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ABSTRACT

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The germination of field-collected pseudosclerotia and the development of apothecia from eight New Jersey populations of the mummy berry fungus *Monilinia vaccinii-corymbosi* were evaluated under controlled conditions in the greenhouse. Development data for apothecia were used to describe the timing of apothecium formation and to estimate broad- and narrow-sense heritabilities of fungal phenology. Mean development times for the formation of apothecia ranged from 35.4 to 54.7 days. The mean development times for populations collected from early-season cv. Weymouth ranged from 35.4 to 39.6 days and were significantly shorter than the development times for three of the four populations collected from late-season cv. Jersey (46.9 to 54.7 days) or for the population collected from mixed stands of cultivated blueberries (42.7 days). The development of populations from late cultivars planted in very close proximity to early cv. Weymouth was early (36.5 to 39.0 days) and not significantly different from the development of populations collected from cv. Weymouth. Phenotypic and genetic variances of apothecium development for individual populations ranged from 18.9 to 44.8 and 7.2 to 30.9, respectively. Broad-sense heritabilities of apothecia development for each fungal population, calculated by partitioning phenotypic variation into genetic and environmental components, ranged from 0.31 to 0.78. Narrow-sense heritabilities of apothecia development, based on parent-offspring regression, ranged from 0.58 to 0.78. These results indicate that populations of *M. vaccinii-corymbosi* differ in phenology and that a significant portion of the phenological variation within populations is genetic. Thus, it is plausible to propose that the phenology of apothecium development is a component of fungal fitness and that host phenology can influence the timing of pathogen development.

Additional keywords: disease avoidance, fungal adaptation, quantitative fungal genetics, *Vaccinium corymbosum*.

The phenology of fungal plant pathogens (i.e., the development of an organism in relation to its environment) is a potentially important component of fitness. Pathogen genotypes that are phenologically coordinated with the development of their host, especially hosts with highly defined periods of susceptibility, have a greater probability of infecting and reproducing than do genotypes with phenologies that are partially or completely displaced from the development of their host. For example, fungal sclerotia of the onion white rot fungus, *Sclerotium cepivorum*, remain dormant until volatile compounds produced in the onion roots are released into the soil and stimulate fungal development (5). This innate dormancy delays fungal development until host tissue is present, thereby increasing the potential survival of the pathogen. Similarly, coordinated host and pathogen phenologies have also been reported in studies of the apple scab fungus (*Venturia inaequalis*) (25) and the mummy berry fungus from blueberry (*Monilinia vaccinii-corymbosi*). In the mummy berry pathosystem, Ramsdell et al. (19), Batra (1,3), and Lehman and Oudemans (15) have all reported a high degree of phenological synchrony between pathogen and host. In both examples, differences in the periods of pathogen infectivity corresponded to the timing of host development. However, none of these studies have investigated the potential for phenological adaptation of pathogen to host.

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The mummy berry pathosystem is an ideal system in which to examine the significance of fungal phenology in pathogenesis. In this pathosystem, the blueberry host (*Vaccinium corymbosum*) and the fungal pathogen (*M. vaccinii-corymbosi)* display highly defined periods of susceptibility and infectivity, respectively, during the initiation of disease in the spring (1,4,14,19). Hence, primary infection occurs only when pathogen spores are produced and disseminated during the period of host susceptibility of the blueberry cultivars. Theoretically, as the host and pathogen develop more synchronously, the probability of wind-dispersed ascospores contacting young breaking buds increases. Assuming all other factors that influence disease remain constant, the incidence of disease is greatest for host and pathogens with the most closely coordinated phenologies. By understanding the relationship between pathogen and host phenology, it is possible to begin developing strategies to breed resistant cultivars based on disease avoidance (14).

The life cycle of *M. vaccinii-corymbosi* has three major components: (i) an overwintering phase, (ii) a primary infection phase (sexual), and (iii) a secondary infection phase (asexual). During the overwintering phase, the fungus exists as a pseudosclerotium and undergoes chilling. After an adequate chilling period, the fungus enters the primary infection phase, during which pseudosclerotia germinate to produce apothecia from which ascospores are forcibly discharged and wind dispersed (1,3,4,16). The wind-blown ascospores can infect only the breaking buds and young elongating shoots of the blueberry host (12,14,19,20). Such juvenile tissue is present for a relatively short period in the development of the host (14). Therefore, pathogen phenology must be coordinated with host phenology for disease development to occur. During the secondary infection phase, pollinators carry conidia produced from these primary infections to flowers, where the conidia germinate and infect the ovary (17,22). These secondary infections may even-

tually colonize the locules and mummify the fruit to produce pseudosclerotia.

The major objective of this research was to test the hypothesis that the sexual phenology of *M. vaccinii-corymbosi*, specifically the formation of apothecia and resulting ascospores, is a heritable genetic trait that can be potentially altered by selection due to host phenology. In this study, the variation in the timing of apothecium development was quantified for eight New Jersey populations from blueberry cultivars of distinct phenologies. The observed variation was partitioned into genetic and environmental components from which estimates of broad-sense heritability could be calculated. In addition, fungal isolates from field-derived apothecia were used in artificial inoculations to produce subsequent generations of apothecia, and narrow-sense heritabilities of apothecium production were estimated from parent-offspring regressions of isolates of *M. vaccinii-corymbosi*.

MATERIALS AND METHODS

Populations of *M. vaccinii-corymbosi*. Eight populations of *M. vaccinii-corymbosi* were collected in 1995 from naturally infected blueberry bushes growing in Burlington County, New Jersey (Table 1). Pseudosclerotia of populations 951 and 952 and populations 953 to 956 were collected under bushes of early-fruiting cv. Weymouth and late-fruiting cv. Jersey, respectively. Pseudosclerotia of population 957 were from a heterogeneous mixture of cultivars located at the Rutgers Blueberry and Cranberry Research and Extension Center. Population 958 was collected from late-fruiting cv. Rubel. Pseudosclerotia collected from these populations were used in the greenhouse to evaluate the development of pseudosclerotium germination and apothecium formation. Collection sites for all populations were 2 to 26 km apart except for populations 951-WT and 958-WT and populations 952-WT and 956-WT, which were 0.09 and 0.15 km apart, respectively (Table 1).

Pseudosclerotium germination and apothecium development. Mummified fruits from individual collection sites were buried below the surface of moist soil in 17 × 38-cm flats. Flats were maintained in a greenhouse at 20 to 27°C with natural lighting for 1 month and then moved to a unlighted environmental chamber at 0 to 7°C. After pseudosclerotia had received ~1,200 h of temperatures between 0 to 7°C, flats were transferred to 15°C to initiate pseudosclerotium germination and apothecium production. In addition to natural lighting, flats received 10 to 12 h of supplemental fluorescent lighting per day.

Mean development times for each fungal population were determined from daily counts of the number of newly formed apothecia. Development times, expressed as days at 15°C, were calculated as the weighted mean time as previously described (15). Analysis of variance was used to compare apothecium developmental times for the populations. The model was a randomized complete block design with eight fungal populations and five replications. Blocks were locations on a greenhouse bench. Each experimental unit was composed of 50 mummies in an individual flat of soil.

Ascospore production. Forty-eight germinating pseudosclerotia in early stages of development were collected from the field at the Rutgers Blueberry and Cranberry Research and Extension Center and placed on moist sand. The germinating fungal structures were moved to a growth chamber at 15°C with a 12-h photoperiod. Spores were collected daily from each developing apothecium onto coverslips, until all apothecia had ceased sporulating. Spores were then washed from the coverslips and counted with a haemocytometer. The average number of spores per apothecium was calculated for each day of collection. The development times for apothecia and the sporulation data were used to estimate the relative daily ascospore production for each fungal population. In the calculation of relative daily ascospore production, we did not quantify differences between early and late populations for the parameters of ascospore production and viability and pseudosclerotial survival. Rather, we used data for these traits based on the average response of a mixed population of pseudosclerotia or the average response of all eight populations used in this study. Estimates were based on apothecia produced from a hypothetical population of 100 pseudosclerotia. Daily spore production for each of the eight populations of apothecia was determined by multiplying the number of sporulating apothecia present on an individual day by the average number of spores per apothecium per day. The number of sporulating apothecia present each day was determined from the phenological data collected in this study. For example, the number of newly formed apothecia on day i (A_i) from a hypothetical population was calculated as A_i = (total number of pseudosclerotia) × (percentage of surviving pseudosclerotia) × (number of apothecia per pseudosclerotium) × (proportion of apothecia formed on day i).

The total number of pseudosclerotia was arbitrarily set at 100. The values for percentage of surviving pseudosclerotia and number of apothecia per pseudosclerotium, determined from data collected in this study, are 40% and 1.8, respectively, averaged across all populations. The relative proportion of the apothecia formed on day i was also determined from phenological data collected in this study and was unique for each population. For example, the numbers of newly formed apothecia produced on day 24 for population 951-WT was 100 × 0.40 × 1.8 × 0.026 = 1.9 apothecia. In contrast, the number of newly formed apothecia produced on day 24 for population 954-WT was 100 × 0.40 × 1.8 × 0.013 = 0.94 apothecia. Because sporulation for individual pseudosclerotia continued for 9 days, the total number of sporulating apothecia present on day i (SA_i) was calculated as the sum of the number of newly formed apothecia and the number of apothecia produced on the eight previous days. To calculate the total number of ascospores produced for each population for day i (T_i), values for SA_i were multiplied by the average number of ascospores per apothecium per day (61,293 spores per apothecium per day) multiplied by the average percentage of spore viability (70%). Values for T_i were expressed as a proportion of the total number of ascospores produced across all days of sporulation.

Genetic variation and broad-sense heritability. Four or five replications of 50 mummies from each population were used to quantify genetic and environmental variation and to estimate broad-sense heritability of apothecium development. Broad-sense heritability represents the extent to which individual phenotypes are de-

TABLE 1. Eight populations of the mummy berry fungus (*Monilinia vaccinii-corymbosi*) collected in New Jersey in 1995 from early and late blueberry cultivars

Collection location (designation)	Blueberry cultivar	Cultivar phenology[z]	Approximate distance between sites (km)						
			951	952	953	954	955	956	957
Speedwell (951-WT)	Weymouth	Early							
Indian Mills (952-WT)	Weymouth	Early	16.76						
Sheep Pen Hill (953-WT)	Jersey	Late	20.12	20.73					
Chatsworth (954-WT)	Jersey	Late	9.45	14.63	10.36				
Sheep Pen Hill (955-WT)	Jersey	Late	21.49	20.42	2.44	11.58			
Indian Mills (956-WT)	Jersey	Late	16.76	0.15	20.73	14.63	20.42		
Jenkins (957-WT)	Mixed	Mixed	5.33	18.59	24.99	14.33	26.37	18.59	
Speedwell (958-WT)	Rubel	Late	0.09	16.76	20.12	9.45	21.49	16.76	5.33

[z] Cultivar phenology was previously reported based on the time of fruit maturation as well as quantification of shoot elongation (6,14,15).

termined by their genotypes. It is described by the ratio of genetic variation to total phenotypic variation. Environmental variances were estimated from the variation between replications for an individual population (i.e., variation among mean development times for an individual population). The replications for an individual population were assumed to be genetically identical, barring sampling error. Therefore, variation among mean development times calculated for each replication (σ^2_{Pm}) consisted of only environmental variation (σ^2_E) and could be described by the equation $\sigma^2_{Pm} = \sigma^2_E/r$ (9), in which r is the number of replications. This equation is based on the known relationship between the variance among individuals and the variance among means of individuals and can be rearranged to determine environmental variance ($\sigma^2_E = r\sigma^2_{Pm}$). Populations were assumed to be genetically heterogeneous such that phenotypic variation in phenology among individual apothecia within replications (σ^2_{Pi}) was composed of genetic (σ^2_G) and environmental (σ^2_E) variance components and could be described by the equation $\sigma^2_{Pi} = \sigma^2_G + \sigma^2_E$ (9). Genetic variation was calculated as $\sigma^2_G = \sigma^2_{Pi} - \sigma^2_E$ or $\sigma^2_G = \sigma^2_{Pi} - r\sigma^2_{Pm}$. Broad-sense heritability (H) was calculated with estimates of σ^2_{Pm} and σ^2_{Pi} as $H = (\sigma^2_{Pi} - r\sigma^2_{Pm})/\sigma^2_{Pi}$.

Narrow-sense heritability. Narrow-sense heritability represents the degree of resemblance between relatives (i.e., the extent to which phenotypes of progeny are determined by the genes transmitted from the parents). Narrow-sense heritability was estimated for populations 951-WT and 954-WT from parent-offspring regressions. In the spring of 1996, pseudosclerotia from each of the populations were collected and given a 1,200-h chilling period. The number of days required to develop mature cups was recorded. Ascospores were then collected from individual apothecia of known development times and used to inoculate susceptible blueberry shoots. After the infected shoots became blighted and began to sporulate, conidia were collected, mixed with pollen, and used to inoculate 50 to 75 newly opened flowers of the blueberry cv. Jersey. Flowers were then allowed to mature and form infected fruits that eventually developed into fully formed pseudosclerotia. Because ascospores collected from each individual apothecium were maintained separately, each pseudosclerotia produced from conidial infections could be traced to a specific apothecium of known development time. We were able to produce populations of pseudosclerotia that could be traced to a common parental apothecium because multiple flowers were inoculated with a common conidial source. In total, 21 populations of pseudosclerotia derived from apothecia with short, intermediate, and long development times were used. These first-generation pseudosclerotia were again given 1,200 h of chilling and allowed to germinate under the same conditions mentioned previously. The resulting apothecia, which represent the offspring generation, were evaluated for development times. Development times for populations of apothecia of the offspring generation were compared with the values for progenitor parental apothecium.

The development times for parent-offspring generations were used to estimate heritability of apothecium development based on parent-offspring regression (9). Mean development time for the collection of apothecia from the same parental apothecium (i.e., offspring value) was plotted against development time for the progenitor apothecium (i.e., mid-parent value). This relationship was plotted for each parental apothecium and the resulting offspring apothecia. Least-square regression was used to describe the relationship for parents and offspring, and the slope of the straight line was used as an estimate of heritability (i.e., the slope describes how closely offspring values resemble the mid-parent value). Differences in slopes between populations 951-WT and 954-WT were compared with Student's t tests (24).

RESULTS

Fungal development. Fungal populations differed significantly for apothecium development times ($P < 0.0001$) (Table 2). Mean development times ranged from 35.4 to 54.7 days. The earliest and latest populations were 951-WT and 954-WT, respectively, which were collected from blueberry cultivars of corresponding early and late phenologies. With the exception of development times for populations 956-WT and 958-WT, the development of all fungal populations corresponded to the phenology of the host cultivar or cultivars from which the fungus was initially collected. The average development times for two populations on early cv. Weymouth and for four populations on late cv. Jersey were 37.5 and 47.2 days, respectively. The development time for population 957-WT, which was collected from a mixed host population, was 42.7 days.

The average phenotypic variances based on differences among fungal isolates within populations (σ^2_{Pi}) ranged from 18.88 to 44.75 (Table 2). Populations 957 and 951 exhibited the greatest and least variation, respectively. Estimates of environmental variances (σ^2_E), calculated from the equation $\sigma^2_E = \sigma^2_P \times$ (the number of replications), ranged from 4.91 to 16.36 (Table 2). Populations 953, 955, and 957 had the greatest environmental variance and either exhibited a bimodal distribution for development times (populations 953 and 955) or were collected from a heterogeneous set of host cultivars (population 957).

Ascospore production. Expanding apothecia produced ascospores for 9 days (Fig. 1). The distribution of spore production was skewed. Generally, spore production peaked early and then slowly decreased over time. On average, 61,293 spores per cup per day were produced. Mature, fully formed apothecia with a distinct margin developed 3 to 4 days after sporulation began.

Sporulation patterns for populations of 100 apothecia were calculated based on fungal development data and sporulation patterns for individual pseudosclerotia and were used to predict the infectious period for the eight populations of *M. vaccinii-corymbosi* (Fig.

TABLE 2. Development times, variances, and estimates of broad-sense heritability for eight populations of the mummy berry fungus *Monilinia vaccinii-corymbosi*

Fungal population	Development time (days)[w]	n[x]	Variances[y]				Heritability (H)[z]
			σ^2_{Pi}	σ^2_{Pm}	σ^2_E	σ^2_G	
951-WT	35.4 e	4	18.88	1.77	7.08	11.81	0.63
952-WT	39.6 d	5	22.32	0.98	4.91	17.41	0.78
953-WT	48.0 b	5	23.58	3.27	16.36	7.22	0.31
954-WT	54.7 a	5	26.24	1.57	7.86	18.38	0.70
955-WT	46.9 b	5	23.35	3.11	15.56	7.79	0.33
956-WT	39.0 d	5	27.21	1.43	7.19	20.02	0.74
957-WT	42.7 c	5	44.75	2.77	13.84	30.91	0.69
958-WT	36.5 e	5	30.78	1.80	9.10	21.68	0.70

[w] Values for development times are means of five replications of 50 mummies, except for population 951-WT, which is based on four replications. Populations with a letter in common do not differ significantly according to Student-Newman-Keuls' test ($\alpha = 0.05$).

[x] Number of replications.

[y] σ^2_{Pi} = Phenotypic variance based on populations of individuals ($= \sigma^2_G + \sigma^2_E$). σ^2_{Pm} = Phenotypic variance based on means of populations of individuals ($= \sigma^2_E$/number of replications). σ^2_E = Environmental variance (= number of replications $\times \sigma^2_{Pm}$). σ^2_G = Genetic variance ($= \sigma^2_{Pi} - \sigma^2_E$ or $= \sigma^2_{Pi}$ – number of replications $\times \sigma^2_{Pm}$).

[z] Heritability = ratio of genetic variance to total phenotypic variance. $H = (\sigma^2_{Pi} - r\sigma^2_{Pm})/\sigma^2_{Pi}$.

2). Sporulation distributions were continuous and populations produced spores across a 38- to 46-day period (Fig. 2). Sporulation patterns for populations 951-WT, 958-WT, 956-WT, and 952-WT were approximately normally distributed and early. Distributions for populations 957-WT, 955-WT, and 953-WT were either bimodal or skewed toward either early or late. The distribution of population 954-WT was the latest of all the distributions and had little of the component for early sporulation that was present in all the other sporulation distributions.

Genetic variation and heritability. All populations exhibited a significant amount of genetic variation, with estimates ranging from 7.2 to 30.9 (Table 2). Populations 953-WT and 955-WT exhibited the least amount of genetic variation, while population 957-WT exhibited the most. With the exception of estimates for populations 953-WT and 955-WT, estimates of broad-sense heritability based on partitioning of phenotypic variation were similar and ranged from 0.63 to 0.78. Estimates for populations 953-WT and 955-WT were lower (0.31 and 0.33, respectively) and were probably influenced by the bimodality of these populations.

Estimates for narrow-sense heritability for populations 951-WT and 954-WT, based on parent-offspring regressions, were 0.78 and 0.58, respectively (Fig. 3), and were similar to those obtained from the previous method. Regressions were significant at $P < 0.05$, and correlation coefficients for populations 951-WT and 954-WT were 0.31 and 0.85, respectively. There was no significant difference between the slopes of the regression lines ($P = 0.61$).

DISCUSSION

There are few studies that describe genetic variation in the phenology of plant-pathogenic fungi or how fungal phenology influences pathogen fitness and evolution. The objectives of the current study were to explore the variation in phenology among eight populations of *M. vaccinii-corymbosi* and to determine whether the formation of apothecia and ascospores is a heritable genetic trait that can be potentially altered by selection.

The eight fungal populations examined in this study exhibited significant variation in phenology. There was a 20-day difference in the mean development times for the earliest and the latest populations. In general, the phenology of the pathogen populations followed the relative phenology of the host. For example, early-developing fungal populations (e.g., 951-WT and 952-WT) were

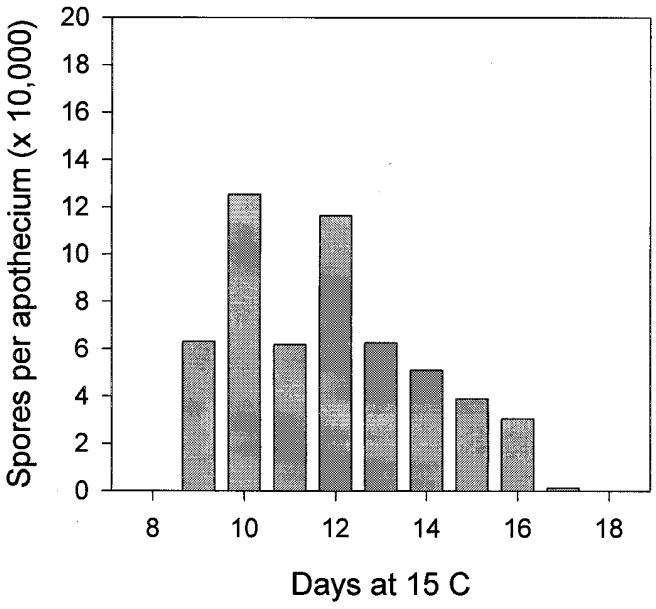

Fig. 1. Mean number of ascospores produced per apothecium of *Monilinia vaccinii-corymbosi.* Values are the averages of 48 apothecia. On average, 61,293 spores per cup per day were produced.

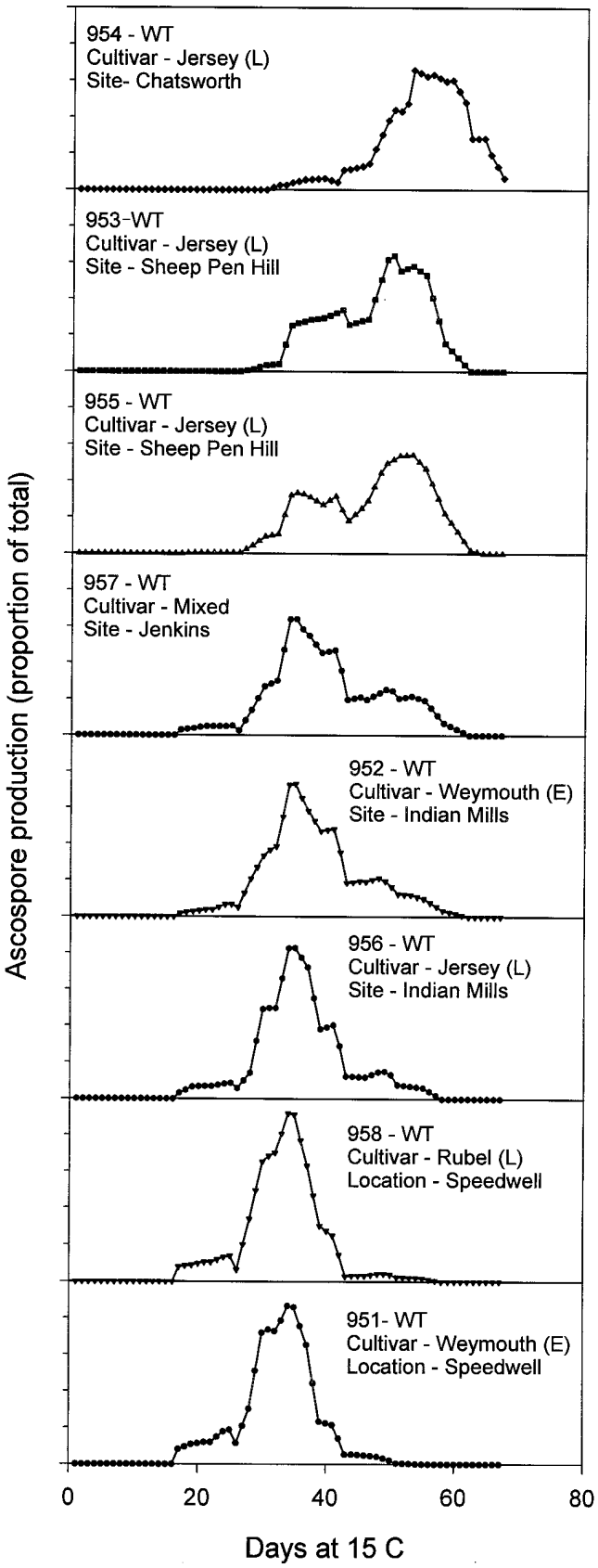

Fig. 2. Relative ascospore production predicted for eight populations of the mummy berry fungus *Monilinia vaccinii-corymbosi*. For each population, the cultivar (E = early and L = late) and site (location) are provided. Ascospore production was calculated based on data for fungal apothecium development and for ascospore production measured in greenhouse studies.

generally collected from the early cv. Weymouth, while late-developing fungal populations were collected from the late cv. Jersey. The development of fungal populations 956-WT and 958-WT were exceptions to this observation. Populations 956-WT and 958-WT were collected from late-developing host cvs. Jersey and Rubel, respectively, but did not exhibit late phenologies. These populations may exhibit early phenologies because their original collection sites were within 90 to 150 m of plantings of the early cv. Weymouth. The close proximity of cv. Weymouth may have influenced the selection of pathogen phenology (i.e., cv. Weymouth favored pathogen genotypes with early phenologies). In terms of pathogen fitness, it is theoretically most beneficial for fungal populations to adapt to the earliest cultivar in mixed blueberry stands assuming all other factors influencing fungal survival and reproduction are constant. In mixed stands, early-developing fungal populations can infect early cultivars through primary and secondary infections. In addition, early populations can infect late cultivars through secondary infections caused by conidia that are produced by blighted shoots of early cultivars. In contrast, late-developing fungal populations located in mixed stands of blueberries will predominantly infect the late cultivar through primary and secondary infection. Early cultivars are less likely to be infected, because the majority of susceptible shoots and flowers were available before the production of ascospores and conidia.

The variation in pathogen phenology for populations 953-WT and 955-WT, as shown in Figure 2 and Table 2, was greater than the variation exhibited by the other populations and appeared to be bimodal. We hypothesize that these populations were heterogeneous mixtures of early and late pathogen genotypes in different relative frequencies. Based on the distribution of development times presented in Figure 2, it appears that the late-developing individuals comprise a larger proportion of the populations than do early-developing individuals.

The results obtained in this study expand upon previous work on phenology of *Monilinia* spp. Lehman and Oudemans (13,15) reported differences in phenology among two *M. vaccinii-corymbosi* populations collected from early and late blueberry cultivars. The differences were described for four defined stages of pseudosclerotial germination. The greatest differences were observed during apothecial cup expansion, which is the stage associated with ascospore production. Phenological differences among fungal populations were comparable to the 14-day difference observed in host shoot elongation between the cultivars from which the pseudosclerotia were collected (15). Similarly, Batra (2,3) found differences (6 to 12 days) in the timing of pseudosclerotial germination between two closely related fungal species, *M. vaccinii-corymbosi* and *M. gaylussacia*, which are pathogens of different species in the subfamily Vacciniodeae. The phenologies of their respective hosts differ by approximately 16 days. These observations support the hypothesis that pathogen development corresponds with host development.

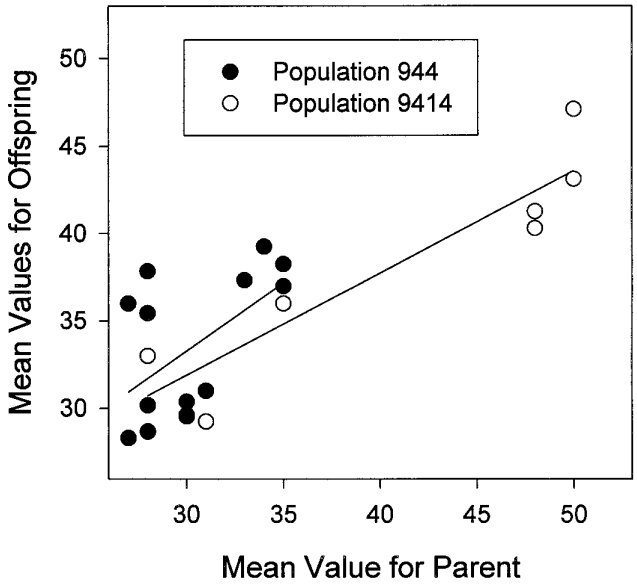

Fig. 3. Parent-offspring regressions and regression equations describing heritability of development times for two populations of *Monilinia vaccinii-corymbosi*.

Knowledge of genetic variation in phenology (i.e., a potentially significant trait in pathogen fitness) is essential for understanding the adaptation and evolution of *M. vaccinii-corymbosi*. Based on the estimates of broad- and narrow-sense heritability, we conclude that phenology is a moderately to highly heritable genetic trait and, therefore, is selectable by host phenology in nature. If fungal phenology in *M. vaccinii-corymbosi* was controlled strictly by environmental factors such as temperature and moisture, very little of the phenotypic variation could be explained by a genetic component. Moderate to high estimates for narrow-sense heritability suggest that the potential for selection of distinct phenological types clearly exists. Although the degree to which phenology affects pathogen fitness has not been quantified, the observed variation does correlate with host phenology, suggesting some level of differential fitness.

The effect of the host on pathogen phenology is the result of the defined period of host susceptibility to primary infection. If the blueberry host were susceptible to *M. vaccinii-corymbosi* throughout the growing season, there would be little merit in the observed degree of host-pathogen synchrony. Previous studies demonstrate that highbush (*V. corymbosum*) and lowbush blueberries (*V. angustifolium*) exhibit defined periods of susceptibility (12,14,19) and that different blueberry cultivars break dormancy and develop at different times throughout the spring season (11). In highbush blueberry, plants are susceptible to ascospore infection from bud break until the vegetative shoots are 50 mm in length (14). Host susceptibility to primary infection declines as shoot length increases beyond 50 mm in length. Similarly, for lowbush blueberry, plant tissue declined in host susceptibility as the tissues matured (12). The incidence of shoot blighting may differ among blueberry cultivars largely because the period of susceptibility is short or displaced with regard to the production of ascospores by a particular pathogen population (i.e., disease avoidance) (14). For example, Pepin and Toms (18) reported that resistant cultivars tended to be late maturing rather than early maturing. Also, Ehlenfeldt et al. (7,8) demonstrated a high correlation between shoot blight incidence and shoot length. These studies indicate that disease avoidance may play a significant role in the expression of resistance.

Dormancy in blueberry is controlled by a chilling requirement and is broken only when the minimum required hours of chilling are fulfilled. This innate dormancy prevents budbreak from occurring midwinter during temporary warm periods. The chilling requirements for *Vaccinium* spp. range from 300 to 1,100 h at 0 to 7°C (6). For example, southern rabbit eye cultivars of blueberry (*V. ashei*) have a low chilling requirement (<300 h), whereas highbush cultivars require longer chilling periods (900 to 1,100 h) (6). Pseudosclerotia also have chilling requirements that appear to correspond with those of their blueberry host. For example, mummies from highbush blueberry in New Jersey require between 900 to 1,200 h of chilling before germination can be initiated (J. S. Lehman and P. V. Oudemans, *unpublished data*), while those from southern rabbit eye cultivars of blueberry from Georgia require only 300 h or less (21). It appears, therefore, that phenology may have a major impact on the geographic distribution of a particular genotype of the pathogen and could lead to development of locally adapted races or biotypes of a pathogen due to temporal separation of fungal isolates. A related mechanism has been reported in certain insect species in which sympatric speciation and sympatric host race formation is associated with differences in insect phenology (10,23).

In pathosystems in which host tissues exhibit a highly defined period of susceptibility, pathogen phenology is likely to be an im-

portant component of fitness and have a significant impact on fungal adaptation and evolution. The highly divergent phenologies exhibited by *M. vaccinii-corymbosi* suggest that fungal populations are temporally separated and, therefore, could incur restricted gene flow. Ultimately, this could lead to adaptation to specific blueberry cultivars or even to different *Vaccinium* species. The phenology of sexual development may be significant for other fungal species that exhibit complicated life histories in which defined periods of host susceptibility can alter the timing of pathogen infectivity (i.e., the apple scab fungus, *Venturia inaequalis*; the cereal ergot pathogen, *Claviceps purpurea*; and the wheat Karnal bunt fungus, *Tilletia indica*).

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