliminary tests, isolates 1–5 and 9 were tested on the host from which they were originally isolated. These particular isolates were suspected of differential adaptation to their original hosts. The absence of sporulation or presence of necrosis which prevented or limited sporulation was considered to be evidence of host resistance to an isolate. The production of sporulating lesions without host necrosis indicated host susceptibility. With the exception of cultivars Pink Parfait and Samantha, each test rose differed from the others in its reaction to the six isolates. Pink Parfait and the remaining four cultivars were selected for use in the identification of races.

Roses were grown in 13-cm-diameter plastic pots in a steam-treated mixture of sand, soil, vermiculite, and peat (1:1:1:1, v/v). The plants were watered biweekly with a commercial soluble fertilizer and were 4–8 mo old when virulence tests were performed.

Virulence tests. Plants selected for virulence tests were defoliated, cut back to approximately 10 cm and surface sterilized in 0.5% sodium hypochlorite for 10 min. Isolation chambers were constructed for each plant to prevent entry of contaminating conidia. Preliminary tests showed that leaves that were no longer expanding were more resistant to powdery mildew. Therefore, plant canopies were thinned prior to virulence tests in order to distribute inoculum uniformly across adaxial surfaces of susceptible leaves. Plants were inoculated approximately 3–4 wk after defoliation when they were in an active growth phase.

Conidia for virulence tests were collected by removing shoots with three to six heavily infected leaves from plants hosting the desired isolate. The number of infected leaves and sporulating area per leaf were adjusted to deliver a similar inoculum load in all tests. Plants were inoculated by vigorously shaking an infected shoot inside the isolation chamber containing plants of the differential cultivar. The air flow to the isolation chambers was withheld 24 hr after inoculation to increase relative humidity (RH) during the spore germination period, after which air flow was gradually restored.

Inoculated plants were maintained in a plant growth room where the temperature was 21 ± 3 C and RH was 45–55%. RH within plant chambers ranged from 40 to 90%. The room was illuminated 12 hr daily with both fluorescent and incandescent lamps with approximately 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at plant level.

Seven days after the introduction of inoculum, the chambers were opened and the reactions of the cultivars were recorded. Only leaflets in an active state of expansion at the time of inoculation were considered in the assessment of isolate virulence. Leaves were examined for necrosis and sporulation. Differences in sporulation were evaluated with an experimental design similar to that used by Rufty et al (15). Host susceptibility was measured as a percentage of susceptible tissue covered with sporulating colonies (mean of eight observations) and was assessed by comparing mildew on adaxial surfaces with standard diagrams (13). Infections that covered more than 50% of the leaf area were estimated visually. Treatments were replicated three times and consisted of inoculating the five cultivars with a given isolate. Percentage values were analyzed according to a split-plot design with isolates as main plots and cultivars as subplots. The data were pooled into a combined analysis over all experiments.

RESULTS

Pathogenic variability. Analysis of variance indicated highly significant differences ($P < 0.001$) in the portion of leaf area which supported sporulating mildew colonies among isolates, cultivars, and in the isolate × cultivar interaction (Table 2). Isolates 1, 2, and 8 sporulated on all five cultivars; isolates 3, 6, 7, and 9 sporulated on four cultivars; and isolates 4 and 5 sporulated on two cultivars. Host resistance prevented sporulation of six isolates on SR 70002/2, two on Pink Parfait, and one isolate on Red Cascade and *R. multiflora*. All isolates sporulated on cultivar Dr. Huey.

Sporulation of isolates 1, 2, and 8 was greater on SR 70002/2 than on the other four roses. Isolates 3, 6, 7, and 9 sporulated most profusely on Pink Parfait, and sporulation of isolates 4 and 5 was greater on Dr. Huey and *R. multiflora*, respectively. These differences in ranking contributed to a highly significant difference in the isolate × cultivar interaction.

Host reactions were rated on a scale which was based on the presence or absence of host necrosis and the degree of fungus sporulation (Table 3). Reaction types 0–2 were regarded as "resistant" responses, and 3–5 as susceptible (Fig. 1). Susceptible and resistant reactions on the rose hosts differentiated the isolates into five pathogenicity groups. The magnitude of differences among these groups suggested that they be classified as separate races.

Race classification. Loegering and Browder's (11) modification of Green's "virulence formulae" (8) was adapted for description of rose powdery mildew races. Red Cascade and Pink Parfait had reacted similarly rather than differentially to the nine isolates (Table 3). Therefore, only four of the rose cultivars were needed to distinguish isolates of the fungus. SR 70002/2, Pink Parfait, Dr. Huey, and *R. multiflora* were numbered sequentially (Table 4) and the virulence formula of each race was described on these cultivars. Virulence formulae 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. Cultivars were listed in order of increasing susceptibility with the most resistant cultivar on the far left and the most susceptible on the extreme right. A race

TABLE 2. Sporulation of nine isolates of *Sphaerotheca pannosa* var. *rosae* on five rose hosts

Isolate	Leaf area covered by sporulating colonies (%)[a]					Mean
	SR 70002/2	Red Cascade	Pink Parfait	Dr. Huey	*Rosa multiflora*	
1	39.5 ± 4.1[b]	1.5 ± 1.3	3.3 ± 3.8	23.0 ± 17.3	17.3 ± 6.3	16.9
2	38.6 ± 4.4	10.5 ± 3.2	17.3 ± 7.0	14.4 ± 5.6	13.6 ± 8.9	18.8
3	0.0 ± 0.1	10.1 ± 2.8	15.8 ± 5.1	3.1 ± 0.4	3.7 ± 0.3	6.5
4	0.0 ± 0.1	1.3 ± 1.2	0.0 ± 0.0	36.1 ± 11.2	0.1 ± 0.1	7.5
5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 3.4	37.3 ± 1.9	9.2
6	0.0 ± 0.1	9.0 ± 2.8	19.0 ± 9.5	4.9 ± 1.4	2.9 ± 1.0	7.1
7	0.1 ± 0.1	11.1 ± 1.5	14.0 ± 3.1	4.2 ± 1.4	2.9 ± 0.6	6.5
8	33.3 ± 8.8	2.5 ± 1.4	2.7 ± 2.4	24.2 ± 7.3	15.1 ± 1.9	15.6
9	0.0 ± 0.0	10.9 ± 4.7	20.2 ± 7.5	4.5 ± 1.5	3.3 ± 1.2	7.8
Mean	12.4	6.3	10.2	13.7	10.7	
LSD for isolates	($P = 0.05$) = 11.2[c] ($P = 0.01$) = 15.5		LSD for cultivars	($P = 0.05$) = 8.0[d] ($P = 0.01$) = 10.7		

[a] Means of three replicates.
[b] Standard error of the mean.
[c,d] LSD values are to be used for comparing isolates within cultivars and cultivars within isolates, respectively.

TABLE 3. Reaction type[a] of five rose hosts to nine isolates of *Sphaerotheca pannosa* var. *rosae*

Isolate	SR 70002/2	Red Cascade	Pink Parfait	Dr. Huey	*Rosa multiflora*
1	5	1–2	1–2	3–5	4
2	5	3–4	3–4	3–4	3–4
3	0	3–4	3–4	3	3
4	0	1–2	1	5	0
5	0	0	1	3–4	5
6	0	3–4	3–5	3	3
7	0	3–4	4	3	3
8	5	2	1–2	4–5	4
9	0	3–4	4–5	3	3

[a] Reaction type rating scale: 0 = no macroscopic evidence of fungus colonization; 1 = necrosis, no production of secondary conidia; 2 = necrosis, slight production of conidia; 3 = no necrosis, sporulating colonies cover 1–10% of leaf area; 4 = no necrosis, sporulation covers 11–25% of leaf area; and 5 = no necrosis, sporulating colonies cover > 25% of leaf area.

abbreviation was assigned to each virulence formula to facilitate discussion of the results.

DISCUSSION

In this study, analysis of variance indicated a highly significant isolate × cultivar interaction ($P < 0.001$), and the ranking of cultivars in order of susceptibility changed with each of the five

TABLE 4. Classification of nine isolates of *Sphaerotheca pannosa* var. *rosae* into five races on four rose hosts

Isolate	Virulence formula (resistant/susceptible)	Race abbreviation
1, 8	2/4,3,1[a]	1
2	/4,3,2,1	2
3, 6, 7, 9	1/4,3,2	3
4	1,4,2/3	4
5	1,2/3,4	5

[a] Rose hosts: 1 = SR 70002/2, 2 = Pink Parfait, 3 = Dr. Huey, and 4 ÷ *Rosa multiflora*.

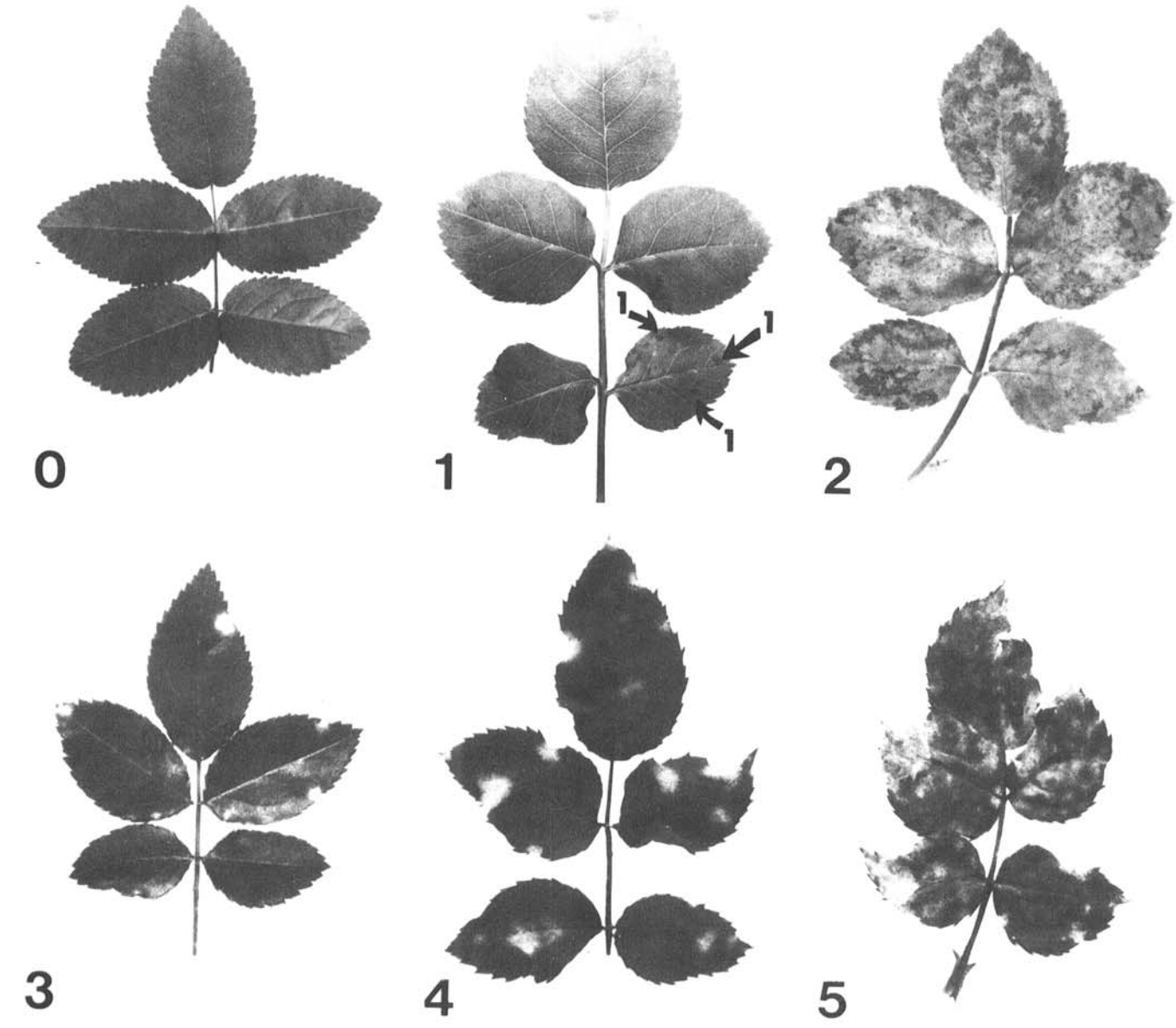

Fig. 1. Classification system for reaction of rose hosts to *S. pannosa* var. *rosae*: Reaction type 0, no macroscopic evidence of fungus colonization; reaction type 1, necrotic lesions (*1*), no production of secondary conidia; reaction type 2, necrotic lesions, slight production of secondary conidia; reaction type 3, sporulating lesions cover 1–10% of leaf area, no host necrosis; reaction type 4, sporulating lesions cover 11–25% of leaf area, no necrosis; and reaction type 5, sporulating lesions cover more than 25% of leaf area, no necrosis.

groups of isolates. The latter is evident in the virulence formula of each race, which enumerates the cultivars in order of increasing susceptibility (Table 4). These two approaches provide evidence of specific resistance in the rose hosts and differential adaptation of the fungus isolates (6,14,17,18).

Field-grown plants of SR 70002/2 remained virtually immune for a period of 8 yr. Therefore, virulence for this host suggested the presence of a "new" race. However, isolates from Tropicana and Red Cascade both attacked SR 70002/2, which demonstrated that virulence for this host was already present in the pathogen population. The identification of race 1 on both Tropicana and SR 70002/2 suggests the transmission of a gene(s) for reaction type from Tropicana to SR 70002/2, from which the latter is descended. It is interesting to note that Tropicana (synonym Super Star) was regarded as highly resistant when first introduced in Britain (19). Although it now becomes substantially mildewed in that country and in many areas of the United States, Tropicana remains very resistant in other locations (1,7,9).

Races 4 and 5, isolated from Dr. Huey and *R. multiflora*, respectively, were both avirulent on SR 70002/2 and Pink Parfait. Rootstocks, unlike modern commercial roses, are not complex interspecific hybrids of seven or eight species. Also, the selection criteria which govern performance in a rootstock and a rose grown for floricultural purposes are quite different. These factors may have contributed to a difference in the genetic susceptibility of the two rootstocks compared with that of the seedling rose and plants of Pink Parfait.

The existence of races of *S. pannosa* var. *rosae* has not previously been conclusively demonstrated. The identification of rose powdery mildew races will be useful in developing cultivars with improved resistance to the fungus. The pathogenicity markers described in this investigation will facilitate future genetic studies of *S. pannosa* var. *rosae*.

LITERATURE CITED

1. Atkiss, L. 1978. Disease resistant rose varieties. Am. Rose Annual 63:99-104.
2. Bender, C. L., and Coyier, D. L. 1982. Identification of five races of *Sphaerotheca pannosa* var. *rosae*. (Abstr.) Phytopathology 72:983.
3. Coyier, D. L. 1961. Biology and control of rose powdery mildew. Ph.D. thesis, University of Wisconsin, Madison. 109 pp.
4. Coyier, D. L. 1973. A portable, single plant isolation chamber. Can. J. Plant Sci. 53:915-916.
5. Coyier, D. L. 1974. Heterothallism in the apple powdery mildew fungus, *Podosphaera leucotricha*. Phytopathology 64:246-248.
6. Day, P. R. 1974. Genetics of Host-Parasite Interaction. W. H. Freeman, San Francisco. 238 pp.
7. Deshpande, G. D. 1980. A note on the varietal reaction of hybrid tea roses to powdery mildew. Indian J. Mycol. Plant Pathol. 9:120.
8. Green, G. J. 1965. Stem rust of wheat, rye, and barley in Canada in 1964. Can. Plant Dis. Surv. 45:23-29.
9. Johnson, L. F. 1972. Susceptibility of rose cultivars to blackspot and powdery mildew. Am. Rose Annual 57:74-87.
10. Kable, P. F., Fried, P. M., and MacKenzie, D. R. 1980. The spread of a powdery mildew of peach. Phytopathology 70:601-604.
11. Loegering, W. Q., and Browder, L. E. 1971. A system of nomenclature for physiologic races of *Puccinia recondita tritici*. Plant Dis. Rep. 55:718-722.
12. Mence, M. J., and Hildebrandt, A. C. 1966. Resistance to powdery mildew in rose. Ann. Appl. Biol. 58:309-320.
13. Price, T. V. 1970. Epidemiology and control of powdery mildew (*Sphaerotheca pannosa*) on roses. Ann. Appl. Biol. 65:231-248.
14. Robinson, R. A. 1969. Disease resistance terminology. Rev. Appl. Mycol. 48:593-606.
15. Rufty, R. C., Hebert, T. T., and Murphy, C. F. 1981. Variation in virulence in isolates of *Septoria nodorum*. Phytopathology 71:593-596.
16. Shepherd, R. E. 1954. History of the Rose. MacMillan, New York. 264 pp.
17. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, New York. 206 pp.
18. Vanderplank, J. E. 1978. Genetics and Molecular Basis of Plant Pathogenesis. Springer-Verlag, Berlin, Heidelberg, and New York. 167 pp.
19. Wheeler, B. E. J. 1978. Powdery mildews of ornamentals. Pages 411-445 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, New York.
20. Yarwood, C. E. 1952. Apricot powdery mildew from rose and peach. Calif. Dep. Agric. Bull. 41:19-25.