Response of Two Fungi in the Apple Sooty Blotch Complex to Temperature and Relative Humidity

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Accepted for publication 15 December 1999.

ABSTRACT

Johnson, E. M., and Sutton, T. B. 2000. Response of two fungi in the apple sooty blotch complex to temperature and relative humidity. *Phytopathology* 90:362-367.

Peltaster fructicola and *Leptodontium elatius*, two of the causal fungi of apple sooty blotch, responded differently to temperature and relative humidity in vitro. Conidia of *L. elatius* germinated from 12 to 32°C at relative humidities $\geq 97\%$, whereas conidia of *P. fructicola* germinated from 12 to 24°C at relative humidities $\geq 95\%$. Germination of conidia of *L. elatius* was optimum at 32°C and 99% relative humidity compared with 24°C and 97 or 99% relative humidity for *P. fructicola*. When *L. elatius* and *P. fructicola* were grown in Parafilm culture, sporulation was greatest at relative humidities of 97 to 99%. In agar culture, mycelia of *L. elatius*

expanded radially from 12 to 32°C, and that of *P. fructicola* at 12 to 28°C. Mycelia of *P. fructicola* did not survive exposure for 7 days or more to temperatures $\geq 32^\circ\text{C}$. Mycelial growth was inhibited at relative humidities $< 95\%$ for both fungi and no growth occurred at 88% relative humidity. Conidia of *P. fructicola* were more sensitive to air drying than were those of *L. elatius*. Conidial viability of *P. fructicola* was reduced significantly after 8 h of air drying and nearly completely inhibited after 12 h. Conidia of *L. elatius* required 24 h of air drying before a significant reduction in conidial viability was observed. These results support the hypothesis that environmental factors influence the temporal and geographical distributions of the fungi associated with the apple sooty blotch disease.

Additional keywords: *Gloeodes pomigena*, *Malus domestica*.

Sooty blotch of apple (*Malus × domestica* Borkh.), associated with at least three unrelated fungi (*Peltaster fructicola* Johnson, Sutton, Hodges; *Gaeastrum polystigmatis* Batista & M.L. Farr; and *Leptodontium elatius* (G. Mangelot) De Hoog) (13) occurs throughout the humid fruit-growing regions of North America, Europe, South Africa, and the Asian Pacific (3,6,11). In North America, sooty blotch is most severe in regions of high rainfall and relative humidity, such as those found in the southeastern United States. Sooty blotch fungi grow epiphytically on the fruit's surface and result in superficial blemishes that are particularly obvious on light-skinned cultivars of apple, although all cultivars are considered to be equally susceptible (8,11,20). Blemishes are dark olive-green to black and vary in appearance from discrete, almost round patches to irregular patches with diffuse margins. Close examination often shows that single blemishes are actually several colonies that have coalesced or are the result of secondary spread (8,13,20). The sizes of the blemishes vary; mature single colonies are generally 0.5 to 1.0 cm in diameter. Economic losses result directly from downgrading of fruit during packout or indirectly from the application of fungicides on a 10- to 14-day schedule to protect fruit from infection (4,10).

Dominance of any one particular species of the fungal genera associated with sooty blotch varies according to its response to environment, sensitivity to fungicides, or competitive abilities on the fruit surface. Temperature and relative humidity have been implied as limiting factors in sooty blotch severity and distribution (6,11, 15,18). Brown and Sutton (5) found that moisture frequency correlated positively with the onset of first sooty blotch symptoms.

Ocamb-Basu et al. (17) reported that the onset of sooty blotch during seasons with normal to above-normal moisture was delayed on fruit on well-pruned trees compared with those on trees that were not pruned. Although differences in pruning regimes may affect drying time, moisture differences were not detected in their studies. Sutton and Sutton (19) found that sooty blotch severity in the orchard correlated positively with hours of relative humidity $\geq 90\%$. This research also showed that the incidence and severity of the mycelial types (ramose, punctate, fuliginous, and rimate [8]) varied in response to environmental variables. Severity of the ramose type was correlated positively with increasing average daily temperature and negatively with hours of $\geq 90\%$ relative humidity, whereas the severity of the punctate type decreased with temperature and increased with hours of relative humidity $\geq 90\%$.

Knowledge of environmental influences on disease development is important to properly time fungicide applications for optimum control of sooty blotch and to reduce unnecessary fungicide use. Precise information could aid in developing models to predict the onset of the disease in the spring. The effect of temperature and moisture on growth and development of each of the fungi in the sooty blotch complex has not been elucidated previously under controlled experimental conditions. Thus, the objective of this study was to compare the influence of temperature and moisture on in vitro growth and development of *P. fructicola* and *L. elatius*, two of the most common fungal species associated with apple sooty blotch in North Carolina.

MATERIALS AND METHODS

Isolates. Isolates were selected from a collection of *P. fructicola* and *L. elatius* isolates that had been maintained on potato dextrose agar (PDA) slants at room temperature in our laboratory. Isolates were collected from Mountain, Piedmont, and Coastal Plain regions in North Carolina and one location in Pennsylvania. Isolates used in the following experiments are listed in Table 1. All isolates were grown in continuous darkness on PDA at 24°C for co-

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Publication no. P-2000-0214-02R

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nidial production and on 2% water agar (WA) at 24°C for mycelial production. Conidial suspensions, used in germination studies, were made by blending 10- to 15-day-old cultures in 0.5% apple juice solution, which was found to enhance germination. The resulting suspension was filtered twice through three layers of cheesecloth to separate conidia from mycelial fragments. The conidial suspension was adjusted to 2.5×10^5 conidia per ml, which provided a minimum of 200 conidia in each 2- μ l drop. The suspension was maintained on ice until used unless noted differently.

Effect of air drying on germination of conidia. The effect of air drying on conidial germination was studied using two isolates of *L. elatius* (D5P11 and 2HC3P9) and two isolates of *P. fructicola* (C3P5 and 2C4F5). A micropipette was used to place 2- μ l drops of a conidial suspension on glass coverslips (25 $\times$ 25 mm) in a laminar flow hood. After the droplets evaporated, coverslips were maintained at 23 to 24°C and at relative humidities of 62 to 74% for 0, 4, 8, 12, or 24 h. Following drying, conidia on coverslips were exposed to constant 98% relative humidity in petri dish incubation chambers (1,2,9). The top and bottom of each petri dish (100 $\times$ 12 mm) was partially filled with 0.59 M sodium chloride (NaCl) agar, which maintained a constant 98% relative humidity at 24°C in the empty space in the center of the dish when it was reassembled (14). Chambers were preconditioned to 24°C for 24 h prior to use, to allow the relative humidity to equilibrate. Coverslips were placed on filter paper, 3 mm below the surface of the agar. Chambers were sealed with Parafilm (American Can Company, Greenwich, CT) and returned to a controlled-temperature incubator at 24°C for 48 h to allow conidia to germinate. After the germination period, coverslips for each treatment combination of isolate $\times$ drying time were inverted on a glass microscope slide with a drop of cotton blue in lactophenol to stop germination and preserve the spores and germ tubes for future observation. The experimental design was a split plot, with isolates of the two fungi as the main plot factor and the drying time as the subplot factor. Three replicates were used for each treatment. Spore germination was determined by examining 50 conidia from each coverslip at $\times 400$ magnification. Microscope fields were chosen arbitrarily, and all conidia, up to 50, were examined for germination. A conidium was considered germinated if the germ tube length was at least one half the length of the spore. The experiment was repeated two times with different dates of initiation.

The proportion of germinated conidia was transformed by the arcsine of the square root prior to conducting an analysis of variance (PROC ANOVA; SAS version 6.1; SAS Institute Inc., Cary, NC). The response of each species to drying time was fit to a linear model (PROC REG, SAS version 6.1) and slopes of the models were compared using student's *t* test.

Germination of conidia in response to temperature and relative humidity. The effects of relative humidity and temperature on conidial germination of isolates of *L. elatius* (ES5Ra2A, ES5P6, and ES5F1) and *P. fructicola* (C4P8, D3P5, and B4Ra2) were determined. A micropipette was used to place 2- μ l drops of a conidial suspension of each isolate on each of three glass coverslips (25 $\times$ 25 mm) in a laminar flow hood. Droplets were allowed to evaporate for 10 min. Coverslips were placed in preacclimated agar dishes using the isopiestic equilibration method previously described to establish relative humidities of 93, 95, 97, 99, and 100% (free water). Each relative humidity was established at 12, 16, 20, 24, 28, 32, and 36°C in controlled-temperature incubators. The experimental design was a split-plot arrangement of three isolates of each fungus species and five relative humidity levels within each temperature (three subsamples for each temperature $\times$ relative humidity combination). All dishes of each temperature $\times$ relative humidity $\times$ isolate combination were wrapped in aluminum foil, sealed within a plastic bag, and incubated in the dark. After 48 h, germination was stopped and percent conidial germination was determined as in the air-drying experiment described previously. The experiment was repeated three times with different dates of initiation.

Data were transformed by the arcsine square root prior to analysis using PROC GLM of SAS (SAS version 6.1). Temperature was tested against plot error (temperature $\times$ replicate) and relative humidity, fungi, and isolates, and interactions tested against the model error.

Radial growth in response to temperature. The effect of temperature on in vitro mycelial expansion was determined for 10 isolates of *P. fructicola* (2HC4Ri3, 2HC1Ra4, HC1P1, C3P5, 2C4F5, HS5P9, 2HS3P11, PaMicroP1, 2ECexF3, and HS1P11) and 4 isolates of *L. elatius* (D5P11, EC5F1, 2HC3P9, and HS5Ri6) (Table 1). Plugs (3 mm in diameter) were removed from the margins of 10- to 15-day-old colonies grown on WA. Individual plugs were placed in the centers of each of four WA dishes for each temperature $\times$ isolate combination. Each dish had been preacclimated at 12, 16, 20, 24, 28, 32, or 36°C in dark environmental incubators. The experimental design was a factorial arrangement of the isolates within each temperature. All dishes of each temperature $\times$ isolate combination were wrapped in aluminum foil and placed in a closed plastic container. Four replicates within each temperature were positioned randomly within the environmental incubators. Radial width at the widest point of the colony was determined 7, 14, and 21 days after inoculation with the aid of a dissecting microscope with an ocular grid. Fungal survival was determined for colonies that did not expand radially at 32 and 36°C after 21 days by incubating them at 24°C for 14 days and observing them for mycelial growth. The experiment was repeated two times with different dates of initiation.

Repeated measures analysis of variance (PROC GLM of SAS; SAS version 6.1) was used to evaluate the effects of replicates (initiation dates), temperature (main plot), fungus, isolate within fungus, and interactions repeated over time on the radial expansion rate (millimeters per day). Radial expansion rates for each isolate at each temperature were calculated by dividing the diameter of the colony by the number of days of incubation in the interval since the last evaluation. The 0- to 7-day expansion rate was excluded from all analyses so that effects were restricted to the linear phase of growth for each colony. Polynomial regression was used to relate the response variable (mean radial expansion rate from 7 to 21 days after inoculation) to the linear, quadratic, and cubic effects of the quantitative variable (temperature). Regression parameters not significantly different from zero ($P < 0.05$) were dropped from the equations and regressions were conducted again. All parameters reported differ significantly from zero ($P < 0.05$). There was not a significant difference among the dates (runs) that the experiments were conducted; therefore, data from both runs were pooled for the regression analysis.

TABLE 1. Isolates of *Leptodontium elatius* and *Peltaster fructicola* used in the relative humidity and temperature response tests

Isolate code	Fungus	Year	Collection location
D5P11	<i>L. elatius</i>	1987	Haywood Co., NC
EC5F1	<i>L. elatius</i>	1987	Johnston Co., NC
ES5F1	<i>L. elatius</i>	1987	Johnston Co., NC
ES5P6	<i>L. elatius</i>	1987	Johnston Co., NC
ES5Ra2A	<i>L. elatius</i>	1987	Johnston Co., NC
2HC3P9	<i>L. elatius</i>	1988	Henderson Co., NC
HS5Ri6	<i>L. elatius</i>	1987	Henderson Co., NC
B4Ra2	<i>P. fructicola</i>	1987	Cleveland Co., NC
C3P5	<i>P. fructicola</i>	1987	Haywood Co., NC
C4P8	<i>P. fructicola</i>	1987	Haywood Co., NC
2C4F5	<i>P. fructicola</i>	1988	Haywood Co., NC
D3P5	<i>P. fructicola</i>	1987	Haywood Co., NC
2ECexF3	<i>P. fructicola</i>	1988	Johnston Co., NC
HS5P9	<i>P. fructicola</i>	1987	Henderson Co., NC
2HC1Ra4	<i>P. fructicola</i>	1988	Henderson Co., NC
2HC4Ri3	<i>P. fructicola</i>	1988	Henderson Co., NC
HS1P11	<i>P. fructicola</i>	1987	Henderson Co., NC
2HS3P11	<i>P. fructicola</i>	1988	Henderson Co., NC
HC1P1	<i>P. fructicola</i>	1987	Henderson Co., NC
PaMicroP1	<i>P. fructicola</i>	1987	York Co., PA

Vegetative and reproductive growth in Parafilm culture in response to temperature and relative humidity. Parafilm strips were attached to microscope slides using mild heat and were disinfested by dipping them in 70% ethanol. Conidial suspensions of isolates of *L. elatius* (ES5P6, ES5F1, and ES5Ra2A) and *P. fructicola* (C4P8, D3P5, and B4Ra2) were prepared as described previously. Conidia were pregerminated at 24°C in a controlled-temperature incubator. A micropipette was used to place 2-µl drops of conidial suspensions containing 1×10^5 conidia per ml on the corners of the Parafilm strips. Water was allowed to evaporate from the droplet, and the slides were placed in chambers with relative humidity controlled by the isopiestic agar method, as described above. Culture dishes were sealed to a maintain constant relative humidity of 88, 95, 98, 99, or 100% (free water) within dark, temperature-controlled incubators at 16, 24, and 32°C. After 21 days, slides were observed at $\times 400$ with a compound microscope utilizing overhead illumination. Individual colonies were scored for vegetative (hyphal elongation and colony expansion) and reproductive (conidiophores and degree of sporulation) development. Vegeta-

tive development was scored on a scale from 0 to 3, in which 0 = no hyphal growth, 1 = short hyphal growth with no extension beyond the inoculation drop, 2 = hyphal extension ≤ 1 mm beyond the inoculation drop, and 3 = hyphal extension > 1 mm beyond the inoculation drop. Reproductive development was rated on a scale of 0 to 3, in which 0 = no development of conidia; 1 = few conidial initials, < 10 conidia per microscope field (0.64 mm^2); 2 = distinct conidiophores, 11 to 40 conidia per microscope field; and 3 = conidiophores extended, > 40 conidia per microscope field. There were three subsamples per treatment and the experiment was repeated two times with different dates of initiation. Because the rating scales were based on qualitative descriptions of vegetative growth and conidiophore development and conidia production, no statistical analyses of the data were performed, and results are presented graphically.

RESULTS

Effect of air drying on germination of conidia. *P. fructicola* was more sensitive to drying time than was *L. elatius* ($P < 0.01$) (Fig. 1). There were no differences ($P > 0.05$) among isolates of the same fungus species and the species $\times$ drying time interaction was not significant. After 12 h of air drying, germination of conidia of *L. elatius* was reduced by approximately 16%, while germination of conidia of *P. fructicola* was reduced by over 65%. The linear regression equation for percent germination of *L. elatius* conidia over the 24-h drying period was $y = 75.58 - 1.168x$, in which x = the duration of the drying time ($R^2 = 0.22$). The linear regression equation for percent germination of *P. fructicola* conidia or the initial 12-h drying period, in which the decline was linear, was $y = 57.116 - 2.959x$ ($R^2 = 0.31$). The slopes of the linear regression models (Fig. 1) for the two species were different ($P < 0.05$).

Germination of conidia in response to temperature and relative humidity. Conidial germination of *L. elatius* and *P. fructicola* differed in response to temperature and relative humidity ($P < 0.01$), and the variance analysis for each fungus was conducted separately (Table 2). Temperature and relative humidity effects accounted for most of the variation explained by the models for each fungus. Generally, both fungi responded similarly to relative humidity across all temperatures; however, *P. fructicola* was much more sensitive to temperatures $\geq 28^\circ\text{C}$ than was *L. elatius* (Fig. 2). There was no difference in percent germination of conidia among isolates of *P. fructicola* ($P = 0.09$), but mean percent germination of conidia of *L. elatius* across temperatures and relative humidity levels was less for isolate ES5P6 than that of the other isolates ($P < 0.001$, mean comparison by Student-Newman-Keuls test; $\alpha = 0.05$).

The temperature $\times$ relative humidity interaction in *L. elatius* was significant at $P < 0.01$ (Table 2). Relative humidity of 99% and temperatures ranging from 20 to 32°C generally were most favorable for conidial germination (Fig. 2). Germination at 100% relative humidity (free water) tended to be greater at cooler (12 to 20°C)

Fig. 1. Mean percent germination after 48 h of incubation at 24°C for conidia of the apple sooty blotch fungi, *Peltaster fructicola* and *Leptodontium elatius*, previously air dried for 0, 4, 8, 12, and 24 h. Bars indicate standard deviation from the mean with the pooled results of two isolates for each fungus. The linear regression equation for percent germination of *L. elatius* conidia over the 24-h drying period was $y = 75.580 - 1.169x$ ($R^2 = 0.22$). The linear regression equation for percent germination of *P. fructicola*, restricted to the initial 12-h drying period in which decline was linear, was $y = 57.116 - 2.959x$ ($R^2 = 0.31$).

TABLE 2. Variance analysis for conidia germination of *Leptodontium elatius* and *Peltaster fructicola* at different relative humidity $\times$ temperature combinations

Source of variation	df	<i>L. elatius</i>			<i>P. fructicola</i>		
		Mean square	F	P > F	Mean square	F	P > F
Model ^z	70	0.2232	20.85	0.0001	0.1941	46.69	0.0001
Replicate (Rep)	2	0.1830	11.50	<0.0500	0.0098	0.96	>0.0500
Temperature (Temp)	6	0.7426	46.70	0.0001	1.7271	169.32	0.0001
Temp $\times$ Rep (Error _a)	12	0.0159	...	...	0.0102	...	...
Relative humidity (RH)	4	1.6571	154.79	0.0001	0.4324	104.02	0.0001
Isolate (within species)	2	0.2038	19.04	0.0001	0.0100	2.41	0.0920
RH $\times$ isolate	8	0.0234	2.18	0.0294	0.0120	2.87	0.0045
Temp $\times$ RH	24	0.1331	12.43	0.0001	0.0476	11.45	0.0001
Temp $\times$ isolate	12	0.0166	1.55	0.1070	0.0079	1.90	0.0355
Error _b	244	0.0107	...	...	0.0042	...	...
Total	314	...	...	...	...	...	...

^z Coefficient of variation for the reduced model with *L. elatius* was 30.6% and for the reduced model with *P. fructicola* was 27.8%. Means were transformed by arcsine ($\times 0.5$) before analysis.

than at warmer temperatures. Very little germination was observed at 93 to 95% relative humidity. Germination of conidia occurred at all temperatures $\leq 32^{\circ}\text{C}$; no germination was observed at 36°C .

The temperature $\times$ relative humidity interaction for *P. fructicola* also was significant ($P < 0.01$) (Table 2). The ranked order of germination response to relative humidity was $97 = 99 > 100 > 95 > 93\%$. Conidial germination was low at all temperatures with 93% relative humidity and all relative humidities at temperatures $\geq 24^{\circ}\text{C}$ (Fig. 2). A temperature of 24°C was most favorable for germination of conidia, and the ranked order of germination response to temperature was $24 > 20 > 16 > 12 > 28 = 32 = 36^{\circ}\text{C}$. In general, conidial germination of *P. fructicola* was numerically greatest at 20 to 24°C and relative humidities of 97 and 99% (Fig. 2).

Radial growth in response to temperature. Radial growth of colonies of the *L. elatius* and *P. fructicola* isolates was greatest from 16 to 28 and 12 to 24°C , respectively (Fig. 3). At each temperature, colony diameters of isolates of *L. elatius* were similar to each other, whereas diameters varied among isolates of *P. fructicola*. *L. elatius* isolates expanded more rapidly than did isolates of *P. fructicola*. After 21 days of incubation at the highest temperature providing optimal growth of each fungus, the maximum colony diameter for *L. elatius* was 20.2 ± 0.9 mm (range 18.5 to 22.5 mm) at 28°C compared with 7.2 ± 1.85 mm (range 4.5 to 12.2 mm) for *P. fructicola* at 24°C . Growth of *L. elatius* was inhibited severely at temperatures $\geq 32^{\circ}\text{C}$ (Fig. 3). Similarly, growth of *P. fructicola* declined rapidly outside the optimal temperature range. *P. fructicola* did not grow at temperatures $\geq 32^{\circ}\text{C}$, and 4 of 10 isolates produced only scant mycelia at 28°C . Mycelial plugs of *P. fructicola* incubated at 28°C , but not at higher temperatures, resumed growth after being transferred to 24°C for 14 days.

Vegetative and reproductive growth on Parafilm in response to temperature and relative humidity. Results of the two runs were similar, and data were combined for the graphical representation (Fig. 4A and B). Growth of *P. fructicola* on Parafilm was characterized by the development of hyphae radiating from the inoculation point. *P. fructicola* produced aerial hyphae infrequently. Growth of mycelia was suppressed at relative humidities $< 97\%$, and no vegetative development occurred at 88% relative humidity (Fig. 4A). At 32°C , vegetative development was reduced at all relative humidity levels except 99%. Under conditions of this test, conidia were produced by *P. fructicola* only by budding in a yeastlike manner, as opposed to being produced in clusters around a conidiogenous locus on the hyphae. Consequently, scores for *P. fructicola* reflect only the number of conidia observed. Conidia production was suppressed at 100 and 93% relative humidity, and no conidia were formed at 88% relative humidity (Fig. 4B).

Growth of *L. elatius* on Parafilm more closely resembled natural symptoms of sooty blotch observed on apples. Aerial hyphae were produced in the most vigorous colonies. Vegetative development in *L. elatius* was generally less at relative humidities $\leq 95\%$; no vegetative development occurred at 88% relative humidity (Fig. 4A). At 16°C and relative humidities other than 99 and 97%, shortened isodiametrical cells were formed that massed upon each other to form a mound. Conidiophores tended to be clustered on the mounds. A gradient between this type of growth pattern and full hyphal extension occurred as relative humidity increased from 93 to 100% and temperature increased from 16 to 32°C (Fig. 4A).

Conidial development of *L. elatius* was extensive at all temperatures when the relative humidity was between 97 and 99% (Fig. 4B). Clusters of approximately 20 to 30 conidia were observed at

Fig. 2. Mean percent germination after 48 h of incubation for conidia of the apple sooty blotch fungi *Leptodotium elatius* and *Peltaster fructicola* in response to temperature and relative humidity. Values are the pooled means of three isolates for each fungus.

each septation and at the tip of the terminal cell of three- to four-celled conidiophores. At 95% relative humidity, conidiophores were single celled, with clusters of conidia at the tips. Clusters contained approximately 10 to 15 conidia at 95% relative humidity and 32°C. The number of conidia per cluster decreased to 5 to 10 at 24°C and 2 to 3 at 16°C. At 93% relative humidity, conidiophores were reduced to slightly elongated cells bearing one to three conidia. No reproductive development occurred at all temperature combinations with 88% relative humidity. At 100% relative humidity (free water), colonies were more diffuse and conidial production was greatest at 16 and 24°C. Conidia were produced only at the tips of conidiophores, which were elongated and extended beyond the water droplet. Conidia were rarely observed within the water droplets in the 100% relative humidity treatment.

DISCUSSION

Baines and Gardner (3) found that *Gloeodes pomigena* grew slowly at temperatures above 27°C and relative humidities <90%, with no growth at relative humidities <86%. Optimum conditions for sooty blotch development were 20°C and >95% relative humidity. Hickey (11) reported that optimum temperatures for four isolates of *G. pomigena* ranged from 20 to 29°C in culture. Similarly, Groves (8) found that growth of isolates of *G. pomigena* in culture varied in response to temperatures in this range and that no growth occurred above 30°C. Our results on the effect of temperature on mycelial growth of *P. fructicola* are similar to those reported for *G. pomigena*. The taxonomic status of *G. pomigena* is in doubt (12), and we believe that much of the data reported in the literature are for *P. fructicola*. In general, mycelial growth of *L. elatius* is more tolerant of higher temperatures than that of *P. fructicola*.

Fig. 3. Average radial expansion rate (millimeters per day) for isolates of the apple sooty blotch fungi *Peltaster fructicola* and *Leptodontium elatius* incubated at 12, 16, 20, 24, 28, 32, and 36°C. Radial expansion rates were calculated from colony diameter measurements 7, 14, and 21 days after inoculation. Values are the pooled means from two experiments. Each dashed line represents a different isolate for each fungal species. Regression equations for *L. elatius* and *P. fructicola* were $y = -1.273 + 0.182T - 0.0040T^2$ ($R^2 = 0.76$) and $y = 0.182 + 0.013T - 0.0005T^2$ ($R^2 = 0.72$).

but is of similar sensitivity to changes in relative humidity. Optimum temperatures for mycelial growth of *P. fructicola* and *L. elatius* are 12 to 24 and 16 to 28°C, respectively. Extended exposure to a temperature of 32°C or higher was lethal to *P. fructicola*, but *L. elatius* was able to survive at these temperatures in vitro. The colony-expansion rate for *L. elatius* was noticeably greater than for *P. fructicola*. This coincides with field observations that mature colonies of *L. elatius* are generally larger and more diffuse than those of *P. fructicola* (13).

There were differences in the reproductive development of *L. elatius* and *P. fructicola* in response to temperature and relative humidity. Conidia of *L. elatius* germinated over a wider range of temperatures (12 to 32°C) than did conidia of *P. fructicola* (12 to 24°C), but conidia of *L. elatius* were more sensitive to low relative humidity than were conidia of *P. fructicola*. Conidia of *P. fructicola* germinated when relative humidity was ≤95%, whereas conidia of *L. elatius* germinated very little at 93 and 95% relative humidity.

Fig. 4. Temperature and relative humidity effects on in vitro vegetative and reproductive development of two apple sooty blotch fungi, *Peltaster fructicola* and *Leptodontium elatius*, cultured on Parafilm strips attached to microscope slides. Environment was controlled by placing slides under sodium chloride-amended water agar within sealed petri dishes inside temperature-controlled incubators. **A**, Vegetative development rating scale: white = no hyphal growth; light-gray stipple = short hyphal growth with no extension beyond the inoculation drop; medium-gray stipple = hyphal extension ≤1 mm beyond the inoculation drop; and dark-gray stipple = hyphal extension >1 mm beyond the inoculation drop. **B**, Reproductive development rating scale: white = no development of conidia; light gray = few conidial initials with <10 conidia per microscope field (×200); medium gray = distinct conidiophores, 11 to 40 conidia per microscope field (×200); and dark gray = conidiophores extended, >40 conidia per microscope field (×200). The rating system for *P. fructicola* reflects only the number of conidia seen in each microscope field because conidiogenous cells were not produced using this technique.

In vitro, *L. elatius* produced conidia over a broad temperature range, but copious conidial production was restricted to relative humidity levels >97%. This result agrees with our field observations that this species sporulates only during periods of extended high relative humidity and fog (13). *P. fructicola* produced spores primarily by budding, using the Parafilm culture technique. We do not know the epidemiological significance of this manner of conidial production on the surface of fruit. We believe that most conidia of *P. fructicola* are produced in pycnothyria and are released and dispersed by rain or dew. However, some conidia may arise from conidiogenous loci on the hyphae or by budding (12).

Conidia of *P. fructicola* were very sensitive to air drying, whereas those of *L. elatius* were less sensitive. *P. fructicola* conidia are borne within a gelatinous matrix in pycnothyria and are protected from desiccation (13). Conversely, conidia of *L. elatius* (a hyphomycete) are borne aurally and viability declines gradually over a 24-h drying period. We hypothesize that dry periods following spore dispersal may severely limit fruit infection by *P. fructicola*. Dry periods would be less restrictive to fruit infection by *L. elatius*.

Groves (8) established four classes of sooty blotch mycelial types: punctate, ramose, fuliginous, and rimate. Previously, we associated the fuliginous type with *L. elatius* and the punctate type with *P. fructicola* (12). Sutton and Sutton (19) reported that distribution of mycelial types of *G. pomigena* on fruit in the orchard was correlated with rainfall, relative humidity, and temperature. They observed the fuliginous mycelial type across a broader range of temperatures and relative humidities than that of punctate and ramose types. Ramose and punctate types were restricted geographically to climates with high moisture. Our results with *L. elatius* and *P. fructicola* corroborate their results.

Sooty blotch usually occurs in the orchard together with flyspeck (*Zygophiala jamaicensis*, teleomorph *Schizothyrium pomi*). Ocamb-Basu et al. (17) observed more sooty blotch than flyspeck in dry years and hypothesized that the development of sooty blotch required less moisture than did flyspeck. Because ascospores of *S. pomi* require 99% relative humidity or greater for germination, and conidial germination for *Z. jamaicensis* does not occur (or is severely limited) at relative humidities below 97% (16), our results confirm their hypothesis. However, both fungi associated with sooty blotch are very sensitive to relative humidity, and other factors also may influence the appearance of visible symptoms.

The data collected in this study may be useful in developing weather-based models to predict infection and development of sooty blotch and flyspeck diseases and subsequent deployment of fungicides. Such a model must predict behavior of all fungi in the sooty blotch complex, as well as flyspeck, because control measures are directed simultaneously toward both diseases. Because there are at least three organisms involved in this complex, refinement of the decision analytic model of Gold and Sutton (7) would be warranted over the development of mechanistic simulation models.

ACKNOWLEDGMENTS

We thank S. Williamson for the review of this manuscript and suggestions for its improvement.

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