Oversummer Survival of *Monilinia vaccinii-corymbosi* in Relation to Pseudosclerotial Maturity and Soil Surface Environment

K. D. Cox and H. Scherm, Department of Plant Pathology, University of Georgia, Athens 30602

ABSTRACT

Cox, K. D., and Scherm, H. 2001. Oversummer survival of *Monilinia vaccinii-corymbosi* in relation to pseudosclerotial maturity and soil surface environment. Plant Dis. 85:723-730.

Pseudosclerotia (infected, mummified fruit) on the orchard floor act as overwintering and overwintering structures and the sole source of primary inoculum of *Monilinia vaccinii-corymbosi*, the causal agent of mummy berry disease of blueberry. Survival of pseudosclerotia may be affected by their maturity (degree of stromatization), which can vary considerably at the time of fruit abscission in early summer, and by variations in the soil surface environment. From July through October in 2 years, survival of pseudosclerotia of varying initial maturity (expressed as the proportion of fruit containing mature, melanized entostromata; immature, nonmelanized entostromata; or undifferentiated mycelia) was investigated in the laboratory relative to soil surface temperature and soil moisture content and in the field in relation to shading (full sun versus 50% shade) and ground cover (bare soil versus grass). In the laboratory, oversummer survival, expressed as the percentage of intact pseudosclerotia at the end of the experiment, was higher for cool soil temperatures (approximately 15°C), soils drier than field capacity, and pseudosclerotia containing mature entostromata. In the field, survival was related solely to initial maturity of pseudosclerotia and was highest for pseudosclerotia containing mature entostromata. Shading or grass ground cover did not significantly ($P > 0.05$) affect oversummer survival, presumably because they did not greatly modify soil temperature or soil moisture. When individual, intact pseudosclerotia were tested for viability using fluorescein diacetate staining, a linear relationship ($r = 0.982$, $P < 0.0001$, $n = 90$) between viable and intact pseudosclerotia was observed, supporting the use of the percentage of intact pseudosclerotia as a measure of oversummer survival.

Additional keywords: *Vaccinium ashei*

The fungus *Monilinia vaccinii-corymbosi* (Reade) Honey is the causal agent of mummy berry disease, an important problem in blueberry (*Vaccinium* spp.) throughout the United States and Canada (3,11,18). The pathogen survives in infected, mummified fruit (pseudosclerotia) on the orchard floor. In late winter to early spring, apothecia, produced by carpogenic germination of pseudosclerotia, release ascospores that infect emerging vegetative host tissues, causing a leaf and shoot blight (primary infection). Conidia produced on blighted shoots subsequently infect fruit via the stigmata and styles of open flowers (secondary infection), followed by internal colonization of the fruit locules (16,21,24). As infected fruit mature during early summer, fungal mycelium in the locules invades the mesocarp and forms a melanized entostroma that encases fungal and host elements (4). Most

pseudosclerotia fall to the ground as the fruit mature, where they act as overwintering and overwintering structures and the sole source of primary inoculum for the following seasons (3,11,24).

Carpogenic germination of pseudosclerotia of *M. vaccinii-corymbosi* is affected by various environmental factors such as temperature, soil moisture, and light. The primary effect of temperature has been described as a chill-hour/degree-day-dependent relationship (19). As for soil moisture, it is well established that carpogenic germination is favored in moist soils (4,15). Indeed, Milholland (15) observed that pseudosclerotia of *M. vaccinii-corymbosi* germinated readily and produced apothecia when placed on soil at 81% of field capacity, whereas no germination occurred at 35% of field capacity. Effects of light are not as well documented, although light is considered an important factor during apothecium maturation (16).

Despite detailed knowledge about environmental effects on carpogenic germination of *M. vaccinii-corymbosi*, little is known about the impact of the environment on survival of its pseudosclerotia during the summer and fall preceding the germination period. In rabbiteye blueberry (*V. ashei* Reade) plantings in Georgia with a high incidence of fruit infection at har-

vest, it commonly is observed that few pseudosclerotia remain in the fall (H. Scherm, *unpublished*). Woronin (24), in the first detailed study of *M. vaccinii-corymbosi*, also observed a large decrease in numbers of pseudosclerotia during the summer, suggesting that oversummer survival of pseudosclerotia is low or variable. A possible explanation could be that biotic or abiotic degradation of pseudosclerotia is affected by environmental conditions. For example, field observations indicate that more intact pseudosclerotia are found in shaded and grass-covered areas (H. Scherm, *unpublished*) which may provide temperature and moisture conditions more conducive to oversummer survival than a bare-soil environment. Another possible explanation is that differences in pseudosclerotial maturity at the time of fruit abscission, which can be very pronounced in rabbiteye blueberry (17), may affect oversummer survival. For example, infected fruit that developed mature, melanized entostromata before fruit abscission may be more apt to survive than those containing only undifferentiated mycelia or immature, nonmelanized entostromata.

Survival of sclerotia in different environments has been investigated in detail for various species of the genus *Sclerotinia*, a close relative of *M. vaccinii-corymbosi*. Sclerotia of *S. sclerotiorum* and *S. minor* survived better in dry soils than in moist soils (1,6,13,22). Similarly, prolonged flooding has been shown to reduce sclerotial viability (7). Temperature effects on survival of sclerotia also have been documented, with temperatures $>30^{\circ}\text{C}$ reducing viability (2,5). Ground cover also can play a role; for example, survival of sclerotia of *S. sclerotiorum* was favored beneath a bean straw mulch (14).

The objective of this study was to investigate the oversummer survival of pseudosclerotia of *M. vaccinii-corymbosi* from July (after fruit abscission) through October (the onset of the chilling period in the field). In the laboratory, effects of soil surface temperature and soil moisture content on survival of pseudosclerotia of different maturity levels were examined. Concurrent field studies focused on the effects of shading and ground cover on survival of pseudosclerotia differing in maturity. Results obtained from this study could explain why relatively few intact pseudosclerotia remain in late fall in commercial blueberry plantings with high incidence of fruit infection.

Corresponding author: H. Scherm
E-mail address: scherm@uga.edu

Supported by Hatch Act and State of Georgia funds.

Accepted for publication 9 March 2001.

Publication no. D-2001-0420-01R
© 2001 The American Phytopathological Society

MATERIALS AND METHODS

Characterization of pseudosclerotial maturity. Pseudosclerotia formed on rabbiteye blueberry cv. Climax (1999) and Tifblue (2000) were collected from within and beneath blueberry plants in heavily infected commercial plantings in Murray County (1999) and Walton County (2000) in northeastern Georgia when fruit were mature in early July. Pseudosclerotia were grouped into three maturity classes based on exocarp color, referred to henceforth as black, gray, and brown. Twenty pseudosclerotia in each class were dissected under a stereo microscope to determine their internal condition based on the presence of mature, melanized entostromata, immature nonmelanized entostromata, or undifferentiated mycelia. Pseudosclerotia in the black

class appeared dark purple to black externally due to the presence of a fully melanized entostroma partly visible through the exocarp. The gray class was characterized by a dried exocarp often associated with surface sporulation of *M. vaccinii-corymbosi*; internally, pseudosclerotia in this class contained either undifferentiated mycelia, nonmelanized entostromata, small pieces of melanized entostromata, or, in some cases, combinations of the three. The brown class appeared tan and water-soaked externally and represented the most immature development stage. Internally, fruit locules were colonized with mycelia or, occasionally, nonmelanized entostromata.

Maturity and temperature effects on survival. Pseudosclerotia were sorted into the three maturity classes and placed, in

groups of 20 (1999) or 40 (2000), on the surface of nonsterile sand in 10.2-cm-diameter clay pots. Pots were arranged in a split-plot design with soil surface temperature (three levels) as the main plot and pseudosclerotial maturity (three levels) as the subplot; each main plot was replicated three times in a randomized complete block design. Individual pots were wrapped with silicon rubber heating tapes (Brisk Heat, Barnstead/Thermolyne, Dubuque, IA), and constant soil temperature treatments of approximately 15, 25, and 35°C were maintained by adjusting the voltage supplied to each tape (20). Internal temperatures of pseudosclerotia were measured at intervals with a type-K thermocouple (Digi-Sense, Cole-Palmer Instrument Co., Vernon Hills, IL); actual internal temperatures of pseudosclerotia in the three treatments averaged 14.2, 23.7, and 33.8°C in 1999 and 15.8, 23.3, and 33.1°C in 2000. The soil moisture content in all pots was maintained at field capacity by daily watering of saucers beneath the pots. After 18 weeks, survival was assessed by determining the percentage of intact pseudosclerotia. Pseudosclerotia were considered intact if they maintained their structural integrity during tactile inspection by attempting to crush the pseudosclerotia between two fingers.

Maturity and moisture effects on survival. Pseudosclerotia were sorted into the three maturity classes and placed on sand in clay pots as described above. Pots were arranged in a split-plot design with soil moisture (three levels) as the main plot and pseudosclerotial maturity (three levels) as the subplot; each main plot was replicated three times in a randomized complete block design. Soil moisture treatments were applied as irrigation thresholds, whereby all pots belonging to a given treatment were watered to field capacity when measured soil water matric potential (Ψ_m) reached a predetermined threshold (20); watering thresholds were approximately 0, -0.16, and -0.8 MPa (wet, intermediate, and dry, respectively). Soil moisture blocks (Model 257; Campbell Scientific Inc., Logan, UT) measured Ψ_m at 5-min intervals. The three watering thresholds yielded average gravimetric soil moisture contents, determined by sampling from the upper 1 cm of soil, of 0.23, 0.04,

Fig. 1. External and internal characterization of pseudosclerotia of *Monilinia vaccinii-corymbosi* assigned to three maturity classes at the beginning of the oversummer survival experiments in early July of **A**, 1999 and **B**, 2000. External classification was based on the color of the exocarp. Internal classification was expressed as the percentage of pseudosclerotia within each maturity class containing mature, melanized entostromata; a combination of mature entostromata, immature nonmelanized entostromata, and undifferentiated mycelia; or undifferentiated mycelia alone. Twenty pseudosclerotia were assessed for each maturity class.

Table 1. Results of a split-plot analysis of variance to determine the effects of soil surface temperature, pseudosclerotial maturity, and their interaction on oversummer survival of pseudosclerotia of *Monilinia vaccinii-corymbosi* in the laboratory^a

Source	df	1999			2000		
		Mean square	F	P > F	Mean square	F	P > F
Replication (block)	2	0.008	0.23	0.8012	0.013	1.28	0.3726
Soil surface temperature (main plot)	2	0.604	17.05	0.0110	0.462	46.37	0.0017
Main-plot error	4	0.035	...	...	0.010	...	...
Maturity class (subplot)	2	0.174	5.65	0.0187	0.835	36.55	0.0001
Soil surface temperature-maturity class	4	0.032	1.04	0.4288	0.025	1.10	0.3997
Subplot error	12	0.031	...	...	0.023	...	...

^a Analysis based on arcsine-square root-transformed proportions of intact pseudosclerotia at the end of the experiment.

and 0.01 g g⁻¹ in 1999, and 0.31, 0.13, and 0.01 g g⁻¹ (wet, intermediate, and dry, respectively) in 2000. Pots were maintained at 25°C for 18 weeks, at which time pseu-

dosclerotia were assessed and survival determined as described above.

Maturity, ground cover, and shade effects on survival. Pseudosclerotia were

sorted by maturity as described above and placed on the soil surfaces of microplots (0.9 by 1.8 m²) at the University of Georgia Horticulture Farm in Oconee County in

Fig. 2. Oversummer survival of pseudosclerotia of *Monilinia vaccinii-corymbosi* in relation to soil surface temperature and pseudosclerotial maturity in the laboratory. Survival was expressed as the percentage of intact pseudosclerotia at the end of the experiment. Pseudosclerotial maturity was classified into three classes based on the color of the exocarp. Values in **A** and **B** are the means and standard errors of three replications with 20 (1999) or 40 (2000) pseudosclerotia assessed per replication. Soil temperatures were held constant at the levels shown in **C** and **D**. Values in **C** and **D** show the main effect of temperature across maturity classes, whereas those in **E** and **F** show the main effect of maturity class across temperatures.

northeastern Georgia. Microplots were arranged in a split-split-plot design with shading (full sun versus 50% shade) as the main plot, ground cover (bare soil versus grass) as the subplot, and pseudosclerotial maturity class (black, gray, and brown) as the subsubplot. Each main plot was replicated three times in a randomized complete block design, and each subsubplot (0.15 by 0.61 m²) received 120 pseudosclerotia at the beginning of the experiment. In the main plots, shade was obtained by covering the plots with shade cloth (blocks 50% light) 9 cm above the plots; in the subplots, the existing Bermuda grass (*Cynodon dactylon* (L.) Pers.) sod was either removed and the soil surface covered with sand or left in place and clipped periodically. Soil surface temperature and soil moisture (at a depth of 3 to 6 cm) were measured at 5-min intervals in at least one replication of each subplot using thermistors and gypsum blocks, respectively. Experiments were initiated in July of 1999 and 2000 and ended in early October in both years. At least 20 pseudosclerotia per subsubplot were sampled for tactile examination at the beginning of each experiment and four times thereafter to determine the percentage of intact pseudosclerotia as described above.

Fluorescein diacetate analysis and viability of intact pseudosclerotia. In 2000, pseudosclerotia were assayed individually using fluorescein diacetate (FDA) to estimate viability and to examine whether structural integrity was a good predictor of viability inferred by FDA staining. Pseudosclerotia, determined to be intact by tactile examination as described above, were collected at the end of the laboratory and field experiments and separated by experiment, treatment, and replication. Pseudosclerotia were rinsed three times in distilled water by vortexing for 1 min. Pseudosclerotia were then surface sterilized in 2% NaOCl (40% household bleach) for 3 min with vortexing, transferred to sterile vials, and rinsed three times in sterile distilled water for 1 min with vortexing. Pseudosclerotia then were rinsed in 70% ethanol for 1 min with vortexing, followed by decanting the ethanol and placing pseudosclerotia on sterile filter paper in a laminar flow hood. After drying for approximately 1 h, individual pseudosclerotia

were transferred to sterile 118-ml whirl-pack bags. Two milliliters of the reaction solution, prepared by diluting an FDA stock solution (5 mg ml⁻¹ FDA [Sigma-Aldrich, St. Louis] in high performance liquid chromatography-grade acetone) 10-fold in sodium phosphate buffer (0.05 M, pH 6.0), was added to each bag containing a single pseudosclerotium. Bags were sealed and the contents crushed with a pestle. Each reaction was rated visually, and reactions producing a color intensity corresponding to an average absorbance at 490 nm of ≥ 0.818 after 6 h were considered to contain viable pseudosclerotia. Preliminary experiments with heat-killed pseudosclerotia yielded an average absorbance at 490 nm of 0.083 after 6 h.

Data analysis. For laboratory experiments, effects of soil temperature or soil moisture and pseudosclerotial maturity were analyzed by analysis of variance (ANOVA) for a split-plot design (SAS version 6.12; SAS Institute Inc., Cary, NC). For field experiments, effects of ground cover, shading, and pseudosclerotial maturity at the various assessment dates were analyzed using repeated-measures ANOVA for a split-split-plot design. The percentage of intact pseudosclerotia, the dependent variable in all ANOVAs, was arcsine-square root-transformed before these analyses. Main effects and interactions were considered statistically significant at $P = 0.05$. The relationship between the percentage of intact pseudosclerotia (determined tactilely) and that of presumably viable pseudosclerotia (inferred by FDA staining) was analyzed by linear regression analysis.

RESULTS

Internal characterization of samples of pseudosclerotia at the beginning of the experiments revealed consistent differences among the three maturity classes in both years. Whereas all pseudosclerotia classified externally as the black class contained exclusively mature, melanized entostromata, the gray and brown classes contained a mixture of undifferentiated mycelia, immature entostromata, and mature entostromata, with the proportion of the latter being higher in the gray class than in the brown class (Fig. 1). A higher proportion of the pseudosclerotia belong-

ing to the gray and brown classes contained mature entostromata in 1999 compared with 2000 (Fig. 1).

Structurally intact pseudosclerotia were recovered from all treatments in all three experiments. In laboratory experiments, survival, expressed as percentage of intact pseudosclerotia at the end of the experiment, ranged from 0 to 60.0% in 1999 and 2.0 to 93.3% in 2000. At the last sampling date in the field experiment, this percentage varied from 13.3 to 75.6% in 1999 and from 1.0 to 70.0% in 2000.

Soil surface temperature and pseudosclerotial maturity were significant factors influencing oversummer survival in both years, whereas the interaction of soil temperature and maturity was not significant (Table 1). Survival was consistently higher in treatments of lower soil temperature, with the percentage of intact pseudosclerotia decreasing with increasing temperature (Fig. 2A–D). Across maturity classes, survival was 41.1 and 41.0% (1999 and 2000, respectively) at the lowest temperature compared with 7.2 and 9.4% at the highest temperature (Fig. 2C and D). Also in both years, survival was higher for the more mature pseudosclerotial classes. Across temperatures, percentage of intact pseudosclerotia at the end of the experiment was 21.7 and 47.8% (1999 and 2000, respectively) for the black class compared with 10.6 and 5.6% for the brown class (Fig. 2E and F).

Soil moisture content and pseudosclerotial maturity were significant factors influencing oversummer survival in both years, whereas the interaction of soil moisture and maturity was not significant (Table 2). Survival was highest in treatments of lower soil moisture, and the percentage of intact pseudosclerotia decreased with increasing moisture (Fig. 3A–D). Across maturity classes, survival was 39.4 and 54.0% (1999 and 2000, respectively) for the driest treatment compared with 15.0 and 2.0% for saturated soil (Fig. 3C and D). As was found in the soil temperature experiments, survival was higher for more mature pseudosclerotia. Across soil moisture levels, percentage of intact pseudosclerotia at the end of the experiment was 42.8 and 68.3% (1999 and 2000, respectively) for the black class compared with 5.6 and

Table 2. Results of a split-plot analysis of variance to determine the effects of soil moisture, pseudosclerotial maturity, and their interaction on oversummer survival of pseudosclerotia of *Monilinia vaccinii-corymbosi* in the laboratory^a

Source	df	1999			2000		
		Mean square	F	P > F	Mean square	F	P > F
Replication (block)	2	0.027	1.10	0.4153	0.017	0.88	0.4832
Soil moisture (main plot)	2	0.327	13.23	0.0172	0.563	28.87	0.0042
Main-plot error	4	0.025	...	...	0.020	...	...
Maturity class (subplot)	2	0.814	68.59	0.0001	1.205	38.91	0.0001
Soil moisture–maturity class	4	0.013	1.06	0.4167	0.019	0.63	0.6518
Subplot error	12	0.012	...	...	0.031	...	...

^a Analysis based on arcsine-square root-transformed proportions of intact pseudosclerotia at the end of the experiment.

13.3% for the brown class (Fig. 3C and F).

In the field experiments, pseudosclerotial maturity and time significantly af-

ected oversummer survival, whereas shading and ground cover did not (Table 3). There were, however, significant interactions for ground cover and maturity class

in 1999, for time and ground cover in 2000, and time and maturity class in 2000. Across shading treatments for both ground covers and all three maturity classes, sur-

Fig. 3. Oversummer survival of pseudosclerotia of *Monilinia vaccinii-corymbosi* in relation to soil moisture content and pseudosclerotial maturity in the laboratory. Survival was expressed as the percentage of intact pseudosclerotia at the end of the experiment. Fluctuating soil moisture levels were obtained by watering all pots belonging to a given treatment to field capacity when soil water matric potential reached a predetermined threshold. Pseudosclerotial maturity was classified into three classes based on the color of the exocarp. Values in **A** and **B** are the means and standard errors of three replications with 20 (1999) or 40 (2000) pseudosclerotia assessed per replication. Mean soil moisture level values are shown in **C** and **D**. Values in **C** and **D** show the main effect of soil moisture across maturity classes, whereas those in **E** and **F** show the main effect of maturity class across soil moisture levels.

vival decreased with increasing time (Fig. 4). Oversummer survival was consistently highest for the black maturity class, with the brown and gray classes having fewer intact pseudosclerotia as time progressed for both ground covers. Generally, the rate of decrease in percentage of intact pseudosclerotia over time was higher for less mature pseudosclerotia compared with the more mature, black class.

FDA staining of individual intact pseudosclerotia assessed by tactile examination suggested that these pseudosclerotia were viable. Across the three experiments, the percentages of viable and intact pseudosclerotia were closely correlated ($r = 0.982$, $P < 0.0001$, $n = 90$) with a slope of 0.959 (Fig. 5). However, the intercept (-2.49) was significantly lower than 0 ($P = 0.0015$) indicating that survival expressed as percentage of intact pseudosclerotia slightly overestimated viability as determined via FDA analysis.

DISCUSSION

Whereas there are several reports on factors affecting carpogenic germination of pseudosclerotia of *M. vaccinii-corymbosi* during early spring (4,15,16,19), this is the first study investigating the survival of pseudosclerotia during the preceding summer and fall. In all three experiments in both years, oversummer survival was most strongly related to pseudosclerotial maturity at the time of fruit abscission. The black maturity class, in which all pseudosclerotia contained fully melanized entostromata at the onset of the experiment, consistently had the highest survival, whereas the immature, brown class, often characterized internally by containing mycelia only, had the lowest survival. The gray maturity class was more variable across years, with differences in proportions of pseudosclerotia containing mature entostromata or a mixture of entostromata and mycelia between years being the likely

cause of this variability. Such differences in internal maturity between years are not surprising because pseudosclerotia were collected from different cultivars each year and because the maturity classes initially were sorted based solely on external characteristics.

Woronin (24) observed a large decrease in the number of pseudosclerotia on the ground following crop harvest but did not suggest a lack of maturity as a potential reason for this decrease. In rabbiteye blueberry, variation in pseudosclerotial maturity at the time of fruit abscission can be very pronounced (17), and it is conceivable that differences in the frequency of various maturity classes between cultivars could lead to differences in survival of pseudosclerotia, with important epidemiological implications. Maturity effects on survival or biological function have been noted previously for sclerotia of other fungal plant pathogens. For example, in *S. sclerotiorum*, immature, nonmelanized sclerotia do not exhibit dormancy and generally undergo myceliogenic germination instead of carpogenic germination (12). Coley-Smith et al. (7), who investigated the survival of sclerotia of *S. cepivorum* of differing maturity and age, found that sclerotia buried for 2 years survived better than immature, nonmelanized sclerotia or newly formed melanized sclerotia.

In the laboratory, soil surface temperature influenced pseudosclerotial survival significantly, with the percentage of intact pseudosclerotia being highest at approximately 15°C and lowest at approximately 35°C when tested on sand in the laboratory. Similar observations have been made for sclerotia of *S. sclerotiorum*, for which a 40% loss in viability occurred when incubated at 35°C for only 3 days (5). For longer exposure periods at 35°C, viability was reduced by 90 and 100% after 6 and 7 weeks, respectively (2). These findings agree with the low levels of survival ob-

served here for pseudosclerotia of *M. vaccinii-corymbosi* at a soil temperature near 35°C.

Soil moisture content also influenced pseudosclerotial survival in the laboratory, with survival being highest in dry soils (-0.8 MPa watering threshold) and lowest in soils maintained at field capacity. Studies on the effect of soil moisture on the survival of sclerotia of *Sclerotinia* spp. yielded similar results. Teo et al. (22) reported that 65% of sclerotia of *S. sclerotiorum* had disintegrated after 2 years at moderate soil moisture ($\Psi_m \geq -0.15$ MPa) and that survival was better at lower soil moisture. Similarly, sclerotia of *S. minor* survived better in dry soils than in wet soils (13). Sclerotia of the latter species failed to survive after 8 weeks in saturated soils, with survival increasing as Ψ_m was decreased (1). Interestingly, soil moisture, while reducing survival of pseudosclerotia and sclerotia by enhancing decay, is essential for carpogenic germination (8,15,23), indicating that there must be a seasonal balance of soil moisture for these propagules to be biologically successful.

In the field experiments, pseudosclerotial maturity and time were the sole factors affecting survival, whereas shading and ground cover did not result in significant differences in survival. The latter two factors were initially hypothesized to affect survival via their effects on soil surface temperature and soil moisture content. However, effects of shading and ground cover on soil temperature and moisture were apparently not pronounced enough to affect survival in these experiments. In the field, the largest drop in survival occurred in July and August and, within this period, there was little difference in soil temperature between treatments. For example, in 2000 there were 51 (shade, grass cover), 50 (shade, bare soil), 53 (full sun grass cover), and 54 (full sun, grass and bare soil) days in July and August during which maximum

Table 3. Results of a repeated measures split-split-plot analysis of variance to determine the effects of shading, ground cover, pseudosclerotial maturity, time, and their interactions on oversummer survival of pseudosclerotia of *Monilinia vaccinii-corymbosi* in the field^a

Source	df	1999			2000		
		Mean square	F	P > F	Mean square	F	P > F
Replication (block)	2	0.119	1.11	0.4739	0.040	0.48	0.6757
Shading (main plot)	1	0.057	0.53	0.5423	0.087	1.08	0.4078
Main-plot error	2	0.107	...	...	0.082	...	...
Ground cover (subplot)	1	0.271	2.91	0.1632	0.156	3.64	0.1291
Shading-ground cover	1	0.008	0.09	0.7791	0.125	2.919	0.1627
Subplot error	4	0.093	...	...	0.043	...	...
Maturity class (subsubplot)	2	3.402	36.48	0.0001	8.753	155.11	0.0001
Shading-maturity class	2	0.100	1.08	0.3637	0.020	0.35	0.7114
Ground cover-maturity class	2	0.470	5.04	0.0201	0.009	0.15	0.8583
Shading-ground cover-maturity class	2	0.119	1.28	0.3052	0.004	0.06	0.9380
Subsubplot error	16	0.093	...	...	0.056	...	...
Time ^b	3	1.828	28.04	0.0001	4.153	142.38	0.0001
Time-shading	3	0.087	1.33	0.2775	0.030	1.04	0.3826
Time-ground cover	3	0.009	0.15	0.8405	0.216	7.42	0.0011
Time-maturity class	6	0.111	1.71	0.1819	0.110	3.79	0.0078
Time error	48	0.065	...	...	0.029	...	...

^a Analysis based on arcsine-square root-transformed proportions of intact pseudosclerotia at the end of the experiment.

^b First sampling date at the beginning of the experiments in early July was omitted from the analysis.

soil temperature exceeded 35°C (*data not shown*), a temperature found detrimental to survival in the laboratory. Soil moisture differences among treatments in the field were more pronounced, but still relatively similar to each other compared with the range of soil moisture contents evaluated in the laboratory. For example, in 2000, average soil water content, determined gravimetrically on 13 days for shade, bare soil; shade, grass; full sun, bare soil; and full sun, grass was 87, 69, 57, and 65% of field capacity, respectively (*data not shown*).

In contrast to this study, shading and ground cover affected sclerotial survival in other pathosystems. For example, more intact sclerotia of *S. sclerotiorum* were recovered from exposed field plots than from sheltered or shaded plots (10). Organic matter content of soils can affect the survival of sclerotia, presumably by enhancing the soil microflora. Sclerotia of *Phymatotrichum omnivorum* were eliminated more readily from soils containing organic matter amendments than from those without amendments during periods of intense soil microbial activity (6). In

Fig. 5. Percentage of viable pseudosclerotia of *Monilinia vaccinii-corymbosi* in relation to that of intact pseudosclerotia at the end of the oversummer survival experiments in 2000. Pseudosclerotia were considered intact if they maintained their structural integrity during tactile inspection. Viability was estimated by fluorescein diacetate staining of individual intact pseudosclerotia. The dashed line indicates a theoretical 1:1 relationship. Each value is based on the assessment of at least 20 individual pseudosclerotia.

Fig. 4. Oversummer survival of pseudosclerotia of *Monilinia vaccinii-corymbosi* in relation to ground cover and pseudosclerotial maturity in the field. Survival was expressed as the percentage of intact pseudosclerotia at each sampling date. Ground cover was either **A** and **B**, grass or **C** and **D**, bare soil. Pseudosclerotial maturity was classified into three classes based on the color of the exocarp. All values are the means and standard errors of three replications with at least 20 pseudosclerotia assessed per replication, combined across two shading treatments (full sun versus 50% shade).

contrast, survival of sclerotia of *S. sclerotiorum* was lower in treatments in which the propagules were exposed directly to the soil compared with those in which they were protected by bean straw (14).

FDA staining has been used previously as an indicator of viability in a microscopic study of sclerotia of *S. trifoliorum*. Dittmer and Weltzien (9) reported that FDA staining was rapid and simple and consistently classified >95% of viable and nonviable sclerotia correctly. In the present study, survival expressed as percentage of intact pseudosclerotia strongly correlated with FDA-measured viability. This supports the use of intact pseudosclerotia as a valid measure of oversummer survival. It should be noted, however, that high levels of pseudosclerotial viability in late fall do not necessarily translate directly into high levels of carpogenic germination during the following spring, given the strong dependence of the latter process on weather conditions during the germination period (4,15,16,19).

In conclusion, maturity at the time of fruit abscission in early summer was the most important factor affecting oversummer survival of pseudosclerotia of *M. vaccinii-corymbosi*. This suggests that, after pseudosclerotia fall to the orchard floor, maturation does not continue and degradation begins. The strong effect of pseudosclerotial maturity may have important implications for certain cultivars that have higher frequencies of mature pseudosclerotia at fruit abscission, which could lead to higher survival and associated disease potential in the following year. Thus, future studies are warranted to determine the frequencies of different maturity classes in different cultivars for different harvest dates.

ACKNOWLEDGMENTS

We thank C. Mims, J. Williams-Woodward, and A. Savelle for reviewing an earlier version of the manuscript.

LITERATURE CITED

1. Abawi, G. S., Grogan, R. G., and Duniway, J. M. 1985. Effect of water potential on survival of sclerotia of *Sclerotinia minor* in two different California soils. *Phytopathology* 75:217-221.
2. Adams, P. B. 1975. Factors affecting survival of *Sclerotinia sclerotiorum* in soil. *Plant Dis. Rep.* 59:599-603.
3. Batra, L. R. 1983. *Monilinia vaccinii-corymbosi* (Sclerotiniaceae): Its biology on blueberry and comparison with related species. *Mycologia* 75:131-152.
4. Batra, L. R. 1991. World Species of *Monilinia* (Fungi): Their Ecology, Biosystematics and Control. *Mycol. Mem. No. 16*, J. Cramer, Berlin.
5. Cartia, G., and Asero, C. 1994. The role of temperature regarding *Sclerotinia sclerotiorum* in the soil solarization method. *Acta Hort.* 366:323-330.
6. Coley-Smith, J. R., and Cooke, R. C. 1971. Survival and germination of fungal sclerotia. *Plant Dis. Rep.* 58:65-85.
7. Coley-Smith, J. R., Mitchell, C. M., and Sanford, C. E. 1990. Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli*. *Plant Pathol.* 38:58-69.
8. Dillard, H. R., Ludwig, J. W., and Hunter, J. E. 1995. Conditioning sclerotia of *Sclerotinia sclerotiorum* for carpogenic germination. *Plant Dis.* 79:411-415.
9. Dittmer, U., and Weltzien, H. C. 1990. A rapid viability test for sclerotia. *J. Phytopathol.* 130:59-64.
10. Harvey, I. C., Foley, L. M., and Savelle, D. J. 1995. Survival and germination of shallow-buried sclerotia of *Sclerotinia sclerotiorum* in pastures in Canterbury. *N. Z. J. Agric. Res.* 38:279-284.
11. Honey, E. E. 1936. North American species of *Monilinia*. I. Occurrence, grouping, and life-histories. *Am. J. Bot.* 23:100-106.
12. Huang, H. C., and Kozub, G. C. 1994. Germination of immature and mature sclerotia of *Sclerotinia sclerotiorum*. *Bot. Bull. Acad. Sin.* 35:243-247.
13. Imolehin, E. D., and Grogan, R. G. 1980. Factors affecting survival of sclerotia, and effects of inoculum density, relative position, and distance of sclerotia from host on infection of lettuce by *Sclerotinia minor*. *Phytopathology* 70:1162-1167.
14. Merriman, P. R., Pywell, M., Harrison, G., and Nancarrow, J. 1979. Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. *Soil Biol. Biochem.* 11:567-570.
15. Milholland, R. D. 1974. Factors affecting apothecium development of *Monilinia vaccinii-corymbosi* from mummified highbush blueberry fruit. *Phytopathology* 64:296-300.
16. Milholland, R. D. 1977. Sclerotium germination and histopathology of *Monilinia vaccinii-corymbosi* on highbush blueberry. *Phytopathology* 67:848-853.
17. Scherm, H., and Copes, W. E. 1999. Evaluation of methods to detect fruit infected by *Monilinia vaccinii-corymbosi* in mechanically harvested rabbiteye blueberry. *Plant Dis.* 83:799-805.
18. Scherm, H., Horton, D. L., NeSmith, D. S., Krewer, G., and Savelle, A. T. 1999. Georgia blueberry pest management survey. Pages 37-45 in: *Blueberry Research at the University of Georgia: 1999 Annual Research Update*. D.S. NeSmith, ed. Res. Rep. 662, University of Georgia, College of Agricultural and Environmental Sciences, Athens.
19. Scherm, H., Savelle, A. T., and Pusey, P. L. 2001. Interactions between chill-hours and degree-days affect carpogenic germination in *Monilinia vaccinii-corymbosi*. *Phytopathology* 91:77-83.
20. Scherm, H., and Yang, X. B. 1996. Development of sudden death syndrome of soybean in relation to soil temperature and soil water matrix potential. *Phytopathology* 86:642-649.
21. Shinnars, T. C., and Olson, A. R. 1996. The gynoeical infection pathway of *Monilinia vaccinii-corymbosi* in lowbush blueberry (*Vaccinium angustifolium*). *Can. J. Plant Sci.* 76:493-497.
22. Teo, B. K., Morall, R. A. A., and Verma, P. R., 1989. Influence of soil moisture, seeding date, and canola cultivars (Tobin and Westar) on the germination and rotting of sclerotia of *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol.* 11:393-399.
23. Twengstrom, E., Kopmans, E., Sigvald, R., and Svenson, C. 1998. Influence of different irrigation regimes on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. *J. Phytopathol.* 146:487-493.
24. Woronin, M. 1888. Über die Sclerotienkrankheit der Vaccinieen-Beeren. *Mem. Acad. Imp. Sci. St.-Petersbourg (VIIe Série)* 36:1-49.