Effect of Leaf Wetness Duration, Temperature, and Conidial Inoculum Dose on Apple Scab Infections

J. R. Hartman, Professor, Department of Plant Pathology, University of Kentucky, Lexington 40546; and L. Parisi, Research Plant Pathologist, and P. Bautrais, Biometeorologist, INRA, Station de Pathologie Végétale, B.P. 57, 49071 Beaucouzé Cedex, France

ABSTRACT

Hartman, J. R., Parisi, L., and Bautrais, P. 1999. Effect of leaf wetness duration, temperature, and conidial inoculum dose on apple scab infections. *Plant Dis.* 83:531-534.

Apple seedlings (2 months old, 'Idared' × 'Golden Delicious') were inoculated with conidia of *Venturia inaequalis* in order to study the effects of inoculum dose and leaf wetness duration on development of apple scab symptoms. For each experiment, the C3 curve (indicating heavy infection levels) was used as the basis for relating infection to temperature and leaf wetness duration. In one series of experiments, seedlings were treated with inoculum doses of 1.5, 5.4, 15.6, 32.2, 81.2, and 250×10^3 conidia/ml and leaves were kept wet during C3 infection periods at temperatures of 6, 11, 16, and 22°C. At all four temperatures, disease incidence (scab lesions/plant) increased with increasing inoculum doses up to about 81.2×10^3 conidia/ml. Disease incidence was lower at 22°C than at the other temperatures. In a second series of experiments, seedlings inoculated with 10×10^3 conidia/ml were kept moist for infection periods ranging from 0.6 to 2.0 times the C3 leaf wetness duration curve at 6, 11, 16, and 22°C. Disease incidence increased with increasing duration of leaf wetness and generally leveled off between 150 and 200% of the C3 curve. At this inoculum dose (10×10^3 conidia/ml), doubling the leaf wetness duration indicated by the C3 curve resulted in high disease incidence, similar to levels obtained with a higher inoculum (250×10^3 conidia/ml) and shorter wetness period (1.0 C3).

Environmental variables such as temperature and duration of leaf wetness have been shown by many investigators to influence infection of apple leaves by ascospores and conidia of *Venturia inaequalis* (Cooke) G. Wint. (4). A table showing the number of hours of leaf wetness needed for primary scab infections at various temperatures was developed by Mills in 1944 (6). Apple growers use Mills-based predictive systems to determine the risks of primary infection by ascospores (2). Similar tables or curves representing the relationship between hours of leaf wetness, temperature, and infection by *V. inaequalis* have been developed by several researchers, and were analyzed by MacHardy and Gadoury (5), who proposed a revision of the Mills table based on these reports. This revision was further modified by Stensvand et al. (11), who determined that the wetness duration required for infection is approximately the same for ascospores and conidia. Parisi et al. (7), using apple seedlings, high inoculum doses (250×10^3

conidia/ml), and favorable controlled environmental conditions, developed three curves (C1 to C3) which described light, medium, and heavy infection levels by conidia. The C3 curve is similar to the Schwabe low-risk curve (9) and represents high levels of seedling infection (more than 50 spots per plant) which are thought to be equivalent to significant, but low, levels of infection in the orchard (7). The relationship between inoculum dose used in the C3 curve experiments and amount of secondary inoculum produced in the orchard which then results in some level of additional scab beyond primary infection is not completely known.

The occurrence of high numbers of apple scab conidia in the orchard has been demonstrated. In Pennsylvania, scab lesions were found to contain up to 95×10^3 conidia (1) and in Michigan, almost 7×10^3 conidia/ml were found in water traps following rainfall (12). MacHardy (4) describes work done by Borecki showing that, in Poland, as many as 160×10^3 conidia/ml were found in water dripping from leaves. Smith and MacHardy (10) studied conidial production on a range of cultivars with different susceptibility to scab. They found significant differences between cultivars. The conidial productivity ranged from 15 to 29×10^3 conidia/ml in the cv. Golden Delicious group to 5 to 10×10^3 conidia/ml in the cv. McIntosh group. Although these studies suggest that high levels of inoculum are potentially available in

the orchard, they do not describe the numbers of viable conidia present on the leaf surface that could be available for new infections.

As reported by MacHardy (4), inoculum dose was shown by Cook and by Palm to be important in determining levels of apple scab infection. Cook found a linear relationship between disease levels and dose up to 80×10^3 conidia per shoot, whereas Palm, using inoculum concentrations ranging from 0.5 to 100×10^3 conidia/ml, obtained increases in disease levels up to a dose of about 50×10^3 conidia/ml, after which the disease levels did not increase further. Temperature relationships to inoculum dose were not described in these experiments.

Employing conidial inoculum, Schwabe (9) studied the relationship between disease development and duration of leaf wetness at five different temperatures using an inoculum dose of between 28.8 and 39.5×10^3 conidia/ml. Scab development increases were generally correlated with increases in leaf wetness duration. Keitt and Jones (3) observed more lesions with increased wetting and surmised that longer periods of wetting increase the chance of infection by less favorably placed conidia or those that germinate in a direction not leading to early contact with the cuticle.

Using apple seedlings and conidia of *V. inaequalis*, we report the relationship between inoculum dose, temperature, and disease development for a leaf wetness duration time equal to the C3 curve of Parisi et al. (7), and the relationship between duration of leaf wetness, temperature, and disease development for a single, low inoculum dose.

MATERIALS AND METHODS

Plants. To provide uniform and susceptible plants, apple (*Malus domestica*) seedlings derived from the cross 'Idared' × 'Golden Delicious' were started in the greenhouse and grown at 20 to 25°C under ambient light for about 2 months. From September through May, actively growing plants with 8 to 10 unfurled, fully or partly expanded leaves were used in all experiments.

Inoculum. A native isolate of *V. inaequalis* indigenous to the Anjou region of France was used in all experiments. Scab-infected apple ('Idared' × 'Golden Delicious') leaves were dried and stored frozen

Corresponding author: J. R. Hartman
E-mail: jhartman@ca.uky.edu

This work was partly supported by a grant from the P.R.I.A., Angers, France.

Accepted for publication 2 March 1999.

Publication no. D-1999-0405-03R
© 1999 The American Phytopathological Society

as a source of inoculum. Conidial suspensions were obtained by agitating infected leaves in distilled water and filtering them through four layers of cheesecloth to remove leaf debris.

For the inoculum dose experiments, fresh stock suspensions were prepared for each experiment, and conidial concentrations were adjusted to 250×10^3 conidia/ml using a Malassez cell (hemacytometer). A cascade dilution scheme was used to obtain additional suspensions intended to contain 100, 50, 25, 10, and 2.5×10^3 conidia/ml. By actual count, however, conidial suspensions averaged 250, 81.2, 32.2, 15.6, 5.4, and 1.5×10^3 conidia/ml, respectively. For the leaf wetness duration experiments, conidial suspensions containing 10×10^3 conidia/ml were prepared and verified by actual count.

To determine inoculum viability for each experiment, six droplets of conidial suspension were placed at well-spaced intervals on malt agar and their locations were noted. The plates were incubated in the same chamber where plants were being held for infection experiments; afterwards, the percent germination of at least 200 conidia representing all of the droplets was determined microscopically.

Inoculation, infection, and incubation.

Conidial suspensions were uniformly applied with a hand-held sprayer over all leaf surfaces of groups of six apple seedlings at

a rate of 25 ml/group. For each experiment at any given temperature, 36 plants were inoculated (six plants each with six different inoculum doses or with six different wetness durations). At any given temperature, the six seedlings collectively represented one replicate for each inoculum dose or leaf wetness duration treatment, and these experiments were repeated five or six times over a several month period.

Inoculated plants were immediately placed in a temperature-controlled (6, 11, 16, or 22°C) chamber or phytotron (Froids et Mesures, Beaucoz, France) equipped with a humidifier or fogger to keep the leaves wet without runoff. Temperatures were continuously monitored at six locations in the chamber using a Campbell 21 Datalogger (Campbell Scientific Ltd., Shepshed, United Kingdom) connected to thermocouple temperature sensors in contact with the surface of representative leaves. Leaf wetness was monitored intermittently by visual observation. For the inoculum dose experiments, plants were kept moist and in the dark for the duration of leaf wetness appropriate to the temperature according to the 1.0 C3 curve (7; Table 1). For the leaf wetness duration experiments, plants were kept moist for different time spans ranging from 0.6 to 2.0 times the C3 curve; the exact values are given in Table 1. For all experiments, 15 min before the end of the desired infection

period, plants were removed and dried for 30 min using an electric heater-fan delivering slow-moving air at 19 to 21°C.

After drying, plants were placed in a growth chamber maintained at 20°C with 16 h of light per day at 70 $\mu\text{E/s/m}$ light intensity and grown for 11 to 14 days while symptoms developed.

Evaluation. After symptom development, plants were evaluated for disease by counting and recording the number of apple scab lesions per plant. The data were analyzed using multiple regression analysis. A log transformation of the response variable was necessary to stabilize nonconstant error variances. For each experiment, values for R^2 and adjusted R^2 , mean square error, and regression equations were determined.

RESULTS

Inoculum dose experiments. Disease incidence increased with increasing inoculum doses up to about 100×10^3 conidia/ml (Fig. 1). The curves for disease incidence based on number of lesions per plant were approximately the same for experiments done at 6, 11, and 16°C. The disease incidence at each of these three temperatures was high, reaching more than 100 lesions/plant (Fig. 1). The disease incidence at 22°C was consistently lower than the incidence at the other temperatures.

The influences of temperature and inoculum dose on lesions per plant were best described by the equation $Y = 0.833 - 0.026T + 0.005ID - 3.814/ID + 0.362DL - 0.003JD$, where Y is the natural logarithm of (lesions/plant + 1), T is the temperature during the time of infection, ID is the number of conidia/ml applied to the plants, DL is the day length or photoperiod (light) at the time of year that inoculations were made (which reflects photoperiod status of greenhouse grown seedlings), and JD is the Julian date or day of the year (1996) or day of the year + 365 (1997) that inoculations were made. The R^2 and adjusted R^2 were 0.7508 and 0.7413, respectively, and the model mean square and error mean square were 229.47 and 76.18, respectively. The estimated regression coefficients were each significant at the 0.05 level or less. By substituting constants for the two variables DL and JD , this equation may be used to calculate relative disease levels at temperatures or inoculum doses within the range of those used in the experiment.

Leaf wetness duration experiments. At all four temperatures, disease incidence increased with increasing duration of leaf wetness (10×10^3 conidia/ml inoculum dose) over the range of 0.6 to 2.0 times the C3 curve leaf wetness time (Fig. 2). The number of spots per plant generally doubled with a 1.5 to 2.0 increase of the C3 leaf wetness duration. Low but significant disease at times of leaf wetness below the C3 curve were observed.

Table 1. Duration of leaf wetness values at the four temperatures included in this study^a

Proportion of C3	Temperature			
	6°C	11°C	16°C	22°C
0.75 C3	15h36 (0.6 C3)	9h48	7h54 (0.8 C3)	5h06
C3	26h00	13h04	9h52	6h48
1.25 C3	20h48 (0.8 C3)	16h20	12h20	8h30
1.5 C3	31h12 (1.2 C3)	19h36	14h48	10h12
1.75 C3	41h36 (1.6 C3)	22h52	17h16	11h54
2.0 C3	52h00	26h08	19h44	13h36

^a Minimum number of hours of wetness required for light infection of apple seedlings by scab conidia is defined by the row labeled C3; other rows give wetness values proportionally lower and higher than the C3 baseline. Times are given in hours (h) and minutes.

Fig. 1. Effect of conidial inoculum dose and infection temperature on apple scab using the C3 (7) leaf wetness duration. At each temperature, times for leaf wetness duration are given in hours (h) and minutes.

The influences of temperature and leaf wetness duration on lesions per plant were best described by the equation $Y = 0.083 - 0.043T - 0.225(LWD)^3 - 5.586/LWD + 0.653DL - 0.003JD$, where Y is the natural logarithm of (lesions/plant + 1), T is the temperature during the time of infection, LWD is the leaf wetness duration as a proportion of the C3 curve, and DL is the day length or photoperiod (light) at the time of year that inoculations were made (which reflects photoperiod status of greenhouse-grown seedlings). The R^2 and adjusted R^2 were 0.8319 and 0.8264, respectively, and the model mean square and error mean square were 233.51 and 47.17, respectively. The estimated regression coefficients were each significant at the 0.05 level or less. By substituting a constant for the variable DL , this equation may be used to calculate relative disease levels at temperatures or leaf wetness times within the range of those used in the experiment.

DISCUSSION

At four different temperatures, as inoculum dose increases up to about 80×10^3 conidia/ml, disease incidence also increases. As reported by MacHardy (4), this confirms the results of Cook done at one temperature, and of Palm at one temperature with the cv. Boskoop. Palm's experiments with the cvs. Gloster and Golden Delicious, however, indicate a leveling off of disease after only 30 to 50×10^3 conidia/ml. When using the C3 curve for leaf wetness duration, very high disease levels are obtained with a high inoculum dose and low disease levels are observed if the inoculum is low. The question of whether curves like C3, obtained with high inoculum in controlled environmental conditions, are valid when applied to orchard conditions remains unresolved.

Nevertheless, the influence of inoculum dose may be important to continued scab disease development. At some point, primary and secondary infections in the orchard may be so severe that an apple crop is lost. However, before that point is reached, and after ascospore production has been exhausted, to prevent further damage to the crop, apple scab predictive and warning systems for secondary infections may benefit from incorporation of conidial inoculum dose into the design of such systems.

The lower disease severities observed at 22°C than at 6, 12, and 18°C in the inoculum dose experiments may be an artifact of the condition of the plants obtained from the greenhouse. During the course of this study, typically five or six replications of the experiments at the four temperatures were done from September to May. We determined that, across all temperatures, plants produced in the greenhouse during the periods of shortest day length were less susceptible to apple scab than plants produced at other times of the year (J. R.

Hartman, unpublished data). In this study, a disproportionate number of experimental replications done at 22°C made use of these winter-grown plants. These plants typically had two leaves that developed fewer lesions, while the other plants had three or more heavily scabbed leaves. Thus, the results at 22°C show lower disease incidence. Nevertheless, the pattern of the relationship between inoculum dose and infection levels was consistent with those obtained at other temperatures. Regression analysis taking day length and time of year into account reaffirms this pattern.

The inoculum doses used in our experiments, especially in the lower ranges, are similar to those that might be found in the orchard (1,12). Our highest inoculum dose of 250×10^3 conidia/ml is probably not frequently observed in commercial orchards; moreover, under orchard conditions, inoculum is not so uniformly applied to all foliage surfaces as was done in our experiments. The dose of 10×10^3 conidia/ml, used in the second experiment, is a relatively low dose for experimental work, but probably represents a more realistic orchard dose. Using the C3 curve for wetness duration, this dose yielded few infections. However, with an increased wetness duration, a high infection level was reached, showing clearly that there is an interaction, confirmed by regression analysis, between these two parameters.

We found that, as leaf wetness duration is increased up to about 1.5 to 2.0 times the C3 curve, disease incidence also increases, a result also supported by regression analysis. Doubling C3 curve leaf wetness duration resulted in a high level of disease, similar to that obtained with a higher inoculum (250×10^3 conidia/ml) and shorter wetness period (1.0 C3).

Schwabe (9) also studied the relationship between scab infection and leaf wetness duration using temperatures of 3.9, 8.5, 14.5, 20, and 25.2°C. Conidial inoculum doses varied between 28.8 and 39.5×10^3 per ml on potted 1- to 4-year-old cv.

MM 106 apple trees. Although increases in scab development occurred at first with increased leaf wetness duration, further increases in leaf wetness duration did not result in increased scab development. In comparison with our results, scab infection levels reported by Schwabe at comparable temperatures were lower. For example, at 14.5°C, leaf wetness duration for a 1.0 C3 level of infection would be 10.64 h; at this time, Schwabe reports very low infection levels. However, levels rapidly increase, so that by 2.0 C3 (21 to 22 h), they are four to five times the 1.0 C3 levels. After 2.0 C3, the infection levels show no further increases. In our work at 11, 16, and 22°C, there are moderate levels of infection at 1.0 C3, and numbers of lesions per plant increase two- to fourfold by 1.5 C3 and level off from there to 2.0 C3. Thus, the patterns are the same, but perhaps shifted. Our apple seedlings probably are more susceptible than the potted apple trees of Schwabe, a possibility suggested by Parisi et al. (7). Also, we use different disease rating systems, which could influence how these results are interpreted. This emphasizes the importance of field validation of these results before incorporation into a risk prediction system.

Our work clearly shows that inoculum dose and leaf wetness duration both have an effect on disease incidence and may need to be taken into account to make disease warning systems for secondary infections more precise. Knowing the relationship between inoculum dose and disease levels could be used to predict risks of continued secondary infections in apple orchards where the disease has already become established. Research is needed to determine what levels of inoculum could be expected in apple orchards with defined levels of infection. Knowledge of varietal susceptibility and differences in numbers of conidia produced on them is also needed.

In our experiments, the number of leaves with lesions increased with inoculum dose and with leaf wetness duration up to about

Fig. 2. Effect of leaf wetness duration as a proportion of C3 (7) and infection temperature on apple scab following inoculation (10×10^3 conidia/ml).

3 leaves (of the 8 to 10 available) per plant (J. R. Hartman, *unpublished data*). This result is consistent with the concept that leaf susceptibility is influenced by their age and stage of growth (3,8,10). However, to obtain maximum disease levels on all susceptible leaves, high inoculum doses or long leaf wetness periods are required. Thus, leaf susceptibility is another important factor to be considered for scab risk prediction systems. The results of our experiments may contribute to development of improved secondary scab risk models.

ACKNOWLEDGMENTS

We thank S. Rousseau for her helpful technical assistance; and M. Barker and M. Smith for their help with statistics.

LITERATURE CITED

1. Albert, J. J., and Lewis, F. H. 1962. Effect of repeated applications of dodine and of captan on apple scab foliage lesions. *Plant Dis. Rep.* 46:163-167.
2. Jones, A. L., Lillevik, S. L., Fisher, P. D., and Stebbins, T. C. 1980. A microcomputer-based instrument to predict primary apple scab infection periods. *Plant Dis.* 64:69-72.
3. Keitt, G. W., and Jones, I. K. 1926. Studies of the epidemiology and control of apple scab. *Wis. Agric. Exp. Stn. Bull.* 73.
4. MacHardy, W. E. 1996. Apple Scab: Biology, Epidemiology, and Management. American Phytopathological Society, St. Paul, MN.
5. MacHardy, W. E., and Gadoury, D. M. 1989. A revision of Mill's criteria for predicting apple scab infection periods. *Phytopathology* 79:304-310.
6. Mills, W. D. 1944. Efficient use of sulfur dusts and sprays during rain to control apple scab. *Cornell Ext. Bull.* 630.
7. Parisi L., Bautrais P., Guillaumès J., Decoret, A., and Huber L. 1997. Conidial infection of apple plants by *Venturia inaequalis* in controlled environmental conditions. Pages 176-179 in: *Proc. 4th Workshop Integrated Control Pome Fruit Dis.* A. M. Berrie, ed. Croydon, England. IOBC/WPRS Bulletin Vol. 20(9):176-179.
8. Schwabe, W. F. S. 1979. Changes in scab susceptibility of apple leaves as influenced by age. *Phytophylactica* 11:53-56.
9. Schwabe, W. F. S. 1980. Wetting and temperature requirements for apple leaf infection by *Venturia inaequalis* in South Africa. *Phytophylactica* 12:69-80.
10. Smith, C. A., and MacHardy, W. E. 1993. The production of conidia by *Venturia inaequalis* on 7 apple cultivars. (Abstr.) *Phytopathology* 83:247.
11. Stensvand, A., Gadoury, D. M., Amundsen, T., Semb, L., and Seem, R. C. 1997. Ascospore release and infection of apple leaves by conidia and ascospores of *Venturia inaequalis* at low temperatures. *Phytopathology* 87:1046-1053.
12. Sutton, T. B., Jones, A. L., and Nelson, L. A. 1976. Factors affecting dispersal of conidia of the apple scab fungus. *Phytopathology* 66:1313-1317.