AN ABSTRACT OF THE THESIS OF

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Title: PATHOGENIC SPECIALIZATION AND HETEROTHALLISM IN SPHAEROTHECA PANNOSA VAR. ROSAE

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Dr. Duane L. Coyier

Studies were conducted on pathogenic specialization and heterothallism in Sphaerotheca pannosa var. rosae, causal agent of rose powdery mildew.

Methods were developed for isolation and identification of Sphaerotheca pannosa var. rosae races on rose. Monoconidial isolates were initiated on detached leaves, increased on host plants, and inoculated to rose cultivars to obtain 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 cultivars.

The susceptibility of detached rose leaves to S. pannosa var. rosae isolates of unknown and known virulence was assessed on physiologically similar tissue and compared with results on attached leaves. The correlation between inoculation to detached and attached leaves varied with the host and host-pathogen interaction. Detached leaves are useful in screening for differences in host specificity, but should not be used exclusively.

Virulence patterns were studied in several conidial populations of S. pannosa var. rosae. Although pathogenic variation was greater in the field, repeated sampling of both field and greenhouse locations demonstrated that rose powdery mildew populations are dynamic. Two populations consisted of more than one race; all others were racially homogenous. In one population, the presence of cleistothecia and ratio of pathotype occurrence suggested the meiotic recombination of two virulence factors.

The sexuality of S. pannosa var. rosae was investigated. Nine monoconidial isolates were coinoculated to four rose hosts in 11 combinations. Heterothallism was demonstrated by the strong fruiting response which occurred when an isolate designated as RR1 was paired with two other isolates. In the nine combinations where cleistothecia did not form, the coinoculated isolates differed in host specificity and were reisolated when the experiment was terminated. The results indicated that the virulence of compatible strains on a mutual host was necessary for ascocarp formation, and that fruiting may require physiologic stimulation by the host.

Pathogenic Specialization and Heterothallism in *Sphaerotheca pannosa* var. *rosae*

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PATHOGENIC SPECIALIZATION AND HETEROTHALLISM IN *SPHAEROTHECA PANNOSA* VAR. *ROSAE*

INTRODUCTION

Rose powdery mildew, which is incited by *Sphaerotheca pannosa* ({Wallr.} Fr.) Lév. var. *rosae* Wor., is a serious disease problem on roses grown for the cut-flower market. The mechanisms and extent of variation in the fungus are unknown. In the performance surveys that are published periodically in the *American Rose Annual*, the powdery mildew resistance ratings of individual cultivars vary both geographically and temporally. The severity of powdery mildew epidemics also varies considerably when the reports of commercial rose growers are compared. Although these differences allude to pathogenic specialization in the fungus, the existence of rose powdery mildew races is not well documented in the literature. Variation in the fungus was first reported by Coyier (1961), who demonstrated the existence of two pathogenic strains, i.e. one which was virulent on *Rosa virginiana* Mill., and a second which attacked modern commercial hybrids. The most recent report of variation in the fungus was published by Mence and Hildebrandt (1966) and is an extension of Coyier's work. Observations in both reports (Coyier, 1961; Mence and Hildebrandt, 1966) were made on detached shoots rather than intact plants and without the use of monoconidial isolates.

Rose breeders need information on pathogenic specialization to improve their efforts in breeding for powdery mildew resistance. New rose cultivars are continually released which are initially very

resistant to powdery mildew. These later succumb to infection, presumably due to the development of new races of the fungus. Knowledge of pathogenic variation is also needed to explain why epidemics vary in severity.

S. pannosa var. rosae is an obligate parasite which cannot be grown on artificial media. This dictates the use of host tissue as a culture substrate. The use of detached leaves is often necessitated due to the logistics of using intact plants. The advantages of detached-leaf culture were reviewed by Yarwood (1946) and include economy of space, host material, and inoculum. However, authors who used detached rose leaves to study pathogenic specialization and host susceptibility to S. pannosa reported difficulties in interpretation of the results (Coyier, 1961; Mence and Hildebrandt, 1966). Therefore, the value of in vitro (detached leaf) results in predicting in vivo (attached leaf) susceptibility needs to be investigated.

Knowledge of pathogenic variation within fungal populations provides valuable information on how changes are occurring and how stable they are. The latter information is especially important to the breeder in interpreting the value of a particular resistance source. The generation of new rose powdery mildew strains through meiotic recombination has not been demonstrated, largely because the pathogenicity of ascospore infections has not been established. Most members of the Erysiphaceae are heterothallic, i.e. two mating types are necessary for production of the cleistothecial (sexual) stage. However, the formation of cleistothecia by S. pannosa is both infrequent and erratic, and they have not been produced under

controlled conditions. The development of methods for cleistothecial induction is necessary if their role in the epidemiology of *S. pannosa* is to be understood.

CHAPTER 1

THE ISOLATION AND IDENTIFICATION OF RACES IN *SPHAEROTHECA PANNOSA* VAR. *ROSAE*

ABSTRACT

Methods were developed for isolation and identification of *Sphaerotheca pannosa* var. *rosae* races on rose. Monoconidial isolates were initiated on detached leaves, increased on host plants, and inoculated to rose cultivars to obtain 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 cultivars. One isolate was virulent on all four differentials and attacked two of the cultivars at levels comparable to simple races. Races isolated from rootstocks were avirulent on hybrid tea roses. The pathogenicity markers described in this investigation will facilitate future genetic studies of rose powdery mildew.

INTRODUCTION

Pathogenic specialization of *Sphaerotheca pannosa* ([Wallr.] Fr.) Lév. var. *rosae* Wor., causal agent of powdery mildew on rose (*Rosa* spp.), has not been extensively studied. Yarwood (1952) reported the occurrence of two strains of *S. pannosa* which differed in the size of lesions initiated on apricot fruit (*Prunus armeniaca* L.). Kable *et al* (1980) suggested the occurrence of a race of powdery mildew 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. On detached shoots and leaves, Coyier (1961) and Mence and Hildebrandt (1966) 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 Rosa rugosa Thunb. These authors did not use monoconidial isolates to establish pathogenic specialization or confirm their observations in vivo 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, Oregon. This seedling, abbreviated SR 70002/2 in this paper, was developed by Walter Lammerts and has been used as a source of resistance in his breeding program. During the eight years prior to the 1980 growing season, infection on this rose had been limited to small lesions on the hypanthium. 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 races of S. pannosa var. rosae which occur on roses. A preliminary report of the results has been published (Bender and Coyier, 1982).

MATERIALS AND METHODS

Isolates. Nine monoconidial isolates of S. pannosa var. rosae were studied intensively. These isolates were obtained from infected field and greenhouse plantings of the rose cultivars and species

listed in Table 1-1. All field collections were made from naturally infected plants in Benton County, Oregon. With the exception of the isolate from Red Cascade, all greenhouse collections were made from infected plants at the Horticultural Crops Research Laboratory in Corvallis, Oregon. The Red Cascade isolate was cultured from infected plants received from a grower in Clackamas County, Oregon.

Selection of these isolates involved consideration of their potential to be genetically different. Isolates on SR 70002/2 and Tropicana were of interest because these roses were genetically related, and both were noted for their mildew resistance when first introduced to most geographic locations. Isolates from Pink Parfait, Mary Devor, and Samantha were collected as representative of races which 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 varieties (Krüssmann, 1981). Mildew isolates from Dr. Huey and R. multiflora Thunb. were studied because these two roses are grown as rootstocks. The different selection criteria governing performance in rootstocks and floricultural varieties could contribute to the genetic difference in susceptibility. The isolate on the miniature rose Red Cascade was selected primarily because it appeared extremely virulent on this cultivar.

Monoconidial isolates of the fungus were cultured on detached leaves of the same cultivar or species from which a collection was made. Leaf material was prepared for detached leaf culture by mounting tissue on waterproof tape with holes (Coyier, unpublished). The tape was then mounted on a microscope slide and placed in a petri

dish. Water was supplied to the tissue through a wick from a reservoir. Single conidia were transferred to detached leaf material using the method described by Coyier (1974). 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 (Coyier, 1973). One isolate from each host was maintained as a stock culture on the original host variety.

Cultivars. In preliminary tests, detached leaves of SR 70002/2, Red Cascade, Pink Parfait, Samantha, Dr. Huey, and R. multiflora were tested for reaction to isolates 1-5 and 9. These particular isolates originated from the six named roses and were suspected of differential adaptation to their original hosts. The absence of sporulation or presence of necrosis which prevented or limited sporulation was considered as evidence of host resistance to an isolate. The production of sporulating lesions without host necrosis indicated host susceptibility. With the exception of Pink Parfait and Samantha, each test rose differed from the others in its reaction to the six isolates. Pink Parfait and Samantha reacted identically to the isolates tested; therefore, Samantha was arbitrarily excluded as a differential cultivar. 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:peat (1:1:1:1, v/v). The plants were watered biweekly with a commercial soluble fertilizer and were 4-8 months 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 minutes. Isolation chambers were constructed for each plant to prevent entry of contaminating conidia. Preliminary tests showed that leaves which 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.

Conidia for virulence tests were collected by removing shoots with 3-6 heavily infected leaves from plants hosting the desired isolate. The number of infected leaves used in performing each test was a function of leaf size, i.e. more leaf material was removed from the miniature variety Red Cascade than the hybrid Pink Parfait. Plants were inoculated by vigorously shaking an infected shoot inside the isolation chamber of the differential cultivar. The air flow to the isolation chambers was withheld 24 hr after inoculation to increase relative humidity during the spore germination period, after which air flow was gradually restored.

Inoculated plants were maintained in a growth room where the temperature was 21° (±3) C and RH was 45-55%. Relative humidity within plant chambers ranged from 40-90%. The room was illuminated 12 hr daily with both fluorescent and incandescent lamps at an intensity of approximately 100 μE measured at plant level.

Seven days after introduction of inoculum, the chambers were opened and the reactions of the cultivars recorded. Leaves were visually examined for presence or absence of necrosis and fungus sporulation was noted. The amount of sporulation was assessed by comparing mildew on adaxial surfaces with standard diagrams (Price, 1970). Infections which covered more than 50% of the leaf area were estimated

visually. Differences in sporulation were evaluated with an experimental design similar to that used by Rufty et al (1981). Host susceptibility was measured as a percentage of susceptible tissue covered with sporulating colonies (mean of 8 observations). Percentage values were analyzed according to a split-plot design with isolates as main plots and cultivars as subplots. Treatments were replicated three times and consisted of inoculating the five cultivars with a given isolate. The data were pooled into a combined analysis over all experiments.

RESULTS

Pathogenic variability. Analysis of variance indicated highly significant differences ($P < 0.001$) among isolates, cultivars, and in the isolate x cultivar interaction (Table 1-2). Isolates differed significantly in their ability to sporulate on the cultivars. Isolates 1, 2, and 8 sporulated on all five cultivars, isolates 3, 6, 7, and 9 sporulated on four, and isolates 4 and 5 sporulated on two cultivars. Cultivars differed significantly in resistance to the isolates. Cultivar 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 Dr. Huey.

Isolates 1, 2, and 8 were more virulent on SR 70002/2 than on the other four cultivars. Isolates 3, 6, 7, and 9 were more virulent on Pink Parfait, and isolates 4 and 5 on Dr. Huey and R. multiflora, respectively. These differences in ranking contributed to a highly significant difference in the isolate x cultivar interaction.

Host reactions were classified into several qualitative categories

when leaves were examined macroscopically: (1) very resistant with no visible sign of fungus infection, (2) moderately resistant with necrosis which prevented or limited sporulation, and (3) susceptible with sporulation and no cell death. Host reaction ratings (Table 1-3) were combined with the sporulation data (Table 1-2) to define each host-parasite interaction.

Reaction types 0-2 were regarded as 'resistant' responses, and 3-5 as susceptible (Fig. 1-1). Some of the isolates caused similar reactions on the five cultivars. Isolates 1 and 8 were avirulent on Red Cascade and Pink Parfait, but virulent on SR 70002/2, Dr. Huey, and R. multiflora. Isolates 3, 6, 7, and 9 were virulent on four of the cultivars, but avirulent on SR 70002/2. Isolates 2, 4, and 5 caused unique reactions on each of the five cultivars and could not be grouped. The cultivars separated the isolates into five pathogenicity groups and the magnitude of differences among the groups suggested they be classified as separate races.

Race classification. Browder et al (1980) suggested that the term "race" describe an abstract group of individuals having specified characteristics in common. For the purposes of this investigation, a race is considered to be a group of individuals with pathogenicity characteristics in common. Loegering and Browder's (1971) modification of Green's "virulence formulae" (1965) 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 1-3). 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 1-4) 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 abbreviation was assigned to each virulence formula to facilitate discussion of the results.

DISCUSSION

Vanderplank (1968) suggested that a significant isolate x variety interaction in the analysis of variance indicates the presence of specific resistance; i.e. the pathogen variants are differentially adapted to specific host cultivars. However, Johnson and Taylor (1976) showed that an isolate x cultivar interaction may be significant in the analysis of variance for purely arithmetical reasons. Therefore, Vanderplank (1978) recommended an analysis of variance to test for the absence of vertical resistance and ranking methods to demonstrate its presence. Evidence for differential interactions exists if a change in ranking of the susceptibility of cultivars results from interactions with pathogen isolates (Robinson, 1969).

In this study, analysis of variance indicated a highly significant isolate x cultivar interaction ($P < 0.001$), and the ranking of cultivars in order of susceptibility changed with each of the five groups of isolates. The latter is evident in the virulence formula of each race, which enumerates the cultivars in order of increasing susceptibility (Table 1-4). These two approaches provide evidence of

specific resistance in the Rosa hosts and differential adaptation of the fungus isolates.

There are also differences among the cultivars in the degree of host-pathogen specificity. The vertical resistance of SR 70002/2 is associated with sharp host-pathogen specificity and there is a clear distinction between susceptible and resistant reactions (Table 1-3). However, the absence of sharply defined host-pathogen specificity within the other cultivars is reflected in the subdivision of resistant and susceptible reaction classes.

Field grown plants of SR 70002/2 remained virtually immune for a period of eight years. 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 the seedling rose, from which the latter descended. It is interesting to note that Tropicana (syn. Super Star) was regarded as highly resistant when first introduced in Britain (Wheeler, 1978). Although it now becomes substantially mildewed in that country and in many areas of the United States, Tropicana remains very resistant in other locations (Atkiss, 1978; Deshpande, 1980; Johnson, 1972).

Race 2 is a complex race with virulence on all four differential cultivars. Reduced aggressiveness and reduced competitive ability have been associated with races which carry unnecessary genes for virulence on hosts lacking the corresponding genes for race-specific resistance (Vanderplank, 1968). The reduced fitness of complex races

contrasts with the increased fitness of simple races with few genes for virulence. Decreased aggressiveness is associated with the unnecessary virulence of race 2 on Dr. Huey and R. multiflora. This compares with races 4 and 5 which are differentially adapted to Dr. Huey and R. multiflora, respectively. Race 2 also carries unnecessary virulence on SR 70002/2 and Pink Parfait. However, this race infected these two differentials at levels comparable to races 1 and 3, which originated from SR 70002/2 and Pink Parfait, respectively. Therefore, the complexity of race 2 is not correlated with reduced aggressiveness on SR 70002/2 and Pink Parfait.

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. Desirable characteristics in a rootstock include compatibility with scions, adaptability to different edaphic and climatic conditions, and ease of propagation (Buck, 1951). However, high yields and longevity, form, and color of blooms are chief considerations in floricultural roses (Krüssmann, 1981). These factors may have contributed to a difference in the genetic susceptibility of the two rootstocks as compared with SR 70002/2 and Pink Parfait.

The "infection type" of individual lesions is often used to assess resistance to biotrophic leaf pathogens (Parlevliet, 1979). The infection type describes the condition of the infection court in terms of colonization by the fungus and reaction of the host. In a

low infection type, fungus sporulation is prevented or restricted in quantity and host tissue may become necrotic or chlorotic. Infection types of higher magnitude describe pustules with decreasing amounts of necrosis and increasing intensities of sporulation.

Resistance is also evaluated in terms of disease severity, which is defined as the area of plant tissue affected by disease as a percentage of the total area assessed (James, 1974). Infection frequency, the proportion of spores that result in sporulating lesions, is a component of disease severity. However, infection frequency is not a component of infection type, which is measured on individual lesions.

The scale used to evaluate host reaction to S. pannosa var. rosae is a combination of both methods of assessment. Classes 0-2 are infection types which define resistant host reactions. In susceptible reactions where sporulation occurs without necrosis, classes 3-5 describe increasing increments of disease severity. The description of susceptibility to rose powdery mildew solely on infection type would fail to describe large differences in disease severity which are a function of infection frequency. Variation in disease severity of S. pannosa var. rosae has been attributed to leaf age (Longrée, 1939; Mence and Hildebrandt, 1966; Rogers, 1959) and host genotype (Deshpande, 1980; Mence and Hildebrandt, 1966). The results of this investigation indicate that pathogen genotype is also a factor in the amount of attack sustained.

Green (1965) used virulence formulae to classify races in terms of resistant/susceptible cultivars. This made possible the description of races before the reaction genotype of a cultivar was known. Differentials could be added or omitted as needed and the formulae

updated. Classic race identification has no means of adding a differential or not using any differential of a closed set (Browder et al, 1980). Virulence formulae provide additional information about the fungus when cultivars are enumerated in order of increasing susceptibility. A change in the ranking of a cultivar could indicate a corresponding change in pathogenicity of the fungus.

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 identification of races is also helpful in studies where genetic markers are needed. The pathogenicity markers described in this investigation will facilitate future genetic studies of S. pannosa var. rosae.

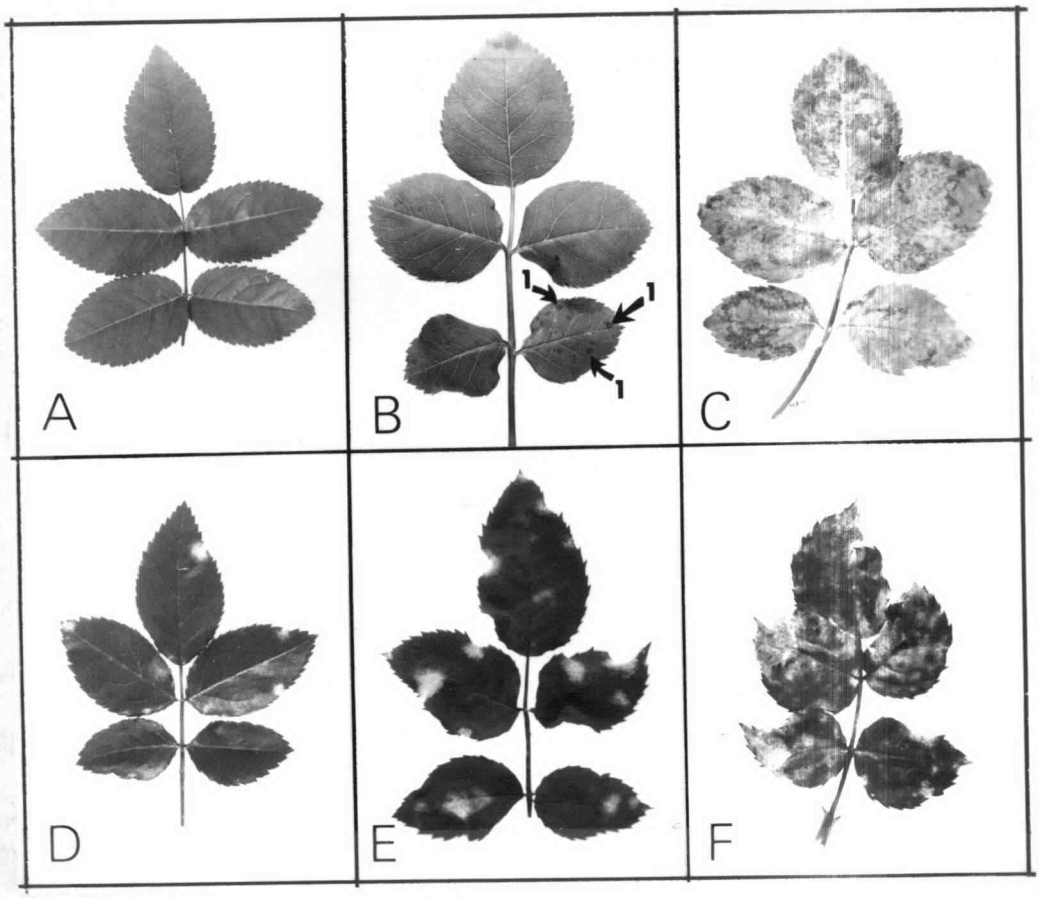

Figure 1-1. Classification system for reaction of Rosa hosts to S. pannosa var. rosae. **A**, reaction type 0, no macroscopic evidence of fungus colonization; **B**, reaction type 1, necrotic lesions (1), no production of secondary conidia; **C**, reaction type 2, necrotic lesions, slight production of secondary condidia; **D**, reaction type 3, sporulating lesions cover 1-10% of leaf area, no host necrosis; **E**, reaction type 4, sporulating lesions cover 11-25% of leaf area, no necrosis; **F**, reaction type 5, sporulating lesions cover more than 25% of leaf area, no necrosis.

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

Isolate No.	Rosa 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

Table 1-2. Sporulation of nine isolates of *Sphaerotheca pannosa* var. *rosae* on five *Rosa* hosts.

Isolate	Percent 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 Isolates: .05 = 11.2
.01 = 15.5

LSD Cultivars: .05 = 8.0
.01 = 10.7

[a]Means of three experiments

[b]Standard error of the mean

Table 1-3. Mean reaction type of five Rosa hosts to nine monoconidial 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

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 10-25% of leaf area

5 = no necrosis, over 25% of leaf area covered by sporulating colonies

Table 1-4. Classification of nine isolates of *Sphaerotheca pannosa* var. *rosae* into five races on four *Rosa* 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] 1 = SR 70002/2
2 = Pink Parfait
3 = Dr. Huey
4 = *Rosa multiflora*

CHAPTER 2

COMPARISON OF SUSCEPTIBILITY OF DETACHED AND ATTACHED ROSE LEAVES TO *SPHAEROTHECA PANNOSA* VAR. *ROSAE*

ABSTRACT

The susceptibility of detached leaves to isolates of unknown and known virulence was assessed on physiologically similar tissue and compared with results on attached leaves. The correlation between inoculation to detached and attached leaves varied with the host and host-pathogen interaction. Detached leaves are useful in screening for differences in host specificity, but should not be used exclusively.

INTRODUCTION

Members of the Erysiphaceae have been cultured on detached leaves in many studies of host range and varietal susceptibility. The advantages of detached leaves over the use of entire plants include economy of space, host material, and inoculum. However, when leaves are detached from plants, translocation is reduced or almost eliminated, and this is responsible for important differences in the physiology of detached and attached leaves (Yarwood, 1946). Studies of the comparative development of powdery mildews on detached leaves and on entire plants indicate that the two methods generally give similar results in regard to virulence of the pathogen and susceptibility of the host (Dundas, 1936; Lim, 1973; Stavely and Hanson, 1966; Wolfe, 1965). However, growth of the Erysiphaceae on detached leaves is frequently more luxuriant than development on the intact host plant (Dundas, 1936; Yarwood, 1946), and the results occasionally conflict (Yarwood, 1936).

Rose (Rosa spp.) leaves gradually increase in resistance to infection by Sphaerotheca pannosa ([Wallr.] Fr.) Lév. var. rosae Wor. as they mature and are almost completely resistant when fully expanded (Longrée, 1939; Mence and Hildebrandt, 1966; Rogers, 1959). Removal of rose leaves from the parent plant decreases or terminates the process of maturation, thereby allowing the fungus to become established more readily. Authors who used detached leaves to study pathogenic specialization and host susceptibility to S. pannosa reported difficulties in interpretation of the results. Coyier (1961) reported inconsistent results in the occurrence of secondary sporulation when inoculations were made to detached leaves. Mence and Hildebrandt (1966) observed variability in reaction type within individual leaves to a single source of inoculum.

In Erysiphe graminis DC., the formation of elongating secondary hyphae indicates a successful primary infection because there is a direct relationship between development of elongating secondary hyphae and functional haustoria (Masri and Ellingboe, 1966). In a preliminary investigation of pathogenic variation in S. pannosa var. rosae, the formation of elongating secondary hyphae was cited as evidence of initial compatibility (Bender and Coyier, 1981). However, hyphal elongation was not a reliable estimate of pathogen virulence in subsequent tests because it did not always correlate with sporulation of the rose powdery mildew fungus.

The reliability of detached rose leaves to predict in vivo susceptibility to S. pannosa var. rosae was studied in this investigation and included: (1) comparison of interactions on detached and attached leaf tissue, (2) definition of criteria useful in assessment

of compatibility on detached leaves, and (3) the ability of detached leaves to differentiate races of the fungus.

MATERIALS AND METHODS

Development of the fungus was studied on detached leaves of an unnamed seedling rose, Rosa multiflora Thunb., and Rosa cultivars, Red Cascade, Pink Parfait, and Dr. Huey. The unnamed seedling rose will be abbreviated SR 70002/2 in the remainder of this paper.

Experimental design. Yarwood (1946) suggested the use of opposite leaves or half leaves as a means of reducing variation among experimental units of plant material. This suggestion was incorporated into the experimental design as a means of reducing 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. Opposite leaflets of the miniature cultivar Red Cascade were paired because size made the use of half leaflets difficult. The susceptibility of each cultivar to isolates of the fungus was assessed by inoculating one member of a pair with a test isolate of unknown compatibility and the other with an isolate of known compatibility which originated from infections on that host. This method made possible comparison of a virulent reaction with an interaction of unknown compatibility and improved the interpretation of test results.

The isolates used in this study and the hosts from which they were obtained are listed in Table 2-1. The interaction of the seven isolates with living host tissue has been previously investigated (Chapter 1), and is included in Table 2-1 for comparison with in

vitro data. Isolates 1, 2, 3, 4 and 5 were used as isolates of known compatibility on SR 70002/2, Red Cascade, Pink Parfait, Dr. Huey, and R. multiflora, respectively, because they originated from infections on these hosts. A control test was performed on each variety by inoculating both members of a tissue pair with the compatible isolate on that host, i.e. isolate 1 on SR 70002/2, isolate 2 on Red Cascade, etc. These experiments were conducted to determine whether or not variability in reaction occurred within pairs to a single isolate. Isolates were maintained on the original host plant in isolation chambers (Coyier, 1973) until inoculum was needed for test on detached leaves.

Inoculation and incubation techniques. In preliminary tests, development of the fungus was evaluated on leaflets in several stages of maturity (Fig. 2-1).

Leaf material for pathogenicity tests was collected from plants which had been maintained free of mildew in isolation chambers. Leaf tissue was mounted on waterproof tape with holes (Coyier, unpublished). The tape was then mounted on a microscope slide and placed in a petri dish. Water was supplied to the tissue from a reservoir through a wick. Members of a leaf pair were mounted on separate slides and assigned a common number. Twenty-four hours prior to inoculation, compressed air was used to remove old conidia from the surface of plants hosting the desired isolates. Leaf disks were inoculated with freshly produced conidia using a soft camel's-hair brush sterilized in 95% ethanol. The brush was moved over the surface of the colony to pick up conidia and then gently stroked across the adaxial surface of the leaf disks to be inoculated. Inoculations

were done with a dissecting microscope. The density of the spore deposit on each leaflet was adjusted to approximately 100-125 conidia per cm^2 of leaf surface. Each test involved simultaneous inoculation of paired leaf tissue with the isolate of known compatibility and one of unknown compatibility to a particular cultivar. Each test involved 6-12 leaf tissue pairs, depending on the availability of leaf tissue. Inoculated disks were incubated in a moist chamber inside a growth room where the temperature was 21° (±3) C and RH 45-55%. The room was illuminated 12 hr daily with both fluorescent and incandescent lamps.

Development of S. pannosa. The initial development of S. pannosa on rose includes spore germination, formation and maturation of appressoria, production of a second germ tube and elongation of the first germ tube, initiation of haustoria, formation of a third germ tube, maturation of haustoria, and branching of germ tubes (Coyier, 1961; Price, 1970; Wheeler, 1978). Lengthening of the primary germ tube is not proof of primary infection compatibility in S. pannosa because hyphal elongation may occur within 8-10 hr after inoculation and prior to the formation of haustoria (Wheeler, 1978). However, hyphal branching occurs after the formation of haustoria (Wheeler, 1978) and indicates establishment of a functional, compatible relationship between host and pathogen. Therefore, the formation of branched hyphae indicated completion of a successful infection in this study.

Germination and infection of compatible and incompatible isolates were assessed 48 hr after inoculation. Germination was evaluated by examining 25 conidia on each disk. A conidium was rated germinated when a germ tube developed to the length of the conidium or greater. Only turgid and hyaline conidia were counted in the assessment of

germination. Conidia which are desiccated and/or crystalline do not normally germinate. As conidia were rated for germination, infection was assessed by recording the number of spores with branched hyphae.

Beginning at 92 hr after inoculation, leaves were examined for signs of sporulation. A quantitative assessment of sporulation was made when secondary conidia became macroscopically visible on leaflets inoculated with isolates of known compatibility. Leaf disks were examined for sporulation at X160 magnification using a Zeiss microscope provided with epi-illumination. Sporulation was assessed by observation of 25 colonies and recording the number which had produced secondary conidia.

Leaflet pairs were examined macroscopically again at ten days, and a qualitative description of isolate-cultivar interactions was made by noting the presence or absence of host necrosis and sporulation.

Statistical analysis. Germination, infection and sporulation of isolates of known and unknown compatibility were statistically analyzed using a two-tailed t-test for paired observations. Each test was performed twice and the results analyzed separately. The results of one test in which the compatibility of isolate 1 on detached leaf tissue of Red Cascade was examined are presented in Table 2-2.

Comparison of in vitro and in vivo inoculations. The inoculation of isolates to detached leaves was compared with results obtained when inoculations were made in vivo. A quantitative comparison of the two techniques was made by contrasting disease severity in vivo with the frequency of sporulation on detached leaves. Qualitative differences were studied by comparing the assignment of reaction type ratings on

living host plants with observations on the presence or absence of necrosis and sporulation on detached leaves. Differences in the development of the isolates were also noted by comparing *in vivo* and *in vitro* inoculations microscopically.

RESULTS AND CONCLUSIONS

Although germination was high (90-100%), sporulation was limited on very young rose leaflets (Fig. 2-1). Both germination and sporulation were reduced on leaflets which had expanded to their maximum size. Germination was high and sporulation luxuriant on leaflets which had unfolded but had expanded to less than one-half their potential size. Leaflets in this developmental stage were selected for use in detached leaf studies.

Quantitative analyses. Germination of test isolates on detached leaf tissue in each experiment ranged from 96-100% and was not significantly different from isolates of known compatibility. Hyphal branching and sporulation were used to quantify the compatibility of test isolates relative to isolates of known virulence (Tables 2-3 and 2-4). The absence of branched hyphae and a significant reduction in branching relative to the virulent isolate were associated with failure to sporulate and reduced sporulation, respectively. For example, the inability of isolate 5 to form branched hyphae on SR 70002/2 and Pink Parfait was followed by failure of this isolate to sporulate on these hosts. The reduced ability of isolate 1 to form branched hyphae on Red Cascade and Pink Parfait was associated with significantly less sporulation relative to virulent isolates on these hosts. There was no significant difference in germination, colony branching or

sporulation in the controls.

Comparison with in vivo inoculations. Inoculations to detached and attached leaves are compared in Table 2-5. A quantitative comparison was made by contrasting the percentage of colony sporulation on excised leaves with the percentage of leaf area supporting sporulation of the fungus in vivo. The significance of sporulation on attached leaves is presented in a manner similar to that for detached leaves, i.e. disease severity of a particular isolate-cultivar combination is compared with the interaction of known compatibility on that host.

The relationship between sporulation on detached leaves and disease severity on plants varied with the rose host. There was a direct relationship between sporulation in vitro and disease severity in vivo in inoculations to Pink Parfait and SR 70002/2. Test isolates which were compatible on these two hosts sporulated at frequencies not significantly different than the virulent isolate, i.e. isolates 2 and 8 sporulated at levels comparable to virulent isolate 1 on both attached and detached leaves of SR 70002/2. A significant reduction in sporulation on detached leaves of Pink Parfait and SR 70002/2 was followed by a reduction in disease severity on plants, i.e. sporulation of isolates 1 and 8 was significantly lower than virulent isolate 3 on attached and detached leaves of Pink Parfait.

Sporulation on detached leaves of the other three cultivars was not as reliable in predicting disease severity in vivo. Sporulation on detached leaves of Red Cascade and Dr. Huey was consistently higher than the amount of attack observed on attached leaves, and the significance of sporulation on detached leaves of Dr. Huey and Rosa multiflora was not consistent in vivo.

The occurrence of host necrosis and fungus sporulation on detached leaves was used to assign isolate-cultivar interactions to one or more qualitative categories (Table 2-5). The similarity of *in vivo* and *in vitro* results varied with the host and host-pathogen interaction. Reaction types on detached and attached leaves were in perfect agreement on SR 70002/2, but the reactions on detached leaves of the other four roses did not always agree with *in vivo* observations. For example, the necrotic lesions elicited by isolates 1 and 8 on attached leaves of Pink Parfait were absent when these inoculations were made to detached leaves. In several isolate-cultivar combinations, the infection type produced on host plants was homogenous, but two or more qualitative responses were observed *in vitro*. For example, the interaction of Red Cascade with isolates 1, 4, and 8 appeared incompatible on detached leaves where sporulation did not occur or necrotic lesions developed, and compatible where sporulation occurred with no cell death.

Identification of races on detached leaves. Reaction types (Table 2-5) were used to evaluate the ability of detached leaves to differentiate races of the fungus. Although the compatibility of certain isolate-cultivar interactions was impossible to assess, the presence of five races was suggested (Table 2-6). Three of the races could be distinguished by their interaction with SR 70002/2 and Pink Parfait. Virulence occurred for SR 70002/2 and Pink Parfait alone in races 1 and 3, respectively, and for both roses in race 2. Although the two remaining races were both avirulent on SR 70002/2 and Pink Parfait, they could be differentiated by their reactions on Dr. Huey and *R. multiflora*. Virulence occurred for Dr. Huey only in race 4 and for

Dr. Huey and R. multiflora in race 5.

Results of the present investigation suggest that inoculations to detached rose leaves be rated for hyphal branching and infection type to predict in vivo susceptibility to S. pannosa var. rosae. Data on hyphal branching are useful in quantifying pathogen development relative to an isolate of known virulence. An assessment of hyphal branching rather than sporulation is recommended for two reasons: (1) hyphal branching is much easier to measure, and (2) the amount of sporulation is a function of colony branching. Quantification of hyphal branching as morphological evidence of compatibility is similar to assessment of elongating secondary hyphae in the cereal mildews.

The occurrence of sporulating and necrotic infection types also aid in assessing host-pathogen compatibility. Necrotic lesions developed on detached leaves in several incompatible inoculations. This response involved large regions of tissue and was distinct from necrosis which involved single cells and was only microscopically visible. The latter reaction occurred in both compatible and incompatible host-parasite interactions and was not significantly higher in response to avirulent isolates. There was a correlation between the consistent occurrence of macroscopically visible sporulation on detached leaves and the compatibility of an interaction in vivo, i.e. compatible isolates sporulated in macroscopically visible quantities on each leaf disk inoculated. Sporulation which occurred on detached leaves in incompatible inoculations either occurred inconsistently in heterogenous reactions or was restricted to only a few conidial chains and not macroscopically visible. These relationships suggest

that leaf disks be rated on an infection-type basis for an improved estimate of pathogen virulence.

The data also indicate differences in the stage of pathogen development at which resistance was expressed in certain host-parasite interactions. Host resistance prevented the development of branched hyphae on SR 70002/2 tissue inoculated with avirulent isolates (Fig. 2-2a) and on Pink Parfait inoculated with isolate 5 (Fig. 2-2b). In the latter interaction, hyphal elongation occurred, but necrotic lesions developed and colony growth ceased. Virulent isolates produced branched hyphae on these hosts within 48 hr following inoculation (Fig. 2-2c). Resistance was effective in reducing but not preventing the formation of branched hyphae in several other host-parasite interactions. The correlation between establishment of branched hyphae and sporulation in most of the interactions suggests a common resistance mechanism. However, the reduced sporulation of several isolates on Dr. Huey was not preceded by lower infection rates, which suggests an additional resistance mechanism.

DISCUSSION

There are indications in the work of Bartlett (1963) of a relationship between amino acid content of rose leaves and their resistance to powdery mildew. Both old leaves and those of resistant cultivars contained higher levels of cysteic acid. Bartlett suggested that a high concentration of cysteic acid could reflect a lack of adequate cysteine for growth of the pathogen, since inhibition of cysteine synthesis could lead to accumulation of cysteic acid. Perhaps the increased susceptibility of detached leaves involves a

relationship between cysteine and cysteic acid which is affected when leaves are detached.

The use of opposite leaflets and half leaflets as experimental units did not eliminate all variability in this study. The significance of colony branching and/or sporulation was not always consistent when an experiment was repeated and variation in infection type occurred in certain isolate-cultivar interactions. However, inoculation of conidia to paired samples of leaf tissue made possible comparison of isolates of unknown compatibility with virulent isolates on physiologically similar tissue. This eliminated doubts about host tissue susceptibility since this could be determined prior to interpreting the compatibility of an unknown.

The infection types elicited by the seven isolates on detached leaves suggested the existence of five races of the fungus, although heterogeneous reactions obscured the compatibility of several isolate-cultivar combinations (Table 2-6). The existence of five races was confirmed in a previous investigation in which the interaction of the same isolates was studied on host plants (Table 2-1).

The utility of detached leaves in studies of pathogenic variation is a function of their correlation with interactions on the plant. Absolute differences in compatibility were lucid in some host-pathogen combinations on detached leaves, but ambiguous in others. Although detached leaves were useful in detecting pathogenic races, they should not be used exclusively. Detached leaves could be used to screen for differences in host specificity which can be evaluated in vivo.

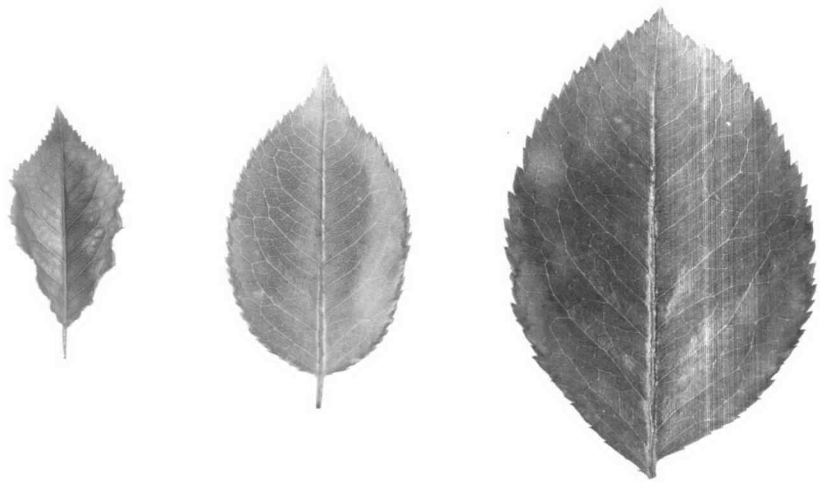

Figure 2-1. Rose leaflets tested for susceptibility to Sphaerotheca pannosa var. rosae. Very young leaflets (left) were not fully open, leaflets of intermediate age (center) were open but still expanding, and older leaflets (right) had expanded to their maximum potential size.

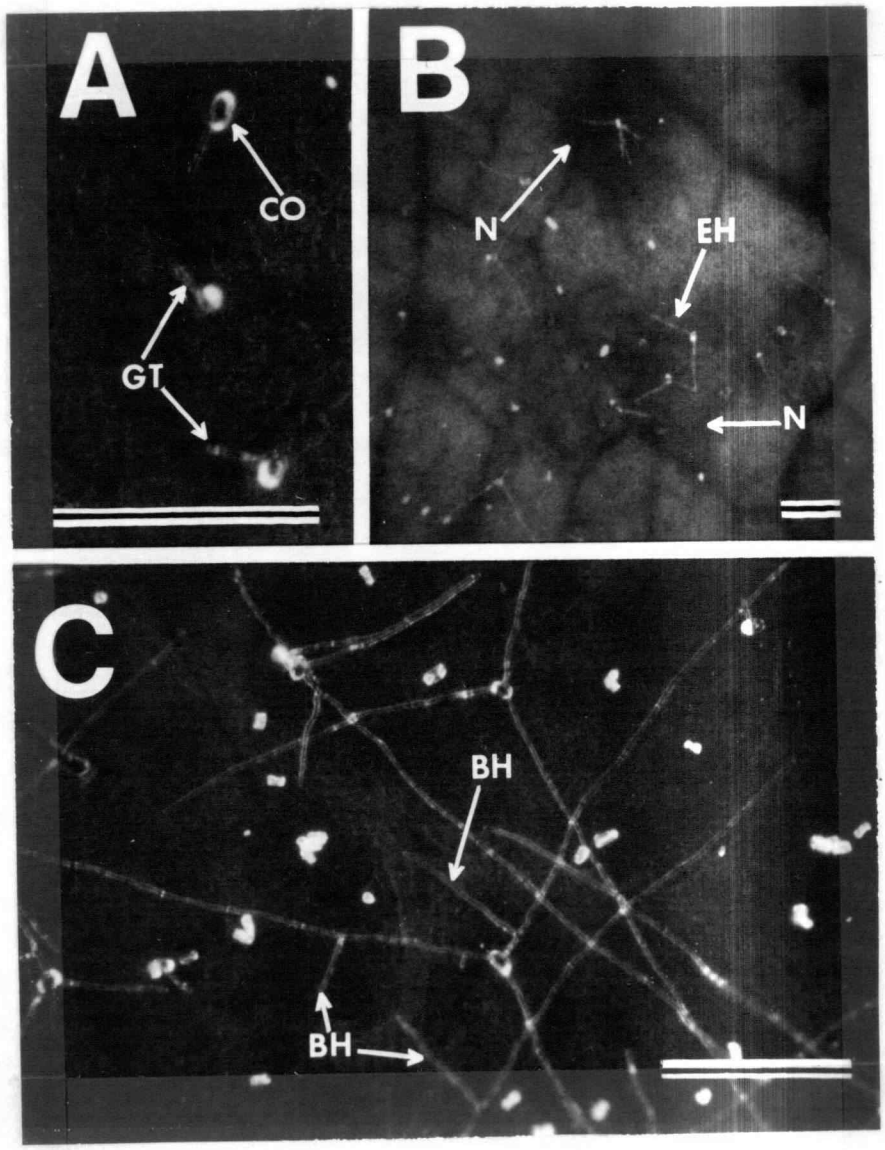

Figure 2-2. Development of avirulent and virulent isolates on detached leaves of SR 70002/2 and Pink Parfait 48 hr after inoculation. **A**, conidia of avirulent isolate 5 formed short germ tubes on SR 70002/2 but did not continue growing; **B**, avirulent isolate 5 conidia produced elongated hyphae on Pink Parfait, but necrotic lesions developed and colony growth ceased; and **C**, virulent isolate 3 conidia formed branched hyphae on Pink Parfait 48 hr after inoculation. Legend: CO = conidium, GT = germ tube, EH = elongated hypha, N = necrotic lesion, and BH = branched hypha. Scale bars represent 200 µm.

Table 2-1. The origin of seven *Sphaerotheca pannosa* var. *rosae* isolates selected for detached leaf studies and their *in vivo* reaction on five test roses.

		Reaction on Test Roses[a]					
Isolate	Original Host	SR 70002/2	Red Cascade	Pink Parfait	Dr. Huey	*Rosa multiflora*	Race Abbreviation
1	SR 70002/2	5	1-2	1-2	3-5	4	1
2	Red Cascade	5	3-4	3-4	3-4	3-4	2
3	Pink Parfait	0	3-4	3-4	3	3	3
4	Dr. Huey	0	1-2	1	5	0	4
5	*R. multiflora*	0	0	1	3-4	5	5
8	Tropicana	5	2	1-2	4-5	4	1
9	Samantha	0	3-4	4-5	3	3	3

[a]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, over 25% of leaf area covered by sporulating colonies.

Table 2-2. Germination, infection and sporulation on detached leaflet pairs of Red Cascade inoculated with isolates 1 and 2, which are of known and unknown compatibility, respectively.

EXPERIMENT 1

Leaflet Pair	No. of germinated conidia[a]		No. of conidia with branched hyphae[a]		No. of conidia with secondary sporulation[a]	
	Iso. 2	Iso. 1	Iso. 2	Iso. 1	Iso. 2	Iso. 1
1	25	25	9	2	12	0
2	25	25	17	3	13	8
3	23	25	18	6	16	3
4	24	25	20	12	10	0
5	25	25	17	4	14	1
6	24	23	7	5	8	6
7	25	25	13	4	8	6
8	25	25	17	7	15	1
9	25	24	6	0	3	0
Frequency (%)	98	99	55	19[b]	44	11[b]

EXPERIMENT 2

Leaflet Pair	No. of germinated conidia		No. of conidia with branched hyphae		No. of conidia with Secondary sporulation	
	Iso. 2	Iso. 1	Iso. 2	Iso. 1	Iso. 2	Iso. 1
1	25	24	12	4	12	4
2	24	25	4	0	2	0
3	24	25	8	12	6	1
4	25	25	12	4	16	0
5	25	25	11	8	11	6
6	24	23	10	7	11	2
7	23	22	12	2	10	0
8	22	25	15	7	9	1
9	25	24	5	6	6	2
Frequency (%)	96	97	40	22[b]	37	7[c]

[a] Out of 25 conidia counted.

[b,c] Significant at P = 0.05 and P = 0.01, respectively.

Table 2-3. The frequency of hyphal branching on detached leaflet pairs of five Rosa hosts inoculated with seven isolates of Sphaerotheca pannosa var. rosae.

	Test Rose[a]									
	SR 70002/2		Red Cascade		Pink Parfait		Dr. Huey		Rosa multiflora	
Isolate	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	72	71	19[b]	22[b]	3[c]	4[c]	51	49	88	48[c]
2	71	70	27	31	37	55	49	45	73	40[c]
3	0[c]	0[c]	33	50	34	46	22	48	45[c]	13[c]
4	0[c]	0[c]	30	12[c]	6[c]	3[b]	80	54	46[c]	55[b]
5	0[c]	0[c]	1[c]	1[c]	0[c]	0[c]	40[c]	37[c]	90	77
8	72	73	17[c]	22	1[c]	7[c]	51	40	4[c]	49[c]
9	0[c]	1[c]	50	47	38	41	37	35	12[c]	6[c]

[a] Values for each experiment are percentages based on observation of 6-12 leaf disks, 25 conidia per disk.

[b,c] Significantly less than the isolate of known virulence at P = 0.05 and P = 0.01, respectively.

Table 2-4. Sporulation frequency of seven Sphaerotheca pannosa var. rosae isolates on detached leaflet pairs of five rose hosts.

	Test Rose[a]									
	SR 70002/2		Red Cascade		Pink Parfait		Dr. Huey		Rosa multiflora	
Isolate	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	42	49	11[b]	7[c]	1[c]	1[c]	50	28	36	15[c]
2	31	36	25	27	19	23	7[c]	17[c]	1[c]	1[c]
3	0[c]	0[c]	20	16[b]	12	20	41	9[c]	1[c]	1[c]
4	0[c]	0[c]	18[b]	6[c]	3[c]	3[c]	63	58	15[c]	15[c]
5	0[c]	0[c]	0[c]	0[c]	0[c]	0[c]	14[c]	17[c]	58	69
8	42	41	14[c]	7[c]	0[c]	2[c]	42	23	0[c]	16[c]
9	0[c]	0[c]	16[c]	28	11	15	14[c]	8[c]	1[c]	1[c]

[a] Values for each experiment are percentages based on observation of 6-12 leaf disks, 25 conidia per disk.

[b,c] Significantly less than the isolate of known virulence at P = 0.05 and P = 0.01, respectively.

Table 2-5. Sporulation and infection type of seven rose powdery mildew isolates on detached and attached leaves of five test roses.

	Test Rose[a]									
	SR 70002/2		Red Cascade		Pink Parfait		Dr. Huey		Rosa multiflora	
Isolate[a]	Detached	Attached	Detached	Attached	Detached	Attached	Detached	Attached	Detached	Attached
1	46[b] (S)[c]	40[d] (S)	9[e] (X)	2[e] (MR)	1[e] (VR)	3[e] (MR)	39 (S)	23[e] (S)	26 (X)	17[e] (S)
2	34 (S)	39 (S)	26 (S)	10 (S)	21 (S)	17 (S)	12[e] (X)	14[e] (S)	1[e] (X)	14[e] (S)
3	0[e] (VR)	0[e] (VR)	18 (S)	10 (S)	16 (S)	16 (S)	25 (X)	3[e] (S)	1[e] (X)	4[e] (S)
4	0[e] (VR)	0[e] (VR)	12[e] (X)	1[e] (MR)	3[e] (MR)	0[e] (MR)	60 (S)	36 (S)	15[e] (VR)	0[e] (VR)
5	0[e] (VR)	0[e] (VR)	0[e] (VR)	0[e] (VR)	0[e] (VR)	0[e] (MR)	16[e] (S)	9[e] (S)	64 (S)	37 (S)
8	42 (S)	33 (S)	6[e] (X)	2[e] (MR)	1[e] (VR)	3[e] (MR)	33 (S)	24 (S)	8[e] (X)	15[e] (S)
9	0[e] (VR)	0[e] (VR)	22 (S)	11 (S)	13 (S)	20 (S)	11[e] (X)	4[e] (S)	1[e] (X)	3[e] (S)

[a]Isolates 1, 2, 3, 4 and 5 originated from the seedling rose, Red Cascade, Pink Parfait, Dr. Huey and R. multiflora, respectively, and were used as isolates of known compatibility on these hosts. Isolates 8 and 9 originated from Tropicana and Samantha, respectively, and were of unknown compatibility on the five test roses.

[b]Sporulation frequency on detached leaves, mean of two replications in which the percentage of sporulating colonies was assessed.

[c]VR = Very resistant, no visible sign of fungus sporulation or leaf necrosis; MR = resistant, necrotic lesions develop which prevent or restrict sporulation; S = susceptible, macroscopically visible sporulation without host necrosis; and X = heterogenous reaction, macroscopically visible sporulation on some leaf disks, no sign of sporulation on others, necrosis in some interactions.

[d]Disease severity on attached leaves, mean of three replications in which the percentage of leaf area supporting sporulation of the fungus was measured.

[e]Significantly less than the isolate of known virulence at P = 0.05.

Table 2-6. Identification of pathogenic races of Sphaerotheca pannosa var. rosae on detached leaves of five rose hosts.

	Fungus				
Test Rose	1,8	2	3,9	4	5
SR 70002/2	S[a]	S	R[b]	R	R
Red Cascade	X[c]	S	S	X	R
Pink Parfait	R	S	S	R	R
Dr. Huey	S	X	X	S	S
R. multiflora	X	X	X	R	S
	↓	↓	↓	↓	↓
	Race 1	Race 2	Race 3	Race 4	Race 5

[a] S = Susceptible

[b] R = Resistant

[c] X = Heterogenous, both resistant and susceptible infection types observed.

CHAPTER 3

VIRULENCE PATTERNS IN POPULATIONS OF ROSE POWDERY MILDEW

ABSTRACT

Virulence patterns were studied in several conidial populations of Sphaerotheca pannosa var. rosae. Although pathogenic variation was greater in the field, repeated sampling of both field and greenhouse locations demonstrated that rose powdery mildew populations are dynamic. Two populations consisted of more than one race; all others were racially homogenous. In one population, the presence of cleistothecia and ratio of pathotype occurrence suggested the meiotic recombination of two virulence factors. Although the pathogenicity of ascospore infections has not been demonstrated in the laboratory, these results imply that the sexual spores are viable and contributing to pathogenic variation in the field.

INTRODUCTION

The crucial information gained from any survey of pathogenic variation is the frequency of certain critical virulence genes in the pathogen population (Day, 1974). There are several disadvantages associated with conducting a race survey, which is the traditional method of studying pathogenic specialization. The culture of monoconidial isolates and their subsequent inoculation to a replicated series of differentials is a time-consuming process. The cultivars used to differentiate races of the fungus may not be relevant to breeding programs, and interpretation of race designations is difficult for the

non-initiated. In order to overcome these difficulties and improve the practical value of information gained from studies of pathogenic specialization, Wolfe and Schwarzbach (1976) proposed a radical change for monitoring variation in mildew populations. These authors describe methods for measuring virulence of a whole population against different sources of resistance. This approach is technically easier and provides data which are more representative of the mildew population.

The purpose of this investigation was to study virulence patterns in rose powdery mildew populations by applying the techniques described by Wolfe and Schwarzbach.

MATERIALS AND METHODS

Collection of rose powdery mildew. Virulence patterns were analyzed in field and greenhouse populations of Sphaerotheca pannosa ([Wallr.] Fr.) Lév. var. rosae Wor., causal agent of rose powdery mildew. Collections from the following field-grown cultivars were made in Benton County, Oregon on the dates indicated: (1) Burgundy, April, 1981; (2) Dr. Huey, Dwarf Crimson Rambler, and Rosa rugosa Thunb., October, 1981; and (3) Tropicana, Honey Favorite and Sundowner, June, 1982. Monoconidial isolates from Dr. Huey, R. rugosa, and Tropicana were previously classified into races 4, 3, and 1, respectively (Chapter 1). Virulence patterns in the populations on these three roses were studied to determine whether the races identified were representative of the entire population on that host or only a portion of it. The population on Dwarf Crimson Rambler was selected for study because both asexual (conidial) and sexual (cleistothecial)

stages of the fungus were present. This suggested the possibility of pathogenic variation within the population due to meiotic recombination of virulence genes. Infections on Burgundy, Honey Favorite, and Sundowner were chosen randomly to represent populations which occur on hybrid tea roses in garden plantings.

Greenhouse populations of rose mildew at two commercial rose-growing operations and at the Horticultural Crops Research Laboratory (HCRL) in Corvallis, Oregon were also collected. One of the commercial operations was located in Washington County, Oregon, and produced roses for the cut-flower market. The population at this range was analyzed in March, 1982, by collecting mildew from four greenhouses. The cultivar Forever Yours was in production in two of these houses; collections in the two remaining houses were taken from Volare and Misty Pink. The other grower was located in Polk County, Oregon, and marketed only miniature roses. The population at this location was analyzed in May, 1982, by collecting mildew from greenhouse plantings of the following cultivars: Marilyn, Sara Jean, Apricot Beauty, Ava, and Butter Scotch. Populations at the HCRL were studied on the following cultivars at the dates indicated: (1) Mary Devor, Samantha, and SR 70002/2, April and October, 1981; and (2) Red Cascade, October, 1981.

Inoculum collected from the field can vary greatly in viability and infection efficiency. Therefore, mildew populations were collected, reinoculated to plants of the same host cultivar, and incubated in a growth room whenever possible. Maintenance of the population in a controlled environment made possible the production of uniform inoculum. Conidia of these populations were synchronized for

development to improve infection efficiency, and quantitative comparisons with isolates of known virulence were made on detached leaf tissue.

Virulence tests. The virulence patterns of rose powdery mildew populations were analyzed on SR 70002/2 and Pink Parfait. SR 70002/2 is resistant to race 3 and susceptible to race 1; Pink Parfait is resistant to race 1 and susceptible to race 3. Therefore, inoculation of mildew populations to these roses will screen for virulence on two distinct sources of resistance. Four possible virulence patterns can be detected on these roses: virulence on Pink Parfait only (race 3), on SR 70002/2 only (race 1), for both roses (race 2), and neither rose (race 4 and 5).

SR 70002/2 and Pink Parfait, which are hybrid tea roses, are sources of resistance directly relevant to a breeding program. Although Dr. Huey and R. multiflora Thunb. are also differential cultivars for identification of rose powdery mildew races (Chapter 1), these two roses may not be of practical value to the breeder because their varietal characteristics are distant from those of modern commercial hybrids.

Detached-leaf inoculations. The virulence patterns in populations from Dr. Huey, Dwarf Crimson Rambler, Rosa rugosa Thunb., Red Cascade, Mary Devor, Samantha, and SR 70002/2 were analyzed on detached leaves of SR 70002/2 and Pink Parfait. Populations were reinoculated to the original hosts by shaking shoots infected with the desired population inside the isolation chamber (Coyier, 1974) of the host plant. Populations were maintained inside a growth room where temperature, relative humidity, and light intensity varied as described previously

(Chapter 1). Sufficient infection for virulence analysis was present on inoculated hosts 1-2 weeks later.

The procedure for analyzing populations from Red Cascade, Dr. Huey, R. rugosa, and Dwarf Crimson Rambler was designed to detect whether or not virulence was complex or simple, and if these populations were heterogenous or homogenous with respect to pathogenic race. The techniques of Wolfe and Schwarzbach (1976) were modified to accomplish these objectives. Half-leaflet pairs of SR 70002/2 and Pink Parfait were prepared as described previously (Chapter 2). Primary tests were conducted by inoculating one member of a pair with conidia from a population (unknown compatibility) and the other with an isolate of known compatibility (isolates 1 and 3 on SR 70002/2 and Pink Parfait, respectively). The population was also inoculated to detached leaves of the original host cultivar (susceptible control). When a population had virulence for both SR 70002/2 and Pink Parfait, a secondary test was conducted by transferring conidia which developed in the primary test to the differential cultivars. Secondary test inoculations were also made to half-leaflet pairs, i.e. Pink Parfait tissue pairs were inoculated with primary test conidia developing on Pink Parfait (known compatibility) and SR 70002/2 (unknown compatibility). The primary test was designed to elucidate virulence of the population for individual sources of resistance, i.e. SR 70002/2 and Pink Parfait. Combinations of virulence are revealed in the secondary test because primary test inoculum developing on one differential cultivar is transferred to the other. Virulence was assessed by counting the number of 30 conidia which produced sporulating colonies and by the infection types present on detached leaves ten days after

inoculation.

The racial composition of the R. rugosa, Dwarf Crimson Rambler, and Red Cascade populations was further investigated by studying the virulence patterns of colonies initiated from these populations. One infected shoot from a plant hosting the desired population was shaken inside the isolation chamber of the original 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 scored for reaction type ten days later. Virulence patterns of 60 Dwarf Crimson Rambler and 30 R. rugosa and Red Cascade colonies were investigated.

The procedure for analyzing virulence at the HCRL was designed to detect the presence of races 1, 2, and 3. Previous results had suggested that race 3 was the predominant form of rose powdery mildew at this location. This pathotype was isolated from Pink Parfait, Mary Devor and Samantha, three cultivars which are grown in large numbers at HCRL greenhouses (Chapter 1). However, infections were also present on greenhouse plantings of SR 70002/2, a host which is resistant to race 3. HCRL populations were analyzed in April, 1981 and again in October, 1981 by collecting mildew from SR 70002/2, Mary Devor and Samantha and inoculating half-leaflet pairs of SR 70002/2 and Pink Parfait. One member of a pair was inoculated with conidia from the population, and the other with an isolate of known compatibility (isolates 1 and 3 on SR 70002/2 and Pink Parfait, respectively). Detached leaves of the original host cultivar were inoculated with the population to function as a susceptible control. Virulence was assessed by counting the number of 30 conidia which had produced

branched hyphae 48 hr after inoculation and by infection types present on detached leaves ten days after inoculation.

Infection and sporulation data on half-leaflet pairs were statistically analyzed using a two-tailed t-test for paired observations. This procedure was described previously (Chapter 2).

In vivo inoculations. However, aseptically-produced leaf material of the original host variety was not always available. In these instances, virulence was rated qualitatively on intact plants, rather than quantitatively on detached leaves. The type of data collected and procedure for interpreting the results varied with *in vivo* and *in vitro* inoculations.

Intact leaves of SR 70002/2 and Pink Parfait were used to analyze virulence patterns in field collections from Burgundy, Tropicana, Honey Favorite, and Sundowner and greenhouse collections from the two commercial operations. Conidia were collected from the desired host and directly inoculated to SR 70002/2, Pink Parfait, and Dwarf Crimson Rambler plants maintained in isolation chambers. Dwarf Crimson Rambler functioned as a susceptible control because all known races of the fungus are virulent on this cultivar. Plants were inoculated by removing shoots hosting the desired population and shaking them inside the isolation chambers of the differential cultivars. Seven days after the introduction of inoculum, the isolation chambers were opened and cultivars were scored for the presence or absence of host necrosis and fungus sporulation.

RESULTS

The *R. rugosa* population was avirulent on Pink Parfait, and

both SR 70002/2 and Pink Parfait were resistant to inoculum from Dr. Huey (Table 3-1). The Dwarf Crimson Rambler and Red Cascade populations were virulent on both differential cultivars. Sporulation of R. rugosa conidia on SR 70002/2 and Dwarf Crimson Rambler conidia on both test roses occurred at levels significantly less than isolates of known virulence. The infection types observed in these interactions were heterogenous, i.e. not uniformly resistant or susceptible. A previous study indicated that virulent isolates sporulate on SR 70002/2 and Pink Parfait at levels comparable to isolates 1 and 3, respectively (Chapter 2). Avirulent isolates do not sporulate on SR 70002/2. Isolates avirulent on Pink Parfait either fail to produce secondary conidia in macroscopically visible quantities or elicit a hypersensitive response which restricts fungal development. Therefore, the reduced sporulation and heterogenous reactions observed in response to Dwarf Crimson Rambler and R. rugosa inoculum suggested that these populations consisted of a mixture of avirulent and virulent pathotypes.

The Dwarf Crimson Rambler and Red Cascade inoculum which sporulated on SR 70002/2 and Pink Parfait was reinoculated to both roses in secondary tests (Table 3-2). Sporulation frequencies in the secondary inoculations also implied heterogenicity in the Dwarf Crimson Rambler population. For example, half-leaflet pairs of SR 70002/2 leaf tissue received primary test inoculum from both SR 70002/2 (compatible inoculation) and Pink Parfait (test inoculation). Inoculum from Pink Parfait sporulated at only 8% on SR 70002/2, as opposed to 26% when compatible conidia were reinoculated to this host. Primary and secondary test inoculations indicated the presence of

three pathogenic strains in the Dwarf Crimson Rambler conidial population, i.e. one which was virulent on SR 70002/2 and avirulent on Pink Parfait, a second which was virulent on Pink Parfait and avirulent on SR 70002/2, and a third which attacked both roses.

In primary inoculations, the Red Cascade population sporulated on the test varieties at levels comparable to virulent isolates 1 and 3. Inoculations at the secondary level indicated that primary test inoculum developing on one test rose was equally virulent on the other. These results suggested that the Red Cascade population was homogenous with respect to SR 70002/2 and Pink Parfait, i.e. the population consisted of a race with combined virulence for the test roses.

The methods described by Wolfe and Schwarzbach (1975) and Wolfe et al. (1976) were used to derive expected and observed virulence frequencies for the sporulation data in primary and secondary tests. Calculation of these values assumes that reassortment of virulence genes is random and independent. Any departure of observed values from expected probability suggests that virulence factors are occurring in combination. In Table 3-2, expected and observed frequencies are similar and dissimilar for the Dwarf Crimson Rambler and Red Cascade populations, respectively. This suggests that virulence factors in the Dwarf Crimson Rambler population occur independently and reassort randomly, whereas virulence factors in Red Cascade population occur in combination. The correlation between observed and expected values could not be statistically verified because of the limited number of data points and the use of percentages rather than actual numbers of avirulent and virulent isolates.

The virulence reactions of individual colonies originating from the Dwarf Crimson Rambler, R. rugosa, and Red Cascade populations are presented in Table 3-3. The reaction of the test roses to individual colonies suggested that the Dwarf Crimson Rambler and R. rugosa populations were heterogenous and consisted of four and two pathotypes, respectively. The Red Cascade population reacted as one pathotype with virulence on both test roses. Monoconidial isolates cultured from the individiual colony types verified these results. The ratio of colony types in the Dwarf Crimson Rambler population is similar to the 9:3:3:1 ratio which would be expected if avirulence was dominant, and virulence for SR 70002/2 and Pink Parfait was segregating as separate genes (.50>P>.30). The colony type ratio in the R. rugosa population resembles a 3:1 ratio (.10>P>.05) which would be expected if avirulence were dominant and virulence on SR 70002/2 was controlled by a single recessive gene.

The interaction of the test roses with populations at the HCRL is presented in Table 3-4. A previous study (Chapter 2) demonstrated that races 1, 2, and 3 can be identified by their ability to form branched hyphae on SR 70002/2 and Pink Parfait. The absence of branched hyphae and hyphal branching at levels lower than virulent isolates characterized the avirulence of race 3 on SR 70002/2 and race 1 on Pink Parfait, respectively. The virulence of race 1 on SR 70002/2, race 3 on Pink Parfait, and race 2 on both roses was indicated by hyphal branching at levels not significantly different from isolates of known compatibility. Therefore, the population collected from SR 70002/2 in April, 1981 had race 1 pathogenicity, whereas Mary Devor and Samantha populations had race 3 pathogenicity. However,

October, 1981 collections from all three roses were uniformly virulent on the differential cultivars, indicating the presence of race 2. The identification of race 1 on SR 70002/2 correlated with the introduction of inoculum from field-grown plants of the same host. The origin of race 2 was traced to the introduction of infected Red Cascade plants in May, 1981.

The results of *in vivo* inoculations to the test roses are presented in Table 3-5. Virulence occurred for Pink Parfait only in all greenhouse collections, which indicated that race 3 predominated at both locations. The virulence patterns from field collections were more variable. Virulence was present for SR 70002/2 only in populations on Tropicana and Sundowner (race 1), for both test roses on Honey Favorite (race 2), and for Pink Parfait only on Burgundy (race 3).

DISCUSSION

Virulence analyses of fungal populations provide a more objective appraisal of pathogenic variability than conventional race surveys. For example, racial heterogenicity in the Dwarf Crimson Rambler population might have been overlooked if only a few monoconidial isolates of the fungus had been cultured. The use of half-leaflet pairs and virulent isolates as compatible controls improved the interpretation of test results. This procedure eliminated doubts about the susceptibility of detached leaves and made statistical analyses possible.

Virulence was assessed in terms of infection or sporulation, depending on the objective of the analysis. Infection data alone were

sufficient to detect races 1, 2, and 3 on detached leaves of the test cultivars. These measurements were confirmed by qualitative ratings ten days after inoculation. When the purpose of the analysis was to detect all possible combinations of virulence by repeated differential testing, quantitative data on sporulation were accumulated. Although *in vivo* inoculations were not rated quantitatively, they did provide information on the occurrence of the four virulence classes.

The virulence of Dr. Huey, Tropicana, and Red Cascade populations on SR 70002/2 and Pink Parfait agreed with the reaction of monoconidial isolates cultured from these hosts. Therefore, these isolates represent the conidial populations on these hosts, and are not chance selections of rare strains. Monoconidial isolates cultured from *R. rugosa* in August, 1981 were identified as race 3 (Chapter 1), i.e. virulent on Pink Parfait only. However, analysis of conidial populations on the same plants in October, 1981 showed that the population consisted of a mixture of two pathotypes, one avirulent on both test roses and the other virulent on SR 70002/2 only. Therefore, during a 2-3 month period, there was a shift in the composition of the *R. rugosa* population. This observation and the population shift at the HCRL demonstrate that populations of rose powdery mildew are dynamic.

A previous study (Chapter 1) demonstrated that the complexity of race 2 is not correlated with reduced aggressiveness on SR 70002/2 and Pink Parfait. The results of the present investigation indicate that race 2 is actually more competitive than races 1 and 3 in a greenhouse environment. The mechanisms which explain the increased competitive ability of race 2 are unknown. The population shift to race 2 was accompanied by increased difficulty in controlling the fungus with

chemicals, which suggests that fungicide tolerance may be a factor.

Race 2 was originally isolated from infections on Red Cascade (Chapter 1) and was identified on field-grown Dwarf Crimson Rambler and Honey Favorite plants in this investigation. Although this race is well-adapted to rose ranges and presents a serious threat to growers, it was not present in populations at two commercial operations. These results indicate that race 2 is not widely distributed in the pathogen population.

The presence of four pathotypes was demonstrated in the Dwarf Crimson Rambler population. The presence of cleistothecia on these plants and the ratio in which the pathotypes occurred suggests the meiotic recombination of two virulence factors. Although the pathogenicity of ascospore infections has not been demonstrated in the laboratory, these results imply that these spores are viable and contributing to pathogenic variation in the field.

Racial heterogenicity was greater in field populations than in commercial greenhouse rose ranges. Less than 5% of available rose species have been exploited in the development of roses grown for floricultural purposes. Therefore, many of these roses share genes for powdery mildew reaction type, and this will limit the number of corresponding pathogen races. However, the genetic basis of species roses and less hybridized cultivars is more expansive and should reflect a greater diversity of pathotypes. Another factor which may contribute to greater pathogenic variation in field populations is the more regular occurrence of cleistothecia on field-grown roses. Pathogenic variation within the fungus may be occurring through sexual recombination of virulence genes. The greater regularity of

cleistothecia on field-grown roses may be a function of host genotype since the ramblers, climbers, and shrub roses that develop ascocarps are not grown in commercial greenhouse ranges. Environmental differences could also influence the formation of cleistothecia because compatible strains may not be equally well-adapted to greenhouse conditions.

Table 3-1. Sporulation and infection type of four *Sphaerotheca pannosa* var. *rosae* populations on detached leaves of SR 70002/2, Pink Parfait and susceptible controls.

Population	Test Rose[a]		Susceptible Control[b]
	SR 70002/2	Pink Parfait	
Dr. Huey	0[c] (VR)[e]	0[d] (MR)	35 (S)
R. rugosa	5[c] (X)	0[d] (MR)	40 (S)
Dwarf Crimson Rambler	10[c] (X)	11[d] (X)	38 (S)
Red Cascade	46 (S)	31 (S)	65 (S)

[a] Values are percentages based on observation of 12-18 leaf disks, 30 conidia per disk.

[b] Host from which the population was collected.

[c,d] Significantly less than isolates 1 and 3, respectively, at P = 0.01.

[e] VR = very resistant, no visible sign of fungus sporulation or leaf necrosis; MR = moderately resistant, necrotic lesions develop which prevent or restrict sporulation; S = susceptible, macroscopically visible sporulation without host necrosis; and X = heterogenous reaction, macroscopically visible sporulation on some leaf disks, no sign of sporulation on others, necrosis in some interactions.

Table 3-2. Sporulation and infection type of Dwarf Crimson Rambler and Red Cascade conidial populations and population subsets on detached leaves of SR 70002/2 and Pink Parfait.

	Dwarf Crimson Rambler[a]				Red Cascade[a]			
Primary Test	SR 70002/2		Pink Parfait		SR 70002/2		Pink Parfait	
	10[b](X)[c]		11[b](X)		64(S)		31(S)	
Secondary Test	SR	PP	SR	PP	SR	PP	SR	PP
	26(S)	11[b](X)	8[b](S)	37(S)	57(S)	48(S)	51(S)	50(S)

	Virulence Combination[d]			Virulence Combination[d]		
	SR	PP	SR + PP	SR	PP	SR + PP
Expected Virulence Frequencies[e]	9	10	1	32	17	14
Observed Virulence Frequencies[f]	9	10	2	24	15	38

[a] Values are percentages based on observation of 12-18 leaf disks, 30 conidia per disk.

[b] Significantly less than interaction of known virulence at P = 0.01.

[c] X = heterogenous reaction, visible sporulation on some leaf disks, no sign of sporulation on others, necrosis in some interactions; and S = susceptible, macroscopically visible sporulation without host necrosis.

[d] Virulence for SR (SR 70002/2) only, PP (Pink Parfait) only, and both roses (SR + PP).

[e,f] Based on sporulation frequencies in the primary test, and primary and secondary tests, respectively.

Table 3-3. Reaction of SR 70002/2 and Pink Parfait to individual colonies initiated from three Sphaerotheca pannosa var. rosae populations.

Population	Virulence Reaction[a]			
	Avirulent on SR and PP	Virulent		
		SR	PP	SR + PP
Dwarf Crimson Rambler	39	11	9	1
R. rugosa	27	3	0	0
Red Cascade	0	0	0	25

[a]Number of 60 Dwarf Crimson Rambler colonies and 30 R. rugosa and Red Cascade colonies which were avirulent on SR 70002/2 and Pink Parfait, and virulent on SR 70002/2 only, Pink Parfait only, and both SR 70002/2 and Pink Parfait.

Table 3-4. Hyphal branching and infection type of Sphaerotheca pannosa var. rosae populations at the Horticultural Crops Research Laboratory in the spring and fall of 1981 on detached leaves of SR 70002/2, Pink Parfait and susceptible controls.

	April, 1981[a]			October, 1981[a]		
Population	SR 70002/2	Pink Parfait	Susceptible Control[b]	SR 70002/2	Pink Parfait	Susceptible Control[b]
SR 70002/2	64 (S)[c]	4[e] (VR)	64 (S)	68 (S)	46 (S)	68 (S)
Mary Devor	0[d] (VR)	40 (S)	54 (S)	88 (S)	84 (S)	43 (S)
Samantha	0[d] (VR)	33 (S)	59 (S)	72 (S)	36 (S)	41 (S)

[a] Values are percentages based on observation of 12-18 leaf disks, 30 conidia per disk.

[b] Host from which the population was collected.

[c] VR = very resistant, no visible sign of fungus sporulation or leaf necrosis; and S = susceptible, macroscopically visible sporulation without host necrosis.

[d],[e] Significantly less than isolates 1 and 3, respectively, at P = 0.01.

Table 3-5. *In vivo* reaction of SR 70002/2 and Pink Parfait to several greenhouse and field populations of *Sphaerotheca pannosa* var. *rosae*.

	Virulence Reaction[a]		
Greenhouse Collections	SR 70002/2	Pink Parfait	Indicated Race
Location 1[b]	VR	S	3
Location 2[c]	VR	S	3
Field Collections			
Tropicana	S	MR	1
Honey Favorite	S	S	2
Sundowner	S	MR	1
Burgundy	VR	S	3

[a]VR = very resistant, no visible sign of fungus sporulation or leaf necrosis; MR = moderately resistant, necrotic lesions develop which prevent or restrict sporulation; and S = susceptible, macroscopically visible sporulation without host necrosis.

[b]Roses grown for cut-flower production.

[c]Miniature roses in production.

CHAPTER 4

HETEROTHALLISM IN *SPHAEROTHECA PANNOSA* VAR. *ROSAE*

ABSTRACT

Nine monoconidial isolates of *Sphaerotheca pannosa* var. *rosae* were coinoculated to four rose hosts in 11 combinations. Heterothallism was demonstrated by the strong fruiting response which occurred when an isolate designated as RR1 was paired with two other isolates. In the nine combinations where cleistothecia did not form, the coinoculated isolates differed in host specificity and were reisolated when the experiment was terminated. Therefore, the failure of these isolates to mate could not be explained by their inability to grow on the inoculated host. The RR1 isolate was avirulent on two hybrid tea roses, but virulent on species roses and less hybridized cultivars. The results indicated that the virulence of compatible strains on a mutual host was necessary for ascocarp formation, and that fruiting may require physiological stimulation by the host. A weak fruiting response was observed on one plant inoculated with RR1 only. Fruitbody formation in this culture could not be explained by the presence of contaminating conidia of other races. Cleistocarps retrieved from this culture were morphologically normal, but asci either contained only two ascospores or undifferentiated cytoplasmic material. The possibility of monokaryotic fruiting in this culture is discussed.

INTRODUCTION

The formation of fruiting bodies in the Erysiphaceae has been

attributed to low temperatures (Bioletti, 1907), alternating high and low temperatures (Cherewick, 1944), the onset of host senscence (Graf-Marin, 1934), unspecified physiologic changes in the host (Jhooty and McKeen, 1962), and heterothallism (Butt, 1978; Byford and Bentley, 1976; Jackson and Wheeler, 1975; Smith, 1970). Most of these reports are not in agreement with Schnathorst (1959). Yarwood (1935) and Jackson and Wheeler (1975) who obtained cleistothecia in detached leaf culture at constant temperatures on young leaves. Smith (1970) found that cleistothecial production occurred over a wide temperature range and was more abundant on fleshy younger leaves, providing the proper mating types were present.

Evidence for heterothallism in the powdery mildews was first reported for Erysiphe cichoracearum DC. ex Mérat on Helianthus annus L. (Yarwood, 1935). Heterothallism has also been demonstrated in other Erysiphe (Hiura and Tomoda, 1959; Morrison, 1960; Powers and Moseman, 1956; Schnathorst, 1959; Smith, 1970). Both homothallism and heterothallism have been reported for E. graminis [DC.] Mérat f. sp. hordei Em. Marchal (Cherewick, 1944; Hiura and Tomoda, 1959), E. graminis tritici Em. Marchal (Cherewick, 1944; Powers and Moseman, 1956), and E. polygoni [DC.] St. Amans (Smith, 1970). Other genera of the Erysiphaceae in which heterothallism has been established include Microsphaera, Uncinula, and Podosphaera (Coyier, 1974; Smith, 1970). Within the genus Sphaerotheca, Homma (1933, 1937) concluded that both homothallic and heterothallic strains occur in S. fuliginea (Schlecht. ex Fr.) Pollacci, and Jackson and Wheeler (1975) demonstrated heterothallism in S. mors-uvae (Schw.) Berk.

Coyier (1961,1962) and Price (1970) surveyed examples of Rosa

spp. and cultivars and found formation of cleistothecia by Sphaerotheca pannosa ([Wallr.] Fr.) Lév. var. rosae Wor. to be infrequent and erratic. Price (1970) studied the occurrence of the sexual stage on outdoor roses over a three-season period and found ascocarp production to be more frequent on ramblers, climbers, and old shrub roses than on modern hybrids. Cleistothecia occurred on some cultivars in successive years but not on adjacent cultivars, and they were not consistently found on the same cultivar in different localities. Coyier (1961) reported abundant ascocarp production on certain cultivars in the greenhouse, but found that cleistothecia were scarce on the same plants the following year. In a survey of infected material from the United States and abroad, ascocarps were found on Rosa spp. and polyantha roses, but not on floribundas or hybrid teas (Coyier, 1962). These reports stated that host factors influence the formation of cleistothecia, but did not present evidence for heterothallism in the fungus.

Cleistocarps formed on R. rugosa Thunb. and cv. Dwarf Crimson Rambler plants in studies designed to investigate virulence patterns in populations of rose powdery mildew (Chapter 3). Cleistocarps developed on both hosts in isolation chambers within a growth room, which suggested that production of ascocarps under controlled conditions was possible. Virulence analyses had demonstrated the presence of two pathogenic races on R. rugosa and four on Dwarf Crimson Rambler. Cleistothecia did not form on plants which were inoculated with populations consisting of only one race.

Heterothallism could explain the absence of ascocarps on plants inoculated with monoconidial isolates of S. pannosa var. rosae. The

occurrence of compatible mating types as separate races was suggested by the presence of cleistothecia in populations of heterogeneous pathogenicity and absence in populations of homogeneous pathogenicity. Therefore, the objective in the present investigation was to determine whether or not heterothallism in S. pannosa var. rosae could be demonstrated by crossing isolates of the fungus which differed in host specificity.

MATERIALS AND METHODS

Monoconidial isolates were cultured from infected leaves and stems of R. rugosa and Dwarf Crimson Rambler plants on which cleistothecia were present. Isolates were initiated on detached leaves of the original host varieties. Two isolates from both R. rugosa and Dwarf Crimson Rambler were selected for further study on the basis of their reaction with SR 70002/2, an unnamed seedling rose used in the differentiation of rose powdery mildew races. Isolates RR1 and DCR1 were avirulent, and isolates RR4 and DCR2 were virulent on detached leaf tissue of SR 70002/2. Isolates with the prefixes RR and DCR originated from R. rugosa and Dwarf Crimson Rambler, respectively. Conidia of these four isolates were maintained as stock cultures on tissue of the original host plants which had been kept free of mildew in isolation chambers (Coyier, 1973).

Pathogenicity tests. The host range of the four isolates was investigated in pathogenicity tests on SR 70002/2, Pink Parfait, Dr. Huey, and R. multiflora, which are differentials for identification of rose powdery mildew races. Plants were inoculated by removing an infected shoot from the stock plant hosting the desired isolate and shaking it

inside the isolation chamber of the differential cultivar. Each test was replicated three times. Seven days after introduction of inoculum, the chambers were opened and the reactions of the cultivars recorded. Leaves were visually examined and the presence or absence of necrosis and fungus sporulation were noted. The amount of sporulation was assessed by comparing mildew on adaxial surfaces with standard diagrams (Price, 1970).

Compatibility tests. Crosses involved five other isolates in addition to those cultured from R. rugosa and Dwarf Crimson Rambler. The host specificity of these isolates had been previously investigated on the four differential rose varieties, and five distinct pathogenic races were identified (Chapter 1). Table 4-1 describes the reaction of the five isolates on the differential rose cultivars and lists the virulence formula and race abbreviation for each isolate.

The plants chosen for this experiment had been maintained in isolation chambers on a greenhouse bench for 6-8 wk prior to testing. Powdery mildew had not been observed on any of the plants for at least one month prior to inoculation. All inoculations were performed in a laminar-flow hood. Dwarf Crimson Rambler, R. rugosa, and R. multiflora Thunb. were chosen as host plants because cleistothecia have been observed on all three roses and previous reports had indicated that host factors influence ascocarp development (Coyier, 1961, 1962; Price, 1970). Crosses involving isolate 4 were made on Dr. Huey rather than the other three hosts because R. multiflora was resistant to this isolate and its compatibility on R. rugosa and Dwarf Crimson Rambler had not been established. The formation of cleistothecia on Dr. Huey has not been reported.

The isolates listed in Table 4-2 were coinoculated to the four rose hosts. Crosses between isolates were performed by simultaneously shaking shoots infected with the two isolates inside the isolation chamber of the host plant. Single inoculations were made by shaking an infected shoot hosting one isolate inside an isolation chamber. Both single and paired inoculations were replicated twice on each host.

Both pathogenicity and compatibility tests were conducted inside a growth room where temperature was 21°C ± 3°C and RH was 45-55%. Relative humidity within plant chambers ranged from 40-90%. The room was illuminated 12 hr daily with both fluorescent and incandescent lamps at an approximate intensity of 100 μE measured at plant level. Colony growth was examined periodically under a dissecting microscope inside a liminar-flow hood. Each inoculation chamber was disassembled at the end of three months and inoculations were assessed for cleistocarp production.

Recovery of isolates. An attempt was made to recover both isolates of a cross by inoculating certain rose hosts to obtain differential reactions. Crosses 2, 6, and 10 involved isolates which could be differentiated by their reaction on Pink Parfait. Each paired inoculation consisted of avirulent and virulent isolates which could be identified on this host by necrotic and sporulating infection types, respectively. The procedure for recovering these isolates was to disperse conidia from the paired inoculation onto plants of Pink Parfait and rate for infection type seven days later.

The isolates paired in crosses 1, 5, 7, 8, and 11 were either avirulent or virulent on SR 70002/2 and could be differentiated by

their development relative to an isolate of known virulence on this host. A previous study had shown that avirulent isolates do not form branched hyphae on detached leaves of SR 70002/2. However, virulent isolates formed branching hyphae at frequencies not significantly different from isolate 1, which originated from SR 70002/2 (Chapter 2). Mixtures of virulent and avirulent isolates were distinguished by formation of branched hyphae at frequencies significantly less than virulent isolate 1 (Chapter 3). Therefore, the procedure for recovering these isolates was to inoculate detached leaves of SR 70002/2 with conidia from the cross and isolate 1, and compare development of branched hyphae.

Crosses 3 and 9 consisted of isolates which could be differentiated on R. multiflora and the miniature cultivar, Red Cascade. Infections which were homogeneous for isolate 4, a participant in both crosses, could be identified by resistant phenotypes on both R. multiflora and Red Cascade. However, if an infection consisted of RR1 or isolate 5 only, R. multiflora and Red Cascade would exhibit susceptible and resistant infection types, respectively. The resistance of Red Cascade to isolate 4 can be differentiated from resistance to isolate 5 and RR1 by the development of necrotic lesions in the former interaction, and the absence of visible infection in the latter. A mixed infection should result in sporulation on R. multiflora and a moderately resistant infection type on Red Cascade. Therefore, the recovery of isolates from crosses 3 and 9 involved dispersal of conidia to intact plants of R. multiflora and Red Cascade and assessment of infection type seven days later.

RESULTS

Pathogenicity tests. The reaction of the differential rose cultivars to the four isolates from R. rugosa and Dwarf Crimson Rambler is presented in Table 4-3. Observations on the occurrence of host necrosis and sporulation were combined with data on the quantity of sporulation to define each host-parasite interaction. Classes 0-2 were regarded as "resistant" reactions, and 3-5 as "susceptible."

Isolates RR4 and DCR2 were both avirulent on Pink Parfait and virulent on SR 70002/2, Dr. Huey and R. multiflora. DCR1 was avirulent on SR 70002/2 and virulent on Pink Parfait, Dr. Huey and R. multiflora. The interaction of the four differentials with these three isolates justified the assignment of RR4 and DCR2 to race 1, and DCR1 to race 3 (Tables 4-1 and 4-3).

Isolate RR1 was avirulent on SR 70002/2 and Pink Parfait, and virulent on Dr. Huey and R. multiflora; therefore, RR1 had pathogenicity characteristics in common with race 5. However, RR1 differed from isolate 5, the type culture for race 5, in disease severity on Dr. Huey and R. multiflora. Isolate 5 was more virulent on R. multiflora than on Dr. Huey (Table 4-1), whereas RR1 was ranked more virulent on Dr. Huey (Table 4-3). When disease severity of RR1 and isolate 5 were analyzed and compared on these two cultivars, the percentage of leaf area colonized by isolate 5 was significantly higher on R. multiflora than Dr. Huey ($P = 0.01$). However, the difference in severity of attack on the two roses was not significantly different when RR1 was used as an inoculum source. Although the differences between isolate 5 and RR1 do not warrant classification of the latter into a

separate race, it should be noted that RR1 can be distinguished from the type culture for race 5.

Compatibility tests. Ascocarps were formed when RR1 was combined with RR4 and isolate 5 (crosses 1 and 4), but cleistothecia failed to develop in the other paired inoculations. Colorless ascocarp initials were observed three weeks after inoculation on plants hosting crosses 1 and 4. These structures were dark brown and macroscopically visible five weeks after inoculation. Cleistothecia formed exclusively on the pedicels and hypanthia of blooms when RR1 and RR4 were paired on Dwarf Crimson Rambler (Fig. 4-1). When these two isolates were combined on R. rugosa, cleistothecia formed abundantly on stems and around thorns (Fig. 4-2a,b). Ascocarps also developed on one set of very young leaflets on this host. Ascocarp formation occurred along stems of R. multiflora coinoculated with RR1 and isolate 5 and was especially frequent in the region immediately below the nodes.

Cleistothecia did not develop in the other nine crosses. The pannose mycelium in which cleistocarps are generally embedded formed in crosses 5 and 10, but fruiting bodies were not initiated. Pannose mycelium also formed along the stems of the R. multiflora which received single inoculations of isolate 5, but cleistothecia did not develop (Fig. 4-2c).

Ascocarp diameters and the dimensions of asci and ascospores were measured when this study was concluded. Cleistothecia were 60-105 µm in diameter, averaging 90 µm; asci were 70-140 x 55-80 µm, averaging 110 x 65 µm; and ascospores were 12-25 x 12-18 µm, usually 21 x 13 µm. Ascocarp diameters in the present study were smaller than those reported by Salmon (1900), but consistent with the measurements of

Coyier (1961). The dimensions of asci and ascospores were more variable than those reported by Salmon. Perhaps this variation can be attributed to the artificial environment in which the tests were conducted.

Several cleistothecia from crosses 1 and 4 were removed at this time and mounted on a microscope slide in distilled water. The asci were discharged by applying pressure to a cover slip which was placed over the cleistothecia. Eight ascospores were well-defined inside each ascus (Fig. 4-3a).

In one of the two single inoculations of RR1 to R. rugosa, cleistothecia were found deeply embedded in mycelium along the stem. The fruiting response was very weak; a total of 20 ascocarps were counted on the entire plant and these were confined to one section of the stem. The ascocarps were dark brown and did not differ in size from those which developed in paired inoculations. When the cleistocarps were mounted on a microscope slide and encouraged to discharge their contents, the majority of asci contained undifferentiated cytoplasmic material (Fig. 4-3b). Two spores were distinguished amongst the undifferentiated material in several asci, but in no instance were eight ascospores observed.

The possibility of contamination in the RR1 culture was explored by dispersing conidia from this plant over SR 70002/2, Red Cascade and Pink Parfait. The absence of visible infection on SR 70002/2 and Red Cascade and development of necrotic lesions on Pink Parfait indicated that cleistothecial formation in the RR1 culture could not be explained by contamination with races 1, 2, 3, and 4.

Cleistothecia did not develop on the other R. rugosa inoculated

with RR1 only or in single inoculations of this isolate to Dwarf Crimson Rambler, R. multiflora and Dr. Huey. Fruiting bodies were also absent in single inoculations of the other eight isolates.

Recovery of isolates. All attempts to recover both isolates from paired inoculations were successful. Inoculation of conidia from crosses 2, 6, and 10 to Pink Parfait resulted in both susceptible and resistant infection types and demonstrated the presence of both virulent and avirulent isolates, respectively. Conidia from crosses 1, 5, 7, 8, and 11 formed branched hyphae at frequencies significantly less than isolate 1 on SR 70002/2, which showed that the inoculum was a mixture of avirulent and virulent isolates. Conidia from crosses 3 and 9 sporulated on R. multiflora and caused a necrotic reaction on Red Cascade. This demonstrated the presence of RR1 and isolate 5 in the former interaction and isolate 4 in the latter.

Isolate 5 and RR1 were both avirulent on SR 70002/2 and Pink Parfait and virulent on Dr. Huey and R. multiflora. Therefore, the recovery of these two isolates from cross 4 could not be readily accomplished by transferring conidia directly to differential cultivars. It was theoretically possible to separate these two isolates by their disease severity on R. multiflora because isolate 5 is significantly more aggressive than RR1 on this host. However, this would have required the culture of monoconidial isolates, their increase on a susceptible host, and subsequent inoculation to R. multiflora in a replicated test. Therefore, no attempt was made to recover RR1 and isolate 5 from cross 4.

DISCUSSION

Homothallic fungi are self-fertile, whereas heterothallic fungi possess self-incompatible and cross-compatible mating types. Heterothallism may be either bipolar with one mating type factor, or tetrapolar with two unlinked factors (Fincham and Day, 1979). In species with bipolar sexuality, prospective mates must have different mating factor alleles in order for compatible mycelia to fuse and form a dikaryon. Sexual morphogenesis then progresses through various stages which include nuclear fusion (karyogamy), meiosis, and spore formation.

The existence of two mating types has been demonstrated for several members of the Erysiphaceae (Coyier, 1974; Hiura, 1962; Morrison, 1960; Schnathorst, 1959; Smith, 1970), and suggests that bipolar sexuality predominates in heterothallic species of this group. The strong fruiting response which occurred when RR1 was combined with RR4 and isolate 5 indicates that S. pannosa var. rosae is heterothallic. The assignment of RR1 to one mating type and RR4 and isolate 5 to its compatible counterpart is implied by the absence of cleistothecia in the RR4 x isolate 5 cross. Crosses between isolate 5 and isolates 1 and 3 were also negative and suggest the inclusion of the two latter isolates in the same mating type as the former. However, isolates 1 and 3 must be combined with RR1 and formation of ascocarps must occur in order to confirm this hypothesis. These preliminary results suggest that the RR1 mating type allele may be relatively rare and its compatible mating type fairly common.

Although two compatible strains were recovered from the population

on R. rugosa, cleistothecia failed to develop when the two isolates cultured from Dwarf Crimson Rambler were crossed on this host. Therefore, DCR1 and DCR2 may not be the compatible strains responsible for ascocarp formation in the population on Dwarf Crimson Rambler. Ascocarp production on the Dwarf Crimson Rambler hosting the population was very scanty and confined to only a few stems at nodal regions. This suggests that at least one compatible strain was present but it was a small part of the population. The virulence patterns in the population had indicated the presence of two races in addition to DCR1 and DCR2 (Chapter 3). These two strains were present in very low frequencies and were not recovered in the limited number of monoconidial isolations made from the population. One of these races was avirulent on both SR 70002/2 and Pink Parfait and elicited a hypersensitive reaction on the latter; these characteristics are identical to those of RR1. Therefore, the RR1 strain may have been present in the population on Dwarf Crimson Rambler and could have combined with the other mating type to produce cleistothecia.

A weak fruiting response was observed on one R. rugosa plant inoculated with RR1. Cleistocarps were not formed on the other R. rugosa, or on Dwarf Crimson Rambler, R. multiflora, and Dr. Huey plants receiving single inoculations of this isolate. Cleistothecial formation in the RR1 culture could not be attributed to contamination with races 1, 2, 3, and 4. Conidia of race 2 were the most likely source of contamination since this race of rose powdery mildew was the predominant form at the Horticultural Crops Research Laboratory during this investigation. It was impossible to screen the RR1 culture for contamination with isolate 5 because both isolates were

identified as race 5 and caused similar pathogenic reactions on test roses.

The production of fruit bodies within a single clone of a heterothallic fungus could be attributed to haploid monokaryotic fruiting, a phenomenon which occurs without the sexual cycle and is separable from the incompatibility factors which control the sexual process (Esser and Graw, 1980; Esser and Meinhardt, 1977; Stahl and Esser, 1976). Bipolar and tetrapolar incompatibility systems are designed to promote outbreeding because the formation of a dikaryon is prevented or restricted when mating type alleles are identical (Fincham and Day, 1979). The phenomenon of monokaryotic fruiting has shown that fruit body morphogenesis can occur without the formation of a dikaryon; therefore, the genes responsible for fruiting are separable from the mating type factor(s). Several characteristics distinguish monokaryotic from dikaryotic fruiters: monokaryotic fruiting is weaker, i.e. the frequency of fruiting is lower (Stahl and Esser, 1976); monokaryotic fruit bodies may be sterile or contain a reduced number of spores, i.e. two mitotic rather than four meiotic spores (Esser and Graw, 1980; Esser and Meinhardt, 1977; Esser et al., 1979; Stahl and Esser, 1976); and fertile mitotic spores of monokaryotic fruiters produce offspring identical to the parent with no segregation for mating type (Stahl and Esser, 1976).

The fruiting response in the RR1 culture of S. pannosa var. rosae was very weak, and asci either contained only two spores or undifferentiated cytoplasmic material. These factors suggest monokaryotic fruiting as a possible explanation for cleistothecial formation in the RR1 culture. Monokaryotic fruiting has not been demonstrated in the

Erysiphaceae, but certain anomalies have been reported when investigators crossed cultures of like mating type. Morrison crossed clones of E. cichoracearum from Helianthus annus and Zinnia elegans Jacq. and obtained cleistothecia which were morphologically normal, but contained no ascospores. Both clones had been previously assigned to the same mating type class (Morrison, 1960a,b). In intervarietal matings of E. graminis on wheat (Triticum compactum L.), Hiura (1962) crossed cultures of the same mating type and reported the formation of white mycelia mats containing the primordia of cleistothecia. Both sets of results are explicable if the activation of morphogenic genes outside the mating-type locus was responsible for fruit-body initiation.

A mutation at the mating type locus could also explain fruiting within a clone of a heterothallic fungus. A mutation from one mating type to a compatible allele could induce fusion of the two unlike nuclei and permit progression through the sexual cycle. Mathieson (1952) cited this phenomenon as an explanation for development of perithecia in homokaryotic cultures of Chromocrea spinulosa (Fuckel) Petch n. comb. Spontaneous mating type interconversions have also been reported in heterothallic strains of Saccharomyces cerevisiae at very low frequencies (Hicks and Herskowitz, 1977).

A study of the segregation patterns of the ascospores in the RR1 culture would clarify this situation. A failure to segregate mating types would suggest monokaryotic fruiting, whereas a segregation for mating type or pathogenic race would suggest a mutational event or contamination, respectively. However, methods for ascospore culture in S. pannosa var. rosae have not been developed. Mence and Hildebrandt (1964) obtained infection of detached rose leaves with

ascospores, but do not report development to secondary sporulation.

A cytological study of the fruiting phenomenon in the RR1 culture would also provide evidence for a mechanism. Karyogamy and meiosis do not occur in monokaryotic fruiters (Esser and Graw, 1980; Stahl and Esser, 1976), and when spores are produced, it is the result of a single mitotic division. However, if fruit-body formation in this culture is caused by a mutation in the incompatibility factors, or by contamination with a compatible race, karyogamy and meiosis should be evident in a cytological examination. However, before a cytological study can be undertaken, methods for inducing the phenomenon must be elucidated. For example, monokaryotic fruiting has been attributed to aging of the mycelium (Raper and Krongelb, 1958), dimunition of nutrition (Niederpruem et al., 1964), mechanical injury of mycelium (Leslie and Leonard, 1979), and to spontaneous (Esser and Meinhardt, 1977) and chemical events (Leslie and Leonard, 1979). Until a method for inducing fruiting in the RR1 culture is discovered, its mechanism will remain an enigma.

Two different types of mycelium may form on roses colonized by S. pannosa var. rosae. Secondary conidia are borne on evanescent, superficial hyphae, whereas cleistothecia form in secondary mycelia characterized by thickened walls and an almost obliterated lumen. Secondary mycelium usually persists in felt-like patches and is the "pannose" mycelium from which the species gets its name (Wheeler, 1978). Coyier classified rose powdery mildew into five groups based on the occurrence of cleistothecia and the persistence and morphology of the mycelium. He found that the formation of secondary mycleium was not always associated with ascocarp production, i.e. pannose

mycelium was present on specimens lacking ascocarps (Coyier, 1961). In the present study, pannose mycelium formed in crosses 5 and 10, in addition to the two crosses where cleistothecia were produced. Isolate 5 was a common participant in both crosses 5 and 10, and when single inoculations of isolate 5 to Dwarf Crimson Rambler and R. multiflora were examined, pannose mycelium was also observed. However, pannose mycelium was not formed when isolates 4 and 5 were combined on Dr. Huey, or when isolate 5 was inoculated singly to this host. These results imply that the differentiation of secondary or pannose mycelium is influenced by host factors and may occur independent of the sexual cycle.

In a study where monoconidial isolates of Podosphaera leucotricha (Ell. & Ev.) Salm. were randomly paired on apple (Malus spp.) seedlings, Coyier (1974) cited four possible explanations for failure of a cross to produce cleistothecia: (i) incompatibility, (ii) similarity of cultures, (iii) lack of intermingling of compatible mycelial strands on the host surface, and (iv) failure of continued growth of one of the cultures. Possibilities (ii) and (iv) can be eliminated in the present study because all crosses involved isolates which differed in pathogenicity and all isolates were recovered from crosses which failed to produce cleistothecia. However, this investigation suggests host physiology as an additional explanation for failure to mate. In the present study, RR1 was assigned to one mating type and isolates RR4 and 5 to an opposite mating type, compatible with the former. In crosses 3 and 9, both mating types were combined with isolate 4 on Dr. Huey, but neither resulted in production of cleistothecia. The absence of ascocarps in these two combinations and the inability of isolate 5

to form pannose mycelium on Dr. Huey, suggest that the physiology of this host may not be conducive for ascocarp formation. Schnathorst (1965) and Jhooty and McKeen (1962) discuss host physiology as a factor in ascocarp formation. Ascocarp production on roses is almost exclusively confined to stems, especially at thorns and nodes, and to the pedicels and hypanthia of blooms. Coyier stimulated ascocarp development on the stems and leaves of apple (Malus spp.) seedlings by applying indolebutyric acid (10 ppm) to these tissues (Coyier, unpublished data). His results suggest possible physiological reasons for the distribution of ascocarps on roses.

Results of the present study indicate that S. pannosa var. rosae is heterothallic. Although fruiting was observed in one culture derived from a monoconidial isolate, it is inappropriate to regard the fungus as homothallic for several reasons: (1) fruiting of the monoconidial isolate occurred in only one of eight inoculations, (2) the fruiting response was weak, and (3) asci did not contain the full complement of ascospores. The heterothallic nature of the fungus was disclosed when compatible isolates were combined. Paired inoculation of compatible isolates resulted in a fruiting response which occurred repeatedly and the formation of asci with eight well-differentiated ascospores.

Powers and Moseman (1956) found that the genes which control pathogenicity in E. graminis f. sp. tritici operated independently of those governing compatibility. Mating was possible between some cultures which gave identical pathogenic reactions on differential varieties. Other cultures, which differed in pathogenicity, were identical in mating potential. In the present study, the formation of

cleistocarps in the RR1 x isolate 5 and RR1 x RR4 crosses demonstrated that mating was possible between cultures of similar and dissimilar pathogenicity, respectively. However, the RR1 strain was avirulent on the hybrid tea roses, SR 70002/2 and Pink Parfait, which may partially explain the infrequent occurrence of cleistothecia on modern hybrids. In order for ascocarp formation to occur, compatible strains must be virulent on a mutual host, and that host must be physiologically conducive for fruit-body formation. Future studies should investigate the host range and mating ability of compatible strains on modern commercial hybrids to explain the infrequency of the sexual stage on these roses.

Figure 4-1. Ascocarp formation (arrow) on pedicel and hypanthium of Dwarf Crimson Rambler coinoculated with isolates RR1 and RR4.

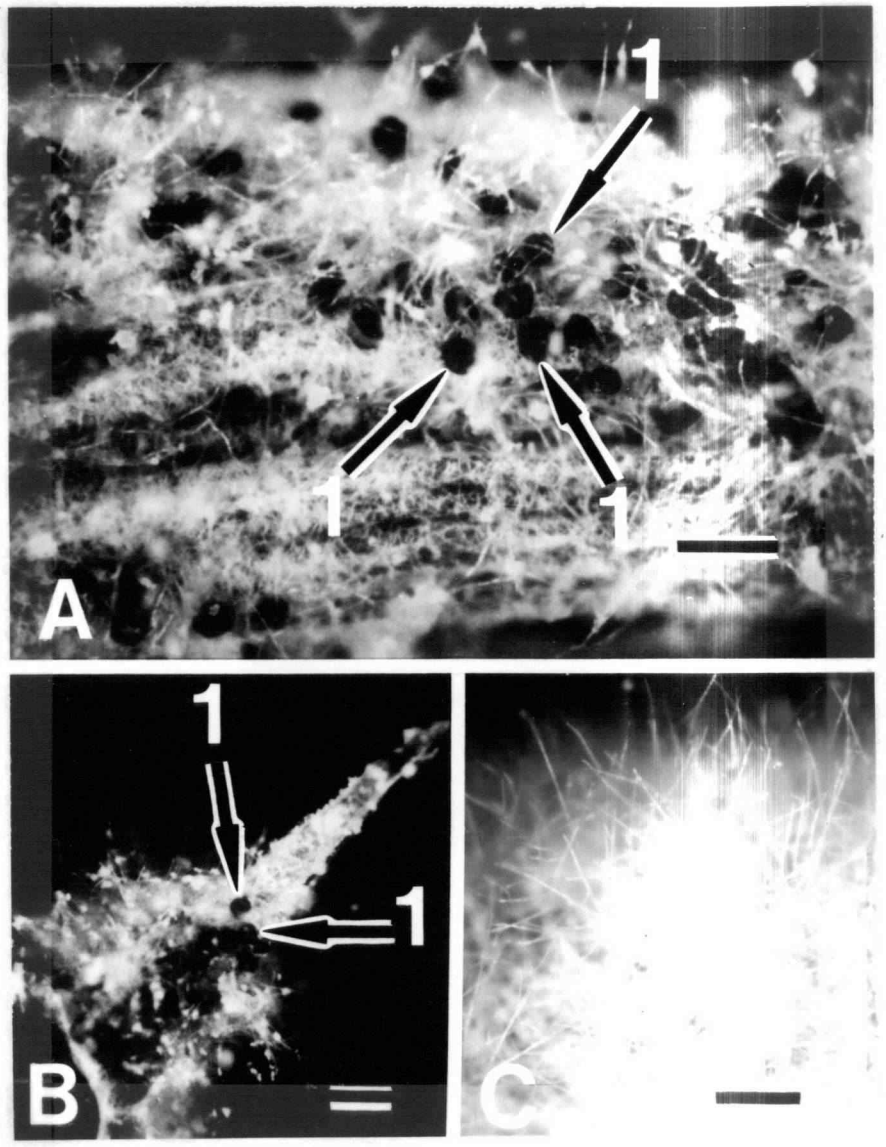

Figure 4-2. Features associated with formation of ascocarps by Sphaerotheca pannosa var. rosae. **A**, cleistothecia (1) on stems, and **B**, thorns of R. rugosa coinoculated with isolates RR1 and RR4; and **C**, formation of pannose mycelium lacking ascocarps on stem of R. multiflora inoculated with isolate 5. Scale bars represent 200 µm.

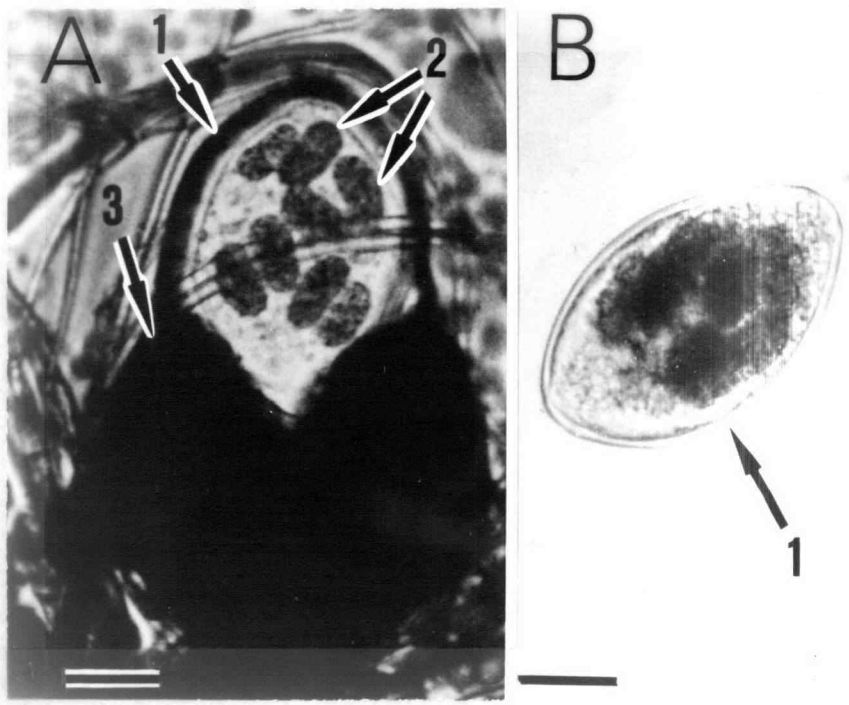

Figure 4-3. Asci produced in single and paired inoculations of Sphaerotheca pannosa var. rosae isolates to rose hosts. **A**, Ascus (1) and ascospores (2) contained in cleistothecium (3) which developed on R. rugosa coinoculated with isolates RR1 and RR4, and **B**, ascus (1) of an ascocarp which formed on R. rugosa inoculated with isolate RR1 only. Scale bars represent 30 µm.

Table 4-1. Host reaction types, virulence formulae and race abbreviations which identify five isolates of *Sphaerotheca pannosa* var. *rosae*.

	Host Reaction Type					
Isolate	SR 70002/2	Pink Parfait	Dr. Huey	*Rosa multiflora*	Virulence Formula resistant/susceptible	Race abbreviation
1	5[a]	1-2	3-5	4	2/4,3,1[b]	1
2	5	3-4	3-4	3-4	/4,3,2,1	2
3	0	3-4	3	3	1/4,3,2	3
4	0	1	5	0	1,4,2/3	4
5	0	1	3-4	5	1,2/3,4	5

[a]Reaction type 0 = no macroscopic evidence of fungus colonization; reaction type 1 = necrotic lesions, 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 over 25% of leaf area, no necrosis.

[b]1 = SR 70002/2; 2 = Pink Parfait; 3 = Dr. Huey; and 4 = *Rosa multiflora*.

Table 4-2. *Sphaerotheca pannosa* var. *rosae* isolates combined in paired inoculations to four rose hosts.

Cross No.	Isolates	Rosa Host
1	RR1 x RR4	*R. rugosa* Dwarf Crimson Rambler
2	DCR1 x DCR2	*R. rugosa* Dwarf Crimson Rambler
3	RR1 x 4	Dr. Huey
4	RR1 x 5	*R. multiflora*
5	RR4 x 5	*R. multiflora*
6	1 x 2	Dwarf Crimson Rambler
7	2 x 3	Dwarf Crimson Rambler
8	1 x 4	Dr. Huey
9	4 x 5	Dr. Huey
10	3 x 5	Dwarf Crimson Rambler
11	1 x 5	*R. multiflora*

Table 4-3. Virulence characteristics of four *Sphaerotheca pannosa* var. *rosae* isolates collected from *R. rugosa* and Dwarf Crimson Rambler plants.

	Host Reaction Type					
Isolate[a]	SR 70002/2	Pink Parfait	Dr. Huey	*Rosa multiflora*	Virulence Formula resistant/susceptible	Race abbreviation
RR1	0[b]	0	4-5	3	1,2/3,4[c]	5
RR4	5	1-2	3-4	3-4	2/4,3,1	1
DCR1	0	4	3	3	1/4,3,2	3
DCR2	5	1-2	4	4	2/4,3,1	1

[a] RR = *R. rugosa*
DCR = Dwarf Crimson Rambler

[b] Reaction type 0 = no macroscopic evidence of fungus colonization; reaction type 1 = necrotic lesions, 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 over 25% of leaf area, no necrosis.

[c] 1 = SR 70002/2; 2 = Pink Parfait; 3 = Dr. Huey; and 4 = *Rosa multiflora*.

DISCUSSION AND CONCLUSIONS

The origin of race variation and dynamics lies in mutations that can combine, undergo selection, and spread (Wolfe and Schwarzbach, 1978). Mutation rates in powdery mildews are probably similar to those in other organisms, i.e. 10^{-6} to 10^{-8} per locus. Manners (1971) estimated that 1 cm of infected barley leaf area produces approximately 10^4 E. graminis conidia per day. If the rate of spore production is similar in Sphaerotheca pannosa var. rosae, then even in a small field or greenhouse there is virtually unlimited possibility for production of mutants virulent against any resistance gene. However, S. pannosa does not appear to have high vertical mutability, the propensity to rapidly produce new vertical pathotypes (Robinson, 1969). For example, greenhouse plantings of SR 70002/2 remained free of infection for several years, although intense selection pressure existed for the production of virulent mutants. This rose did not succumb to infection until virulent race 1 was introduced to greenhouses at the Horticultural Crops Research Laboratory (HRCL). The sexual recombination of mutated genes with other virulence genes would be unlikely under glass. Cleistothecia are rarely formed in greenhouses and are generally found only on ramblers, climbers, and old shrub roses, rather than on hybrid tea roses grown for floricultural use (Price, 1970). Therefore, the low vertical mutability of S. pannosa and absence of the sexual stage on hybrid tea roses may indicate that

pathogenic variation in greenhouse populations will be relatively stable and homogenous. This hypothesis is supported by the population surveys at two commercial greenhouses where only race 3 pathogenicity could be detected (Chapter 3). A low vertical mutability may also indicate that the fungus will be slow in developing other genetic variants, such as fungicide-resistant strains.

The shift from race 3 to race 2 at HCRL greenhouses demonstrated that changes can occur rapidly. In both instances where a change in pathogenicity was detected, it could be traced to the introduction of infected plant material (Chapter 3). Therefore, growers should quarantine any new material they receive and disinfest it thoroughly before introducing it to greenhouses.

The role of cleistothecia in the epidemiology of S. pannosa is unclear. It has generally been assumed that cleistothecia serve a dual function, i.e. reassortment of the variation generated by mutation and a means for perennation of the fungus. The results of Price (1970) contradict the latter hypothesis. Over-wintering cleistocarps showed no dehiscence and very few contained asci with ascospores. Perennation of the fungus as mycelium in dormant buds was demonstrated. However, data in Chapter 3 suggest that cleistothecia do function in the recombination of genetic material. This is the first experimental evidence indicating the role of cleistothecia in the life cycle of S. pannosa.

The identification of virulence genes in S. pannosa and resistance genes in rose hosts has been limited by several difficulties:

(i) the polyploid nature of most rose cultivars and their complex ancestry, (ii) failure to generate the sexual stage of S. pannosa in the laboratory, and (iii) unsuccessful efforts to demonstrate the pathogenicity of ascospore infections. Limitations (ii) and (iii) have made it impossible to obtain the F_1 from matings of S. pannosa isolates. However, compatible isolates of each mating type have now been identified (Chapter 4), which eliminates the barrier imposed by restriction (ii). If future studies succeed in proving the pathogenicity of ascospore infections, the F_1 of S. pannosa matings could be studied for segregation of virulence on rose hosts. This would make possible the identification of specific virulence genes in the pathogen.

This study suggests the existence of two separate resistance factors in hybrid tea roses (Chapter 1; Bender and Coyier, unpublished data). For example, one group of hybrids possesses resistance to race 1 and includes Pink Parfait, Samantha, and Red Cascade. Most floricultural roses can be assigned to this group. A second class of hybrids is resistant to race 3 and includes SR 70002/2, Tropicana, and Sarabande. The resistance of the two groups is mutually exclusive, i.e. roses in each group have resistance to one, but not both races. The resistance factors in the two groups may be members of an allelic series at a locus for powdery mildew resistance. Therefore, it may not be possible to combine resistance to both races in a hybrid tea rose. This theory is substantiated by the fact that a cultivar with resistance to both

races has not been identified. Theorhetically, such a rose would also be resistant to race 2, which is virulent on hosts in both groups. Efforts to identify race 2 resistance have also been unsuccessful. Therefore, the screening efforts of breeders should concentrate on identification of resistance or tolerance to race 2.

BIBLIOGRAPHY

Atkiss, L. 1978. Disease resistant rose varieties. American Rose Annual 63:99-104.

Bartlett, Allan B. 1963. Relation of nitrogenous substances in rose leaves to powdery mildew resistance. Ph.D. thesis, Oregon State University, 75 pp.

Bender, C. L. and Coyier, D. L. 1981. Identification of two biotypes of Sphaerotheca pannosa var. rosae on four rose cultivars. (Abstr.) Phytopathology 71:860.

Bender, C. L. and Coyier, D. L. 1982. The isolation and identification of races in Sphaerotheca pannosa var. rosae, powdery mildew of rose. (Abstr.) Phytopathology 72:983.

Bioletti, F. T. 1907. Oidium or powdery mildew of the vine. Calif. Agric. Exp. St. Bull. 186:315-352.

Browder, L. E., Lyon, R. L., and Eversmeyer, M. G. 1980. Races, pathogenicity phenotypes, and type cultures of plant pathogens. Phytopathology 70:581-582.

Buck, G. J. 1951. Varieties of rose understocks. American Rose Annual 36:101-116.

Butt, D. J. 1978. Epidemiology of powdery mildews. Pages 51-79. In: D. M. Spencer, ed., The Powdery Mildews. Academic Press, New York.

Byford, W. J. and Bentley, D. 1976. Cleistothecia of Erysiphe betae in England. Trans. Br. Mycol. Soc. 67:544-545.

Cherewick, W. J. 1944. Studies on the biology of Erysiphe graminis DC. Can. J. Res., Sect. C., Bot. Sci. 22:52-86.

Coyier, D. L. 1961. Biology and control of rose powdery mildew. Ph.D. thesis, University of Wisconsin, 109 pp.

Coyier, D. L. 1973. A portable, single plant isolation chamber. Can. J. Plant Sci. 53:915-916.

Coyier, D. L. 1974. Heterothallism in the apple powdery mildew fungus, Podosphaera leucotricha. Phytopathology 64:246-248.

Coyier, D. L. and Hildebrandt, A. C. 1962. Characteristics of rose powdery mildew fungi in Wisconsin. (Abst.) Phytopathology 52:6-7.

Day, P. R. 1974. Genetics of Host-Parasite Interaction. W. H. Freeman, San Francisco. 238 pp.

Deshpande, G. D. 1980. A note on the varietal reaction of hybrid tea roses to powdery mildew. Indian J. Mycol. Plant Pathol. 9:120.

Dundas, B. 1936. Inheritance of resistance to powdery mildew in beans. Hilgardia 10:243-253.

Ellingboe, A. H. 1972. Genetics and physiology of primary infection by Erysiphe graminis. Phytopathology 62:401-406.

Esser, K. and Graw, D. 1980. Homokaryotic fruiting in the bipolar-incompatible ascomycete Podospora anserina. Mycologia 72:534-541.

Esser, K. and Meinhardt, F. 1977. A common genetic control of dikaryotic and monokaryotic fruiting in the basidiomycete Agrocybe aegerita. Mol. Gen. Genet. 155:113-115.

Esser, K., Saleh, F. and Meinhardt, F. 1979. Genetics of fruit body production in higher basidiomycetes. II. Monokaryotic and dikaryotic fruiting in Schizophyllum commune. Current Genetics 1:85-88.

Fincham, J. R. S. and Day, P. R. 1979. Fungal Genetics. University of California Press, Berkeley. 636 pp.

Graf-Marin, A. 1934. Studies on powdery mildew of cereals. Cornell Agric. Exp. St. Mem. 157:1-48.

Green, G. J. 1965. Stem rust of wheat, rye, and barley in Canada in 1964. Can. Plant Dis. Surv. 45:23-29.

Hicks, J. B. and Herskowitz, I. 1977. Interconversion of yeast mating types. II. Restoration of mating ability to sterile mutants in homothallic and heterothallic strains. Genetics 85:373-393.

Hiura, U. 1962. Hybridization between varieties of Erysiphe graminis. Phytopathology 52:664-666.

Hiura, U. and Tomoda, H. 1959. On the transmission of pathogenicity by Erysiphe graminis [in Japanese]. (Abst.) Ann. Phytopathol. Soc., Jpn. 24:10-11.

Homma, Y. 1933. Homothallism in Sphaerotheca fuliginea (Schlecht.) Pollacci. Proc. Imp. Acad., Tokyo 9:186-187.

Homma, Y. 1937. Erysiphaceae of Japan. J. Fac. Agric., Hokkaido Univ. 38:183-461.

Jackson, G. V. H. and Wheeler, B. E. J. 1975. Formation of cleistothecia in Sphaerotheca mors-uvae. Trans. Br. Mycol. Soc. 65:491-496.

James, W. C. 1974. Assessment of plant diseases and losses. Annu. Rev. Phytopathol. 12:27-48.

Jhooty, J. S. and McKeen, W. E. 1962. The perfect stage of Sphaerotheca macularis on strawberry plants. Plant Dis. Rep. 46:218-219.

Johnson, L. F. 1972. Susceptibility of rose cultivars to blackspot and powdery mildew. American Rose Annual 57:74-87.

Johnson, R., and Taylor, A. J. 1976. Spore yield of pathogens in investigations of the race-specificity of host resistance. Annu. Rev. Phytopathol. 14:97-119.

Kable, P. F., Fried, P. M., and MacKenzie, D. R. 1980. The spread of a powdery mildew of peach. Phytopathology 70:601-604.

Krüssmann, G. 1981. The Complete Book of Roses. Timber Press, Portland, Oregon. 436 pp.

Leslie, J. F. and Leonard, T. J. 1979. Three independent genetic systems that control initiation of a fungal fruiting body. Mol. Gen. Genet. 171:257-260.

Lim, T. M. 1973. A rapid laboratory method of assessing susceptibility of Hevea clones to Oidium heveae. Exp. Agric. 9:275-279.

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.

Longrée, K. 1939. The effect of temperature and relative humidity on the powdery mildew of roses. Mem. Cornell Univ. Agric. Exp. St. No. 223. 43 pp.

Manners, J. G. 1971. Spore formation by certain pathogens in infected leaves. Pages 339-352. In: T. F. Preece, C. H. Dickinson, eds. Ecology of Leaf Surface Microorganisms. Academic Press, London.

Masri, S. S. and Ellingboe, A. H. 1966a. Germination of conidia and formation of appressoria and secondary hyphae in Erysiphe graminis f. sp. tritici. Phytopathology 56:304-308.

Masri, S. S. and Ellingboe, A. H. 1966b. Primary infection of wheat and barley by Erysiphe graminis. Phytopathology 56:389-395.

Mathieson, M. J. 1952. Ascospore dimorphism and mating type in Chromocrea spinulosa (Fuckel) Petch n. comb. Ann. Bot. (Lond.) 16:449-466.

Mence, M. J. and Hildebrandt, A. C. 1964. Rose leaflet susceptibility in vitro to powdery mildew infection. (Abst.) Am. J. Bot. 51:680.

Mence, M. J. and Hildebrandt, A. C. 1966. Resistance to powdery mildew in rose. Ann. Appl. Biol. 58:309-320.

Morrison, R. M. 1960a. Studies of clonal isolates of Erysiphe cichoracearum on leaf disk culture. Mycologia 52:388-393.

Morrison, R. M. 1960b. Compatibility of several clonal lines of Erysiphe cichoracearum. Mycologia 52:786-794.

Moseman, J. G. 1956. Physiological races of Erysiphe graminis f. sp. hordei in North America. Phytopathology 46:318-322.

Mukhopadhyay, A. N. and Russell, G. E. 1979. Development of Erysiphe betae on leaves of four sugar beet varieties. Phytopathol. Z. 96:15-20.

Nair, K. R. S. and Ellingboe, A. H. 1965. Germination of conidia of Erysiphe graminis f. sp. tritici. Phytopathology 55:365-368.

Niederpruem, D. S., Hobbs, H., and Henry, L. 1964. Nutritional studies of development in Schizophyllum commune. J. Bacteriol. 88:1721-1729.

Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.

Powers, H. R. and Moseman, J. G. 1956. Heterothallism in Erysiphe graminis tritici. (Abstr.) Phytopathology 46:23.

Price, T. V. 1970. Epidemiology and control of powdery mildew (Sphaerotheca pannosa) on roses. Ann. Appl. Biol. 65:231-248.

Raper, J. R. and Krongelb, G. S. 1958. Genetic and environmental aspects of fruiting in Schizophyllum commune. Mycologia 50:707-740.

Robinson, R. A. 1969. Disease resistance terminology. Rev. Appl. Mycol. 48:593-606.

Rogers, M. N. 1959. Some effects of moisture and host plant susceptibility on the development of powdery mildew of roses,

caused by Sphaerotheca pannosa var. rosae. Mem. Cornell Univ. Agric. Exp. St. No. 363. 38 pp.

Rufty, R. C., Hebert, T. T., and Murphy, C. F. 1981. Variation in virulence in isolates of Septoria nodorum. Phytopathology 71:593-596.

Salmon, E. 1900. A monograph of the Erysiphaceae. Torrey Bot. Club, Mem. 9. 292 pp.

Schnathorst, W. C. 1959. Heterothallism in the lettuce strain of Erysiphe cichoracearum. Mycologia 51:708-711.

Schnathorst, W. C. 1965. Environmental relationships in the powdery mildews. Annu. Rev. Phytopathology 3:343-366.

Smith, C. G. 1970. Production of powdery mildew cleistocarps in a controlled environment. Trans. Br. Mycol. Soc. 55:355-365.

Stahl, U. and Esser, K. 1976. Genetics of fruit body production in higher basidiomycetes. I. Monokaryotic fruiting and its correlation with dikaryotic fruiting in Polyporus ciliatus. Mol. Gen. Genet. 148:183-197.

Stakman, E. C., Levine, M. N., and Loegering, W. Q. 1944. Identification of physiologic races of Puccinia graminis tritici. U. S. Dep. Agric. Res. Bull. E 617. 27 pp.

Stavely, J. R. and Hanson, E. W. 1966. Identification and maintenance of races of Erysiphe polygoni from Trifolium pratense. Phytopathology 56:795-798.

Toxopeus, H. J. 1958. Note on the variation in expression of hypersensitivity to Phytophthora infestans in potato seedlings. Euphytica 7:38-40.

Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, New York. 206 pp.

Vanderplank, J. E. 1978. Genetics and Molecular Basis of Plant Pathogenesis. Springer-Verlag, Berlin, Heidelberg, and New York. 167 pp.

Wheeler, B. E. J. 1978. Powdery mildews of ornamentals. Pages 411-445. In: D. M. Spencer, ed., The Powdery Mildews. Academic Press, New York.

Wolfe, M. S. 1965. Physiologic specialization of Erysiphe graminis f. sp. tritici in the United Kingdom. Trans. Br. Mycol. Soc. 48:315-326.

Wolfe, M. S., Barrett, J. A., Shattock, R. C., Shaw, D. S., and Whitbread, R. 1976. Phenotype-phenotype analysis: field application of the gene-for-gene hypothesis in host-pathogen relations. Ann. Appl. Biol. 82:369-374.

Wolfe, M. S. and Schwarzbach, E. 1975. The use of virulence analysis in cereal mildews. Phytopath. Z. 82:297-307.

Wolfe, M. S. and Schwarzbach, E. 1978. Patterns of race changes in powdery mildews. Ann. Rev. Phytopathol. 16:159-180.

Yarwood, C. E. 1935. Heterothallism of sunflower powdery mildew. Science (Wash. D. C.) 82:417-418.

Yarwood, C. E. 1936. Host range and physiologic specialization of red clover powdery mildew, Erysiphe polygoni. J. Agric. Res. 52:659-665.

Yarwood, C. E. 1946. Detached leaf culture. Bot. Rev. 12:1-56.

Yarwood, C. E. 1952. Apricot powdery mildew from rose and peach. Bull. Calif. Dept. Agric. No. 41:19-25.