A Model for Predicting Ascospore Maturation of *Venturia inaequalis*

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ABSTRACT

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A model was developed to predict pseudothecial development of *Venturia inaequalis* from the time asci begin to develop until ascospores mature. An equation, $\hat{y} = 0.0031 + 0.0546\,(\text{TEMP}) - 0.00175\,(\text{TEMP})^2$ (equation 1) in which $\hat{y}$ = daily change in pseudothecial development and TEMP = temperature (C), was developed from laboratory incubation studies to predict daily change in pseudothecial development when moisture was not a limiting factor. February 1 was used as a biofix date (biological reference date) to initiate the model in North Carolina. Pseudothecial development was best described by using equation 1 and daily average temperature and threshold levels of rainfall $\geqslant$ 0.25 mm or hours of 100% relative humidity $\geqslant$ 12 as indicators of leaf wetness. If daily average temperature $\leqslant$ 0 C, or if rainfall $\leqslant$ 0.25 mm and hr of 100% relative humidity $\leqslant$ 12, there was no predicted increase in stage of development. Predicted stage (st) of development = $\Sigma\hat{y}$ + st 5, in which st 5 is the overwintering dormant stage and st 12 is when the ascospores are mature. The $\hat{y}$'s are summed over time. Equation 1 was also evaluated on an hourly basis by dividing all regression coefficients by 24, yielding the equation $\hat{y} = 0.00013 + 0.0022\,(\text{TEMP}) - 0.0000729\,(\text{TEMP})^2$ (equation 4). Equation 4 best described pseudothecial development by using hourly temperature and relative humidity $\geqslant$ 85% as the threshold moisture level. Equations 1 and 4 were tested at three overwintering sites in NC during a 3-yr field study.

Additional key words: apple scab, epidemiology, *Malus sylvestris*.

In 1974, Massie and Szkolnik (4) developed a model from 17 yr of field data to predict the maturity of *Venturia inaequalis* (Cke.) Wint. ascospores. The model satisfactorily predicted ascospore maturity in the Geneva, NY, area. However, in North Carolina the model predicts ascospore maturity much earlier than it occurs in nature (7). Its failure in NC was apparently due to certain aspects of the biology of *V. inaequalis* not taken into account by the model (eg, dormancy [2]) as well as the limited environmental data base from which it was derived.

In 1977, we initiated a study to quantify the environmental factors favoring pseudothecial development of *V. inaequalis* (2). Pseudothecial ontogeny of *V. inaequalis* could be separated into two distinct phases. Ascogonia developed after leaf fall until the lumina of the pseudothecia were filled with pseudoparaphyses. Development of asci and ascospores was initiated in the spring only after a dormant period during which no development was discernible in the lumina of the pseudothecia. Laboratory and field observations indicated that the dormant period lasts ~45 days and dormancy requirements appeared to be met by approximately 1 February in NC. Pseudothecia were capable of rapid maturation during periods of favorable temperature and moisture after that date.

Moisture was the limiting factor for development of *V. inaequalis* (2). In laboratory studies, no pseudothecial development occurred in air-dried apple leaves and in the field pseudothecial development was most highly correlated with rainfall or high relative humidity. In leaves in which moisture was not limiting, temperature had a major influence on pseudothecial development. The optimum temperature range for ascogonial development was 8–12 C; 16–18 C was the optimum range for ascospore maturation. Little pseudothecial development occurred at 0 C.

In NC, temperature and moisture had a greater influence on pseudothecial maturation during February, March, and April than temperature and moisture during October, November, and December. Date of leaf fall or environmental conditions in the fall had little or no influence on date of ascospore maturation the following spring (2).

This paper describes two models which predict pseudothecial development of *V. inaequalis* under North Carolina conditions.

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MATERIALS AND METHODS

Data used in deriving and testing the model. Environmental data and data on pseudothecial maturation were obtained as previously described (2). Data used in model development were from the Mountain Horticultural Crops Research Station at Fletcher, NC (MHCRS, 1978–1980); Boone, NC (BOONE, 1978–1980); the Walter Pace Orchard at Saluda, NC (PACE, 1978) and near the North Carolina State University Campus at Raleigh (NCSU, 1980). In addition to temperature (TEMP), relative humidity (RH), and rainfall (RAIN), leaf wetness was measured during 1979 and 1980 at MHCRS and during 1980 at NCSU with a DeWit leaf wetness meter (Valley Stream Farms, Orono, Canada L0B 1M0).

Stages (st) of pseudothecial ontogeny (2) referred to in this paper are: st 5—lumen of the pseudothecium filled with pseudoparaphyses; st 6—appearance of asci; st 7—asci about one-half mature size; st 8—asci formed, but contents not differentiated; st 9—asci with spores in the process of formation; st 10—asci with ascospores being formed, usually septate; st 11—asci with ascospores formed, but not pigmented; st 12—ascospores pigmented and mature; and st 13—asci empty.

To relate mean stage of pseudothecial development to percent mature ascospores, at each sample date during the 3-yr field study, the logit transformation, $\log_e x/(1\text{-}x)$, in which x = proportion of mature ascospores at each sample date, was regressed with mean stage of pseudothecial development. Regression analysis yielded the equation $\hat{y}$ = 1.6 (mean stage) − 16.6 with an R^2 value for goodness-of-fit of 0.86 ($P = 0.01$).

Model development. Because environmental conditions in the fall or date of leaf fall had no influence on date of ascospore maturation, we decided to model pseudothecial development of *V. inaequalis* during the late winter and early spring. Rates of pseudothecial development during daily periods were derived for 2-wk incubation periods (2) by subtracting the mean stage of the field control at the beginning of each incubation period from the final stage at the end of the incubation period and dividing the difference by the number of days in the incubation period (14 days). Regression analyses were used to describe daily rate of pseudothecial development during each 2-wk incubation period as a function of temperature. Because the rate of development during the 2-wk incubation periods was so rapid, it was difficult to determine the rate of pseudothecial development during each of the various stages. Therefore, a constant rate of development during st 6–12 was assumed.

Equations for daily rate of pseudothecial development from incubation periods beginning on 6 March 1978 and 26 February 1979 were chosen to predict pseudothecial development during st 6–12. During these two incubation periods, even though pseudothecia had developed beyond the dormancy period, maturation into st 12 was not extensive. These equations had the highest R^2 values for goodness-of-fit between daily rate of pseudothecial development and temperature when moisture was not limiting. They are:

$$\hat{y} = 0.0031 + 0.0546\,(\mathrm{TEMP}) - 0.00175\,(\mathrm{TEMP})^2 \qquad (1)$$

and

$$\hat{y} = 0.0370 + 0.0599\,(\mathrm{TEMP}) - 0.00255\,(\mathrm{TEMP})^2 \qquad (2)$$

in which $\hat{y}$ = predicted daily change in pseudothecial stage and TEMP = degrees Celsius. The coefficients of determination for equations 1 and 2 were 0.60 and 0.63, respectively. All coefficients were significantly different from 0 at $P = 0.01$. Equations 1 and 2 predict daily change in pseudothecial development as a function of temperature when moisture is not limiting. Equations 1 and 2 were tested with MHCRS environmental data during the spring of 1978 and 1979 to determine what measures of moisture in the field reflect that of wet leaves in the laboratory.

Based on results of the previous study (2), equations 1 and 2 were first tested by using daily average TEMP (AVGTEMP) and different measures of daily RAIN (millimeters) and/or hours of RH ≥ 100% (RH100) per day as threshold moisture levels. If daily AVGTEMP < 0 C or if moisture was less than threshold levels, then no increase in stage of development was predicted.

For initial equation evaluation, 1 February was used as a biofix date (biological reference date) for NC. On 1 February in NC, pseudothecia are usually in st 5; however, laboratory and field studies during 1978 and 1979 indicated the dormancy requirements had been met and pseudothecia are capable of development under favorable temperature and moisture conditions.

After 1 February, the predicted stage of pseudothecial development (Y) is determined by

$$Y = \Sigma\hat{y} + \mathrm{st}\ 5, \qquad (3)$$

in which $\hat{y}$ = daily predicted change in pseudothecial development and st 5 is the overwintering stage. For each combination of environmental variables, goodness-of-fit (R^2) was determined by comparing observed and predicted stages of pseudothecial development.

Equation 1 was also evaluated based on hourly determinations of temperature when threshold moisture requirements were fulfilled. An hourly rate equation was derived from equation 1 by dividing regression coefficients by 24, yielding the equation

$$\hat{y} = 0.00013 + 0.0022\,(\mathrm{TEMP}) - 0.0000729\,(\mathrm{TEMP})^2 \qquad (4)$$

Equation 4 was tested with MHCRS environmental data during the springs of 1979 and 1980, using hourly TEMP and the following indirect measures of leaf wetness as threshold moisture values: leaf wetness from rain, fog, or dew, as determined by a DeWit leaf wetness meter (WET 1); leaf wetness from rainfall as determined by the DeWit leaf wetness meter (WET 2); RH = 100; RH ≥ 95; RH ≥ 90; RH ≥ 85; and RH ≥ 80. If hourly TEMP < 0 C or if moisture measurements were less than threshold levels, then there was no predicted increase in stage of development. Predicted stage of pseudothecial development is determined by

$$\Sigma\hat{y} + \mathrm{st}\ 5.$$

For each combination of environmental variables, goodness-of-fit (R^2) was also determined.

RESULTS

Evaluation of equations 1 and 2. Predicted stage of pseudothecial development with different threshold values of RAIN and RH100 at MHCRS during the spring of 1978 by using equation 1 are given in Table 1. During a cold, dry February and early March, all the models correctly predicted no early pseudothecial development. Therefore, only predicted stages of development after 3 April were compared with observed stages of the field check.

Varying threshold levels of RAIN had the largest effect on predicted values (Table 1). Values of R^2 ($P = 0.01$) for RAIN ≥ 0.25, 2.54, or 12.7 mm were 0.83, 0.69, and 0.40, respectively. The addition of threshold levels of RH100 ≥ 8 hr or RH100 ≥ 12 hr to the threshold levels of RAIN markedly increased the accuracy of prediction. Daily RH100 ≥ 12 hr were normally associated with rainy periods in the field.

Using equation 1 at MHCRS during 1978, predicted values best fitted observed values when threshold levels of moisture were RAIN ≥ 0.25 mm or RH ≥ 12 hr ($R^2 = 0.90$, $P = 0.01$) (Table 1; Fig. 1). During 1978, similar predictive values were obtained using equation 2 with daily AVGTEMP and varying threshold levels of

TABLE 1. Comparisons of observed and predicted values of pseudothecial developmental stages[a] of *Venturia inaequalis* obtained by using equation 1 and different threshold levels of RAIN (mm) and RH100 during 1978 at MHCRS

Date	Obs. devel. stage	Rain ≥0.25	Rain ≥2.54	Rain ≥12.7	Rain ≥0.25 or RH100 ≥ 8 hr	Rain ≥0.25 or RH100 ≥12 hr	Rain ≥2.54 or RH100 ≥8 hr	Rain ≥2.54 or RH100 ≥12 hr	Rain ≥12.7 or RH100 ≥8 hr	Rain ≥12.7 or RH100 ≥12 hr
6 Feb	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
20 Feb	5.0	5.0	5.0	5.0	5.2	5.2	5.2	5.2	5.2	5.2
6 Mar	5.0	5.0	5.0	5.0	5.3	5.3	5.3	5.2	5.2	5.2
20 Mar	5.3	6.0	6.1	5.2	6.9	6.5	6.7	6.3	6.2	5.7
3 Apr	6.3	7.0	6.8	6.0	7.9	7.5	7.7	7.3	7.3	6.7
17 Apr	8.2	7.4	6.8	6.0	8.3	7.9	7.7	7.7	7.7	7.2
1 May	10.1	8.4	7.6	6.7	9.8	9.4	9.3	8.9	8.9	7.9
15 May	12.8	11.3	10.4	8.4	13.4	12.6	12.2	11.7	12.1	10.8
R^{2}[b]		.83	.69	.40	.88	.90	.86	.87	.88	.77

[a] See text and (2) for definition of stages. Equation 1: $\hat{y} = 0.0031 + 0.0546(\mathrm{TEMP}) - 0.00175(\mathrm{TEMP})^2$ in which $\hat{y}$ = predicted stage and TEMP = temperature (C).

[b] R^2 value for goodness-of-fit between observed and predicted stage of pseudothecial development for 3 and 17 April and 1 and 15 May.

RAIN and RH100. Pseudothecial development at MHCRS during 1979 was also best described by equation 1 (Fig. 2) and equation 2 by using daily AVGTEMP and threshold levels of moisture of RAIN $\geqslant$ 0.25 mm or RH100 $\geqslant$ 12 hr.

Pseudothecial development of *V. inaequalis* at MHCRS during 1979 was best described by equation 4 when RH85 was used as an indication of leaf moisture (Table 2). R^2 values for goodness-of-fit between observed and predicted values of pseudothecial development determined by using equation 4 and hourly threshold values of RH85 were 0.97 and 0.99, respectively, for MHCRS 1979 and 1980 (Figs. 2 and 3; Table 3).

Model validation and use. Because no significant difference was observed between equations 1 and 2, equation 1 was chosen for further evaluation. To establish validity of the model, predicted values of st of development using equation 1 were compared to observed ratings of pseudothecial development at the overwintering sites during the 3-yr field study except for MHCRS

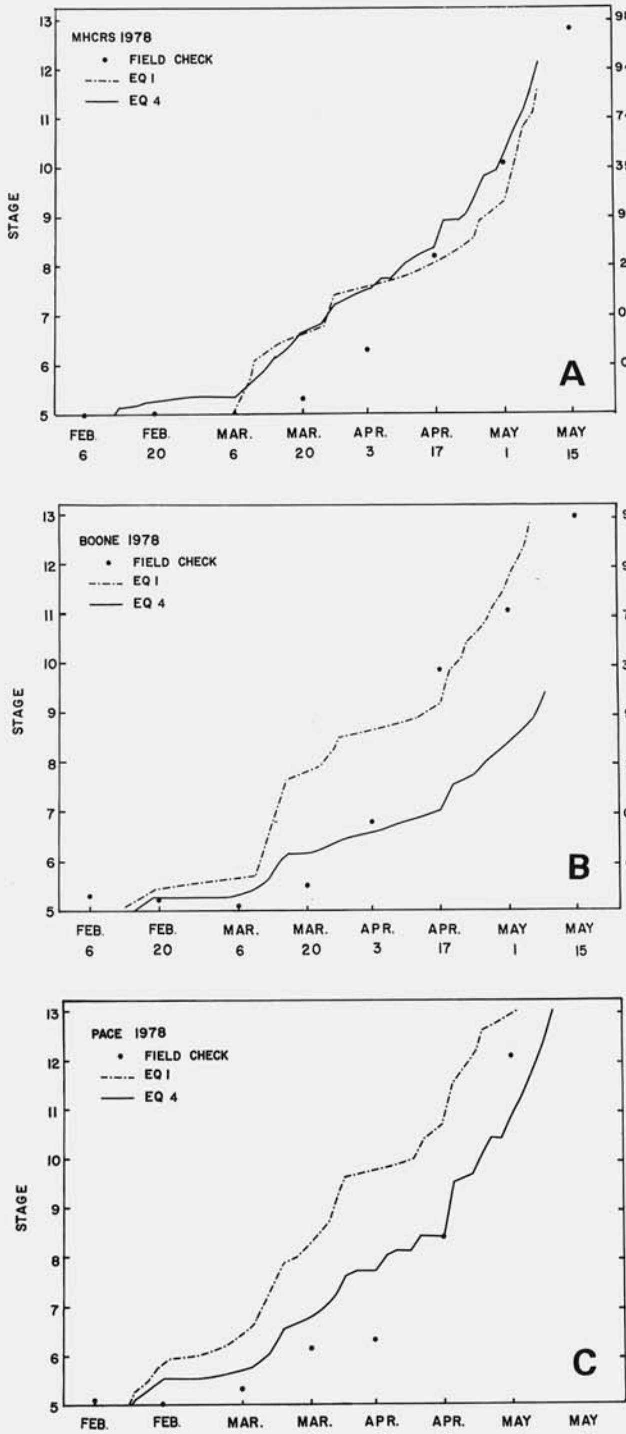

Fig. 1. Comparison of observed and predicted values of pseudothecial development of *Venturia inaequalis* at MHCRS, BOONE, and PACE during the spring of 1978.

TABLE 2. Comparisons of observed and predicted values of pseudothecial developmental stages[a] of *Venturia inaequalis* using equation 4 and different threshold levels of wetting during 1979 at MHCRS[b]

Date	Obs. stage	WET 2[b]	WET 1 + WET 2	RH 100	RH 95	RH 90	RH 85	RH 80
12 Feb	5.0	5.0	5.2	5.1	5.1	5.2	5.2	5.3
20 Feb	6.0	6.1	6.6	6.4	6.5	6.7	6.9	7.0
12 Mar	8.3	7.1	7.6	7.3	7.5	8.0	8.2	8.4
26 Mar	9.9	7.7	8.5	8.0	8.3	8.8	9.2	9.6
9 Apr	11.6	8.7	10.4	9.4	10.0	10.9	11.5	12.1
23 Apr	13.0	9.5	12.0	10.7	11.3	12.4	13.3	14.0
R^2[c]		0.60	.91	.76	.86	.96	.97	.91

[a] See text and (2) for definition of stages. Equation 4: $\hat{y} = 0.00013 + 0.0022\,(\text{TEMP}) - 0.0000729\,(\text{TEMPT})^2$ in which $\hat{y}$ = predicted stage and TEMP = temperature (C).

[b] WET 1 and WET 2 refer to leaf wetness as determined by a DeWit leaf wetness meter. WET 2 indicates leaf wetness during rain and WET 1 includes leaf wetness caused by dew, fog, etc.

[c] R^2 value for goodness-of-fit between observed and predicted stage of development.

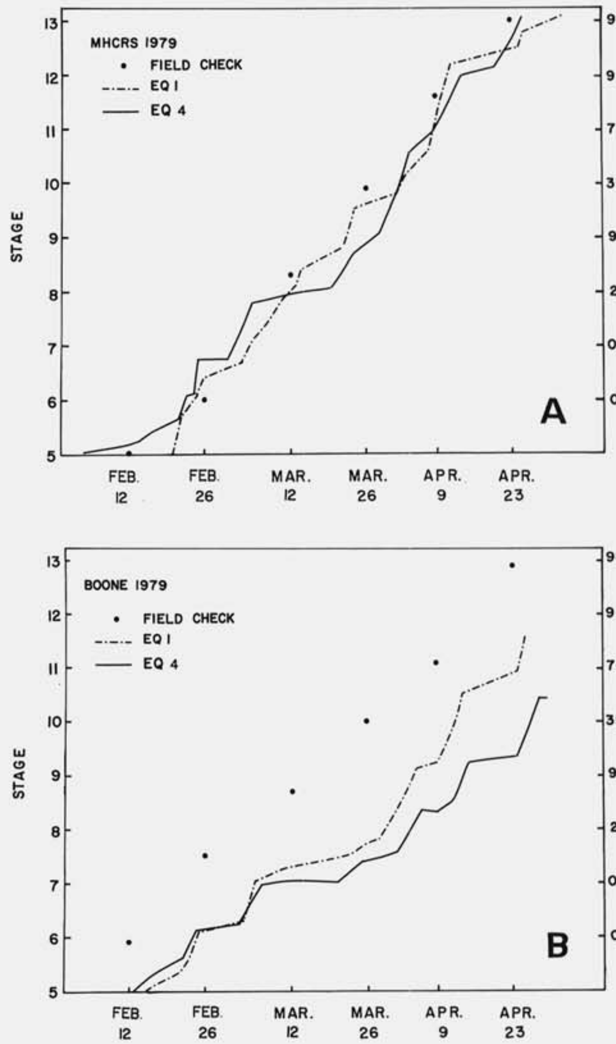

Fig. 2. Comparison of observed and predicted values of pseudothecial development of *Venturia inaequalis* at MHCRS and BOONE during the spring of 1979.

1978 and 1979. Predicted values from equation 4 were compared to observed ratings of pseudothecial development at sites other than MHCRS during 1979 and 1980.

R^2 values for goodness-of-fit between predicted and observed values of pseudothecial development at MHCRS and NCSU during 1980, and BOONE during 1978, were $\geqslant 0.90$ ($P = 0.01$) for equation 1 (Table 3). Equation 1 underpredicted pseudothecial development during 1979 and 1980 at BOONE (Figs. 2 and 3). At PACE during 1978, equation 1 overpredicted pseudothecial development during March and April (Fig. 1).

Equation 4 generally did not predict pseudothecial development as well as equation 1. R^2 values for goodness-of-fit between predicted and observed values of pseudothecial development were 0.93 and 0.87 ($P = 0.01$) for MHCRS and PACE during 1978, respectively (Table 3; Fig. 1). However, equation 4 underpredicted pseudothecial development at BOONE during all years (Table 3; Figs. 1, 2, and 3). Equation 4 also underpredicted pseudothecial development at NCSU during 1980 ($R^2 = 0.58$, Fig. 3).

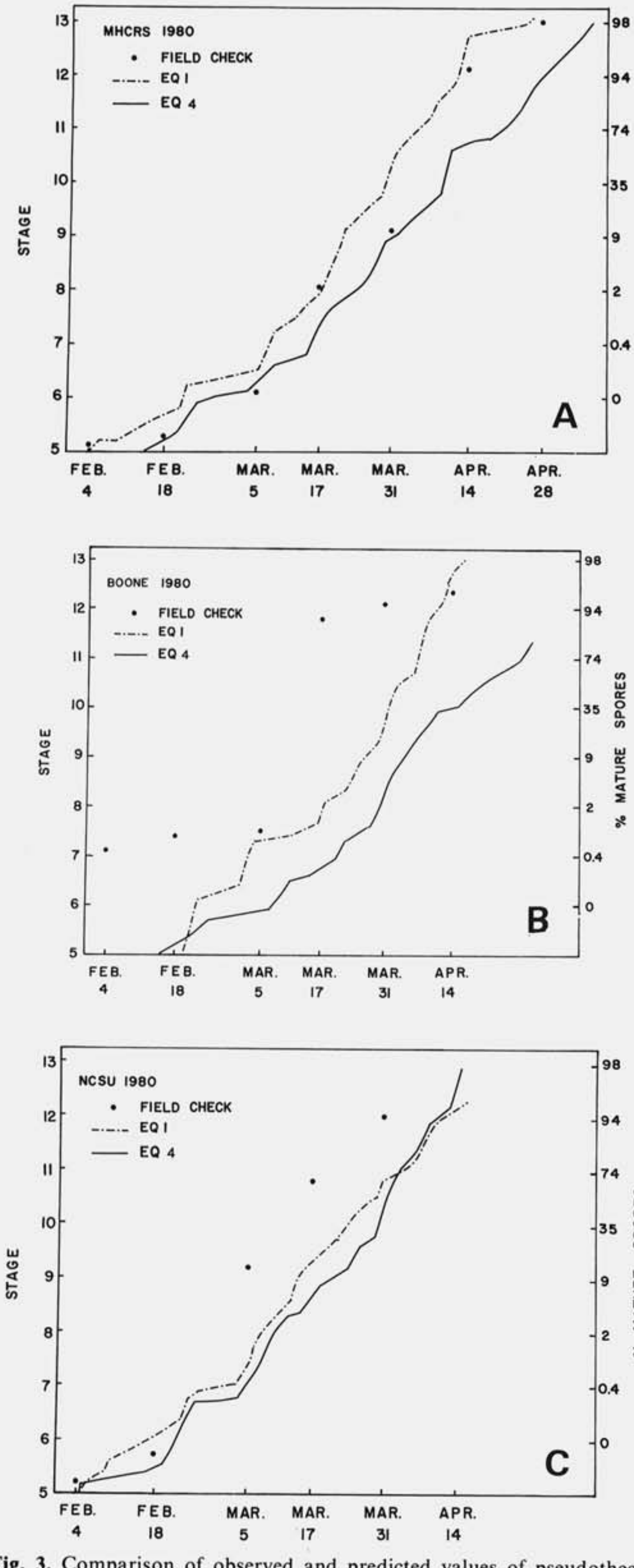

Fig. 3. Comparison of observed and predicted values of pseudothecial development of *Venturia inaequalis* at MHCRS, BOONE, and NCSU during the spring of 1980.

DISCUSSION

In this paper, two models for predicting pseudothecial development of *V. inaequalis* are presented. From these studies it was difficult to determine whether the model based on 24-hr periods or hourly periods was the better predictor and, therefore, both should be further evaluated. Because the developmental equations were derived from a broad data base (2), they should be applicable over a wide range of environmental conditions, assuming that other populations of *V. inaequalis* respond similarly to temperature and moisture.

For the most part, daily determinations provided a satisfactory prediction of stage of pseudothecial development. However, in the orchard, development is undoubtedly related to moisture availability and temperature during periods not necessarily delineated by calendar days. Equation 4 utilized hourly determination of moisture and temperature in order to predict pseudothecial development and should more accurately reflect the response of *V. inaequalis* to changes in temperature and moisture in the orchard.

Equation 1 satisfactorily predicted pseudothecial development at all locations except PACE in 1978, BOONE in 1979 and 1980 when RAIN $\geqslant 0.25$ and RH100 $\geqslant 12$. The lack of satisfactory prediction by equation 1 at BOONE during these 2 yr may be attributed to moisture availability due to melting snow. There was little snow cover at the other sites. During 1980 at BOONE, pseudothecia developed out of the dormancy period (st 5) in mid-January and a mean stage of 7.1 was observed on 4 February. There was no apparent explanation for the appearance of st 7 so early in the season, but subsequent development could be related to it.

Predicted stage of pseudothecial development obtained by using equation 4 with an hourly moisture threshold of RH85 was similar to that predicted by equation 1 at MHCRS during all 3 yr. At PACE, equation 4 was more accurate than equation 1 in 1978. However, at BOONE during 1978 and 1979 and BOONE and NCSU during 1980, equation 4 underpredicted the rate of pseudothecial development when compared to the field check and equation 1. The use of some measure of rainfall with hourly RH might improve predictions with equation 4.

The greatest difficulty encountered in evaluating the model was trying to determine what variables most accurately reflect leaf wetness and how they should be used in the model. Several indirect measures of leaf wetness were evaluated with equation 1; leaf

TABLE 3. R^2 values for goodness-of-fit between observed values of pseudothecial development of *Venturia inaequalis* and those predicted by equations 1 and 4 during the 3-yr field study[a]

Year	Site	Equation 1	Equation 4
1978	MHCRS	0.93	0.93
	BOONE	0.93	0.69
	PACE	0.63	0.87
1979	MHCRS	0.95	0.97
	BOONE	0.80	0.29
1980	MHCRS	0.96	0.99
	BOONE	0.73	0.00
	NCSU	0.91	0.58

[a] All values are significant, $P = 0.01$, except for those predicted by equation 4 at BOONE, NC in 1980.

wetness as determined by the DeWit sensor, RAIN, and threshold values of RH. RAIN $\geq$ 0.25 mm and/or 12 hr of 100% RH was selected as daily measures of leaf wetness because when used with equation 1 they provided the best estimate of stage of development. In addition, these two parameters would be readily obtainable in many apple-growing regions. Based on these tests, we do not believe rainfall alone is a satisfactory predictor of leaf wetness. A direct measure of leaf wetness should permit a more accurate prediction of pseudothecial development. Methods are available for determining leaf wetness on living leaves and could possibly be adapted to leaves on the orchard floor (5,6). A measure of moisture availability during melting snow might also improve the prediction of pseudothecial development where snow cover is common.

The selected biofix date was 1 February for model initiation in NC because previous studies (2) indicated that pseudothecia generally had met the dormancy requirements by then. However, until more is learned about the dormancy requirements and factors influencing it, the model could be initiated by collecting infected leaves, crushing pseudothecia, and determining the developmental stage. This is possible because a constant rate of pseudothecial development was assumed within each 2-wk period. We chose to use the model to predict pseudothecial maturity from 1 February and st 5; however, it could be used to predict development during shorter periods or specific developmental stages (eg, ascospore maturity). Short-term predictions should be more accurate and make the model more valuable as a management tool. Precise knowledge of ascospore maturity is important to scab management programs because fungicide sprays are often timed to coincide with first ascospore maturity and periods of peak maturity (1), and the spray program is relaxed when most of the spores have been released.

Because the developmental rate equations derived are not complex, they can be evaluated without the aid of a computer. They should also be easily adapted to field instruments for predicting disease development (3).

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