Etiology

Effects of Temperature and Relative Humidity on Germination, Growth, and Sporulation of *Zygophiala jamaicensis*

C. M. Ocamb-Basu and T. B. Sutton

Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.
We thank Dr. L. A. Nelson for assistance with the statistical analysis.
Use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.
Paper No. 10906 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.
Accepted for publication 15 July 1987 (submitted for electronic processing).

ABSTRACT

Ocamb-Basu, C. M., and Sutton, T. B. 1988. Effects of temperature and relative humidity on germination, growth, and sporulation of *Zygophiala jamaicensis*. Phytopathology 78:100-103.

In vitro studies of *Zygophiala jamaicensis*, the cause of flyspeck on apple, demonstrated that conidia germinated more rapidly and over a wider temperature range than did ascospores. Some conidia germinated at 20 to 28 C after 2 hr, but no ascospores germinated until 4 hr had elapsed. Ascospores germinated at temperatures from 16 to 28 C, whereas conidia germinated at 8 to 28 C. Conidia required relative humidity of 96.2% or greater for germination, whereas ascospores had a higher minimum relative humidity threshold (>96.2%) for germination. Mycelial growth of *Z. jamaicensis* occurred from 12 to 28 C, with an optimum of 16 to 24 C. Production of conidia occurred from 12 to 24 C and was greatest at 16 and 20 C. Relative humidity of 96.2% or greater was necessary for mycelial growth and sporulation. It is hypothesized that the higher relative humidity requirement of *Z. jamaicensis* limits its development in the orchard compared to that of *Gloeodes pomigena* (the cause of sooty blotch).

Flyspeck, caused by *Zygophiala jamaicensis* Mason (teleomorph, *Schizothyrium pomi* (Mont. & Fr.) v. Arx), is one of the most common apple diseases in the southeastern United States. In North Carolina, it affects approximately 8 to 15% of the fruit annually (9,10), resulting in a downgrading of the fruit and lower market prices. Ascospores cause initial infections in spring (3); secondary infections arise from conidia, which are produced throughout much of the growing season (8). *Z. jamaicensis* is commonly found on fruit in association with *Gloeodes pomigena* (Schw.) Colby, the cause of sooty blotch. *Z. jamaicensis* and *G. pomigena* grow on the surface of apple fruit, utilize the waxy cuticle, and penetrate only the epidermal layer (2,5). The optimum temperature for growth of *Z. jamaicensis* was reported to range from 20 to 25 C (11) and from 15 to 24 C (1). The optimum temperatures for germination of ascospores and conidia and for sporulation of *Z. jamaicensis* have not been reported.

The incidence and development of sooty blotch and flyspeck in apple orchards have been correlated with the amount of rainfall (7). Sharp and Yoder (14) reported that flyspeck required a longer period of relative humidity (RH) greater than 95% than sooty blotch for symptom appearance. Ocamb-Basu (12) found that a lack of rainfall did not limit sooty blotch development, but flyspeck severity was comparatively greater in wetter seasons, indicating that *Z. jamaicensis* may be more sensitive to moisture than *G. pomigena*. Thus the objective of this investigation was to quantify the effects of temperature and relative humidity on germination of ascospores and conidia, mycelial growth, and production of conidia of *Z. jamaicensis*.

MATERIALS AND METHODS

Inoculum preparation. Unless indicated otherwise, ascospores were obtained by crushing mature pseudothecia collected from a wild *Rubus* sp. in deionized water to liberate spores from the asci. *Rubus* stems were surface-sterilized in 0.525% NaOCl for 10 min, and pseudothecia were removed with a dental file, placed in a tissue homogenizer containing approximately 3 ml of water, and homogenized for approximately 40 sec. Conidia were produced in vitro on V-8 juice agar (200 ml of V-8 juice, 17 g of agar, 3 g of $CaCO_3$, and 800 ml of deionized water) in darkness at 20 C. A conidial suspension was made by blending a 9- to 12-day-old culture in deionized water for 30 sec. An isolate of *Z. jamaicensis* (FS-A-1) collected from a wild *Rubus* sp. was used in all tests.

Effect of temperature on ascospore germination. Pseudothecia were crushed on glass coverslips (18 × 18 mm) in deionized water to release ascospores. Four coverslips each were placed in plastic petri plates (100 × 12 mm) that contained moist filter paper. The plates were wrapped in Parafilm (American Can Company, Greenwich, CT), sealed in plastic bags, and placed arbitrarily in incubators at 12, 16, 20, 24, 28, and 32 C. Two plates at each temperature (eight coverslips) were removed arbitrarily after 2, 4, 8, 16, 24, and 36 hr, and spores were stained with cotton blue in lactophenol. Percent germination was determined by examining all ascospores on each coverslip (about 25 to 50 ascospores each). An ascospore was considered germinated if the germ tube length was equal to or greater than the spore diameter. Germ tube length was determined from measurements of 10 ascospores per coverslip. The experiment was repeated twice.

Effect of temperature on germination of conidia. Glass tubes containing 10 ml of a deionized water suspension of conidia

(approximately 4×10^3 conidia per milliliter) were capped tightly and incubated at 8, 12, 16, 20, 24, 28, and 32 C. Four tubes of the spore suspension were incubated at each temperature, and each tube was sampled after 2, 5, 9, 12, and 18 hr. The tubes were agitated prior to sampling, and a 0.01-ml drop of spore suspension was removed and added to a drop of cotton blue in lactophenol. The percent germination and germ tube length were recorded for 100 and 40 conidia, respectively, per replication. A conidium was considered germinated if the germ tube length was equal to or greater than the diameter of the conidium. The experiment was repeated two times.

Effect of relative humidity on germination and germ tube length of ascospores and conidia. Relative humidity chambers were made by the agar dish isopiestic equilibration technique described by Harris et al (6). Potassium chloride concentrations, based on data of Robinson and Stokes (13), were used to achieve humidity levels of 99.8 to 94.9% RH. Forty milliliters of medium was poured into the base of plastic petri dishes (100 × 12 mm), leaving a 6-mm air space, and the plates were cooled for 4 to 6 hr. Drops of suspensions of freshly harvested ascospores (about 20 ascospores per drop) or conidia (about 25 conidia per drop) were placed on glass coverslips (18 × 18 mm) and dried for approximately 0.5 hr in a laminar flow hood. Four coverslips were placed in each plate, and the plates were wrapped in Parafilm and sealed in plastic bags. The ascospores were incubated at 99.8, 99.7, 99.0, 98.1, 97.1, and 96.2% RH (equivalent to −0.23, −0.45, −1.32, −2.62, −4.15, and −4.37 MPa, respectively) at 20 C. The conidia were incubated at 99.7, 97.1, 96.2, and 94.9% RH (equivalent to −0.45, −4.15, −4.37, and −7.08 MPa, respectively) at 12, 20, and 28 C. The ascospores were sampled after 12 and 24 hr of incubation. The conidia were sampled after 9, 12, 16, and 20 hr of incubation. Coverslips from two plates per treatment (eight coverslips) were sampled arbitrarily each time by staining the spore suspension with cotton blue in lactophenol and noting percent germination and germ tube length. Each study was repeated twice.

Effect of temperature on mycelial growth and production of conidia. A 0.1-ml suspension of conidia and mycelium (1×10^3 conidia per milliliter) was placed on petri plates (50 × 12 mm) containing 10 ml of V-8 juice agar. The plates were wrapped with Parafilm, sealed in plastic bags, and incubated in darkness at 8, 12, 16, 20, 24, 28, and 32 C. Three plates at each temperature were sampled arbitrarily 6, 8, 10, and 12 days after inoculation. Five colonies were selected arbitrarily from each plate, blended in 40 ml of deionized water for 1 min, and centrifuged at 10,000 rpm for 10 min. Water was decanted to give a resulting volume of 5.0 ml. Four 0.5-μl aliquot samples from each suspension were mixed with lactophenol and cotton blue, and the number of conidiophores in each aliquot was determined. Conidia rapidly germinated on conidiophores in culture and were difficult to differentiate from mycelium; consequently, the production of conidia was estimated by the number of conidiophores in each aliquot. Conidiophores have a characteristic morphology (2) that is easily recognized, and scars on conidiogenous cells are evidence of production of conidia. The diameter of each colony was recorded before blending. This experiment was repeated twice.

Effect of relative humidity on mycelial growth and sporulation. Filter paper disks (120 mm^2) were autoclaved in V-8 juice for 20 min. The disks were dried in a laminar flow hood for 6 hr; then a 0.1-ml drop of conidial and mycelial suspension was placed on top of each disk and dried for 1 hr. Three disks were placed in each relative humidity chamber, at 99.7, 97.1, 96.2, and 94.9% RH, and incubated at 12, 20, and 28 C. For all disks of two plates per treatment, the percent area colonized 6 days after inoculation was determined by video image analysis, using the Image Plus+ System (Dapple System, Sunnyvale, CA). Conidiophore production was determined by maceration of each disk in 3.5 ml of deionized water, and conidiophores were counted with the aid of a hemocytometer. The experiment was repeated twice.

Data analysis. The experimental design of each of the experiments was a randomized complete block design, with blocks consisting of multiple runs (of which there were three in each experiment). The number of replications in each experiment varied as indicated above. An analysis of variance was used in the analysis of the data, with treatments tested by plot error (run × treatment). Polynomial regression was used to relate the response variables to powers of the quantitative variable (temperature or relative humidity). The temperature variable was transformed to place the origin through the temperature at which no germination or growth occurred. For the effect of temperature on ascospore germ tube length and on conidiophore production, the transformation was temp = (temp − 12)/4. For all other temperature experiments, the transformation was temp = (temp − 8)/4. Fitting was then done using uncorrected sums of squares and cross products. Data collected over time (e.g., after 2, 5, 9 hr, etc.) were analyzed separately for each time period. A combined analysis was not warranted, because of unequal error variances for the different times. In addition to characterizing the form of the response curves, the optimum levels of the input variables were estimated as part of the analysis for each experiment.

RESULTS

Effect of temperature on germination of ascospores and conidia. No ascospores germinated during the first 2 hr; germination was observed at 16 and 20 C after 4 hr. Ascospore germination was highly variable between temperatures as well as between replications at the same temperature. We suspect this was due to differences in ascospore maturity between and within pseudothecia. Consequently, we found no difference ($P = 0.05$) in the percent germination after 36 hr at 16, 20, 24, and 28 C. Ascospores did not germinate after 36 hr at 12 or 32 C. The predicted mean length of ascospore germ tubes was greatest at 21.8 C after 16 hr (Fig. 1).

Conidia germinated at temperatures ranging from 8 to 28 C (Fig. 2). Some germination had occurred after 2 hr at 20 to 28 C. After 5 hr, over 80% of the conidia at 24 and 28 C had germinated. After 12 hr, 100% germination had occurred at 16 to 28 C. Percent germination dropped sharply at 8 C, and no germination occurred at 32 C (Fig. 2). There was little difference in germ tube length at 16, 20, 24, or 28 C after 5 or 9 hr (Fig. 2). After 12 hr of incubation, the predicted optimum germ tube length occurred at 20.8 C.

Effect of relative humidity on spore germination. After 12 hr, nearly all ascospores had germinated at 99.0% RH or greater; the percent germination dropped at 98.1 and 97.1% RH (Fig. 3). After 24 hr, there was no germination at 96.2% RH, and germination was approximately 66% at 97.1% RH. The mean germ tube length was greatest after 12 hr at 99.8% RH; it declined to approximately 5 μm at 97.1% RH (Fig. 3). After 24 hr of incubation, the mean length of ascospore germ tubes at 97.1% RH was 60 μm; at RH > 99%, it was 150 μm. The regression of the data on germ tube length for the 24-hr incubation was not significant.

After 9 hr, conidia at 20 C had germinated only at RH 99.7%, and those at 28 C had germinated only at 97.1 and 99.7% RH (Table 1). After 12 hr, some conidia at 12 and 20 C had germinated at 99.7 and 97.1% RH, respectively. After 20 hr, more than 90% of the conidia incubated at 20 and 28 C had germinated at 99.7% RH. Percent germination was lowest at 97.1% RH at 20 and 28 C.

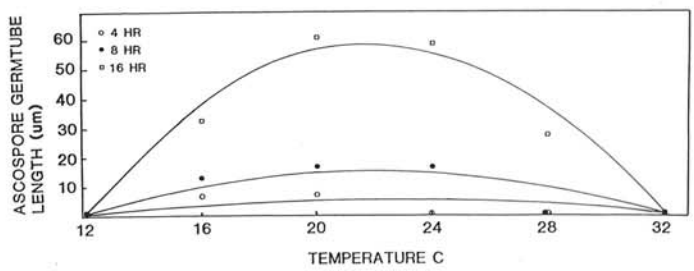

Fig. 1. Effect of temperature on mean length (in micrometers) of ascospore germ tubes of *Schizothyrium pomi*. Regression equations for 4-, 8-, and 16-hr incubations were $y = 3.047X - 0.674X^2$, $y = 12.196X - 2.594X^2$, and $y = 46.754X - 9.484X^2$, respectively. For 4-, 8-, and 16-hr incubations, r^2 was 0.24, 0.75, and 0.87, respectively.

Conidia germinated at 96.2% RH only at 28 C; this was thought to be due to the formation of condensation, as some free moisture was observed on the coverslip. Conidia at 12 C germinated at 99.7% RH but not at 97.1% RH after 20 hr. Germ tube length at 12 C never exceeded 10 μm (Table 2). At 20 and 28 C, the mean length of conidial germ tubes was greater at 99.7% RH than at 97.1% RH.

Effect of temperature on mycelial growth and conidiophore production. On day 6, colonies of similar size were visible at 16, 20, and 24 C; somewhat smaller colonies were present at 28 C. Colonies were visible at 12 C by day 8. After day 12, colony diameter was greatest at approximately 20 C (Fig. 4), but there was little difference in diameter from 16 to 24 C. Colony diameter was consistently smaller at 12 and 28 C; no colonies were visible at 8 and 32 C. After 12 days of incubation, the predicted optimum temperature for conidiophore production was 17.3 C, and abundant conidiophores were produced from 16 to 24 C (Fig. 5). No sporulation occurred at 28 C.

Effect of relative humidity on mycelial growth and sporulation. No colonization of the disks occurred at any relative humidity after 6 days at 12 C. At 20 C, more than 90% of the disk surface area was colonized at 99.7% RH, less than 50% was colonized at 97.1% RH,

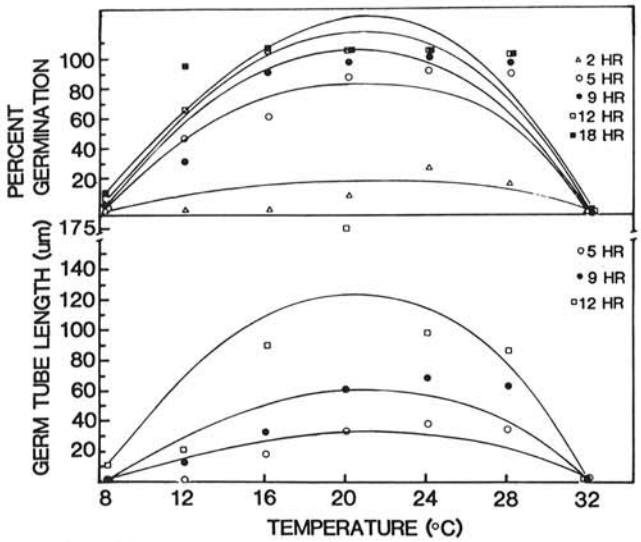

Fig. 2. Effect of temperature on percent germination and mean germ tube length (in micrometers) of conidia of *Zygophiala jamaicensis*. Regression equations for percent germination in relation to temperature in 2-, 5-, 9-, 12-, and 18-hr incubations were $y = 6.350X - 0.860X^2$, $y = 50.061X - 7.621X^2$, $y = 64.498X - 10.202X^2$, $y = 73.352X - 11.817X^2$, and $y = 77.579X - 12.624X^2$, respectively. For 2-, 5-, 9-, 12-, and 18-hr incubations, r^2 was 0.39, 0.84, 0.93, 0.96, and 0.95, respectively. Regression equations for germ tube length in 5-, 9-, and 12-hr incubations were $y = 17.392X - 2.611X^2$, $y = 35.376X - 5.394X^2$, and $y = 80.760X - 13.143X^2$, respectively. For 5-, 9-, and 12-hr incubations, r^2 was 0.77, 0.81, and 0.79, respectively.

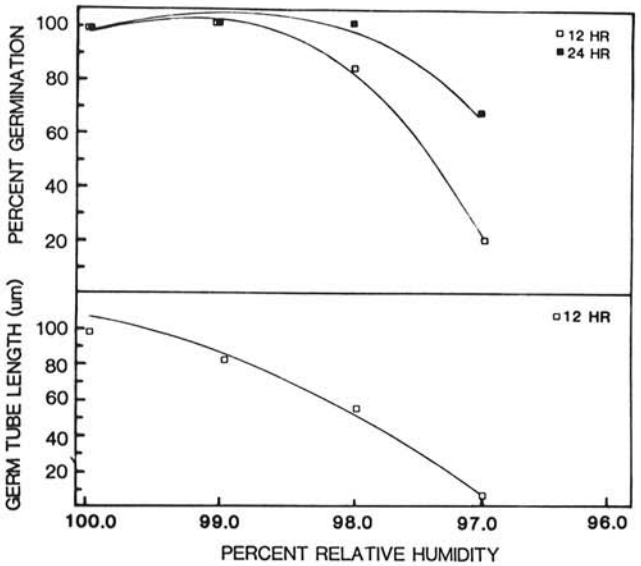

Fig. 3. Percent germination and germ tube length (in micrometers) of ascospores of *Schizothyrium pomi* in 99.8 to 96.2% relative humidity at 20 C. Regression equations for percent germination in 12- and 24-hr incubations were $y = -180{,}027.594 + 3{,}630.537X - 18.294X^2$ and $y = -97{,}131.589 + 1{,}963.941X - 9.917X^2$, respectively. For 12- and 24-hr incubations, r^2 was 0.87 and 0.91, respectively. The regression equation for germ tube length at 12 hr was $y = -54{,}752.866 + 1{,}071.254X - 5.224X^2$, and $r^2 = 0.079$.

TABLE 1. Percent germination of conidia of *Zygophiala jamaicensis*

Temp (C)	% RH	Incubation period (hr) 9	12	16	20
12	99.7	0.00	10.00	26.25	37.50
	97.1	0.00	0.00	0.00	0.00
	96.2	0.00	0.00	0.00	0.00
	94.9	0.00	0.00	0.00	0.00
20	99.7	85.00	87.50	93.75	97.50
	97.1	0.00	1.25	23.75	28.70
	96.2	0.00	0.00	0.00	0.00
	94.9	0.00	0.00	0.00	0.00
28	99.7	67.50	70.00	72.50	92.50
	97.1	12.50	20.00	76.25	67.50
	96.2	0.00	0.00	0.00	27.50
	94.9	0.00	0.00	0.00	0.00

TABLE 2. Mean germ tube length (μm) of conidia of *Zygophiala jamaicensis*

Temp (C)	% RH	Incubation period (hr) 9	12	16	20
12	99.7	0.0	10.0	10.0	10.0
	97.1	0.0	0.0	0.0	0.0
	96.2	0.0	0.0	0.0	0.0
	94.9	0.0	0.0	0.0	0.0
20	99.7	40.0	60.0	80.0	110.0
	97.1	0.0	10.0	20.0	20.0
	96.2	0.0	0.0	0.0	0.0
	94.9	0.0	0.0	0.0	0.0
28	99.7	30.0	80.0	70.0	120.0
	97.1	10.0	20.0	30.0	70.0
	96.2	0.0	0.0	0.0	3.0
	94.9	0.0	0.0	0.0	0.0

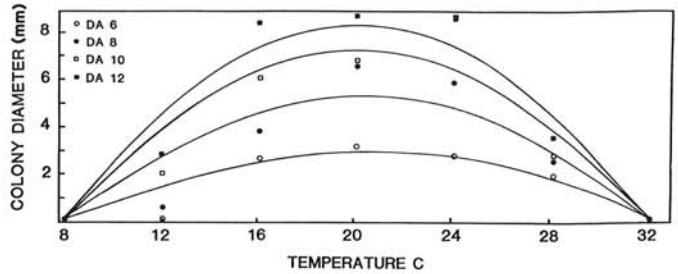

Fig 4. Effect of temperature on colony diameter (in millimeters) of *Zygophiala jamaicensis*. Regression equations for 6-, 8-, 10-, and 12-day incubations were $y = 1.902X - 0.310X^2$, $y = 3.427X - 0.565X^2$, $y = 4.773X - 0.797X^2$, and $y = 5.578X - 0.939X^2$, respectively. For 6-, 8-, 10-, and 12-day incubations, r^2 was 0.84, 0.83, 0.93, and 0.95, respectively.

only slight colonization occurred at 96.2% RH, and no growth occurred at 94.9% RH. At 28 C, no growth occurred at 94.9% RH (Fig. 6). Percent colonization at 28 C was not greatly different at 99.7 and 97.1% RH; it decreased through 96.2% RH (Fig. 6). Conidiophore production was greatest at 97.1% RH and above. Production at 20 and 28 C decreased through 96.2% RH (Fig. 6).

DISCUSSION

Mycelial growth of *Z. jamaicensis* occurred from 12 to 28 C, with the optimum being 16 to 24 C. Production of conidia occurred from 12 to 24 C and was greatest from 16 to 20 C. These results are similar to those obtained by Nasu et al (11) and Baines (1). Nasu and co-workers reported that the optimum temperature for growth of *Z. jamaicensis* ranged from 20 to 25 C. Baines reported that the optimum temperature for growth of the fungus ranged from 15 to 24 C and that little growth occurred at 27 C. We observed some differences in the response of ascospores and conidia to temperature and relative humidity. Conidia germinated more rapidly and over a wider temperature range than did ascospores. Conidia required 96.2% RH or greater for germination; ascospores required a higher relative humidity, as no germination occurred at 96.2% RH. Some of the differences observed between ascospores and conidia may be due to differences in the physiological maturity of spores. Since ascospores had to be collected in vivo, ascospore maturity varied between asci in the same pseudothecium and between pseudothecia, whereas abundant conidia were harvested in vitro.

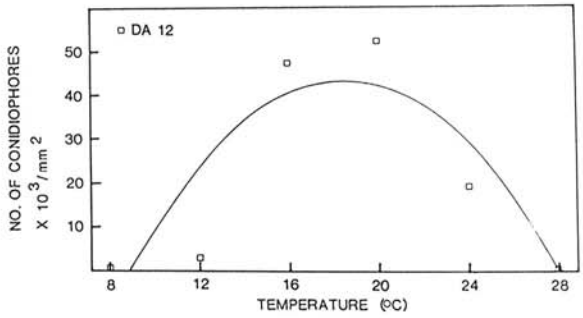

Fig. 5. Effect of temperature on conidiophore production (number of conidiophores per square millimeter of colonized area) of *Zygophiala jamaicensis* after 12 days of incubation. The regression equation was $y = -11{,}727.230 + 1{,}734.510X - 47.125X^2$, and $r^2 = 0.72$.

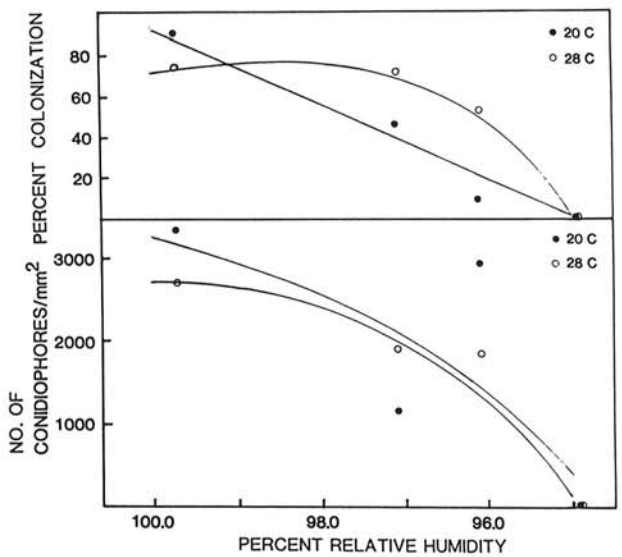

Fig. 6. Effect of relative humidity on percent surface area of filter paper disk colonized at 20 and 28 C and conidiophore production (per square millimeter of colonized area) by *Zygophiala jamaicensis* at 20 and 28 C at 99.7, 97.1, 96.2, and 94.9% RH, 6 days after inoculation. Regression equations for percent surface area colonized at 20 and 28 C were $y = 1{,}258.275 - 44.862X + 0.332X^2$ and $y = -71{,}767.078 + 1{,}461.854X - 7.435X^2$, and r^2 was 0.95 and 0.77, respectively. Regression equations for conidiophore production at 20 and 28 C were $y = -825{,}970.883 + 16{,}437.660X - 81.444X^2$ and $y = -1{,}513{,}587.809 + 30{,}634.08X - 154.723X^2$, and r^2 was 0.54 and 0.90, respectively.

Flyspeck and sooty blotch usually occur together in the orchard. However, we have observed in dry seasons that sooty blotch severity is often proportionally greater than flyspeck severity. This may be because the moisture requirement for at least certain growth components of *G. pomigena* is broader than that for *Z. jamaicensis*. Once the moisture requirements for both *G. pomigena* and *Z. jamaicensis* have been determined, it may be possible to predict periods when the likelihood of the development of each of these diseases is greatest. This has the potential to be a useful disease management tool. Currently, six to eight fungicide sprays are targeted for the control of these diseases, as well as summer rot diseases. Even so, losses to sooty blotch and flyspeck are great (9,10). A model to enable more timely fungicide application could result in substantial savings in fungicide use and improved disease control. A framework for this model has been proposed (4).

LITERATURE CITED

1. Baines, R. C. 1940. Pathogenicity and hosts of the fly-speck fungus of apple. (Abstr.) Phytopathology 30:2.
2. Baker, K. F., Davis, L. H., Durbin, R. D., and Snyder, W. C. 1977. Greasy blotch of carnation and flyspeck of apple: Diseases caused by *Zygophiala jamaicensis*. Phytopathology 67:580-588.
3. Durbin, R. D., Davis, L. H., Snyder, W. C., and Baker, K. F. 1953. The imperfect stage of *Microthyriella rubi*, cause of flyspeck of apple. (Abstr.) Phytopathology 43:470-471.
4. Gold, H. J., and Sutton, T. B. 1986. A decision analytic model for chemical control of sooty blotch and flyspeck diseases of apple. Agric. Syst. 21:129-157.
5. Groves, A. B. 1933. A study of the sooty blotch diseases of apples and causal fungus *Gloeodes pomigena*. Va. Agric. Exp. Stn. Bull. 50:1-43.
6. Harris, R. F., Gardner, W. R., Adebayo, A. A., and Sommers, L. E. 1969. Agar dish isopiestic equilibration method for controlling the water potential of solid substrates. Appl. Microbiol. 19:536-537.
7. Kirby, R. S. 1954. Relation of rainfall to occurrence of apple scab and sooty blotch. (Abstr.) Phytopathology 44:495.
8. Latham, A. J., and Hollingsworth, M. H. 1973. Incidence and control of sooty blotch and flyspeck on apples in Alabama. Auburn Univ. Agric. Exp. Stn. Circ. 208. 11 pp.
9. Main, C. E., and Byrne, S. V., eds. 1986. 1985 Estimates of Crop Losses in North Carolina Due to Plant Diseases and Nematodes. Dep. Plant Pathol. Spec. Publ. 5. N.C. State Univ., Raleigh. 183 pp.
10. Main, C. E., and Nusser, S. M. 1985. 1984 Estimates of Crop Losses in North Carolina Due to Plant Diseases and Nematodes. Dep. Plant Pathol. Spec. Publ. 4. N.C. State Univ., Raleigh. 152 pp.
11. Nasu, H., Fuhii, S., and Yokoyama, T. 1985. *Zygophiala jamaicensis* Mason, a causal fungus of flyspeck of grape, Japanese persimmon, and apple. Ann. Phytopathol. Soc. Jpn. 51:536-545.
12. Ocamb-Basu, C. M. 1987. Cultural and environmental factors affecting the epidemiology of sooty blotch and flyspeck of apple. M.S. thesis, North Carolina State University, Raleigh. 58 pp.
13. Robinson, R. A., and Stokes, R. H. 1955. Electrolyte Solutions. Butterworths Scientific Publications, London. 512 pp.
14. Sharp, W. L., and Yoder, K. S. 1985. Correlation between humidity periods and sooty blotch and flyspeck incidence in Virginia apple orchards. (Abstr.) Phytopathology 75:628.