A Model to Estimate the Maturity of Ascospores of *Venturia inaequalis*

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Scientific Contribution 1123 from the New Hampshire Agricultural Experiment Station.

Portion of an M.S. thesis submitted by the senior author to the Graduate School, University of New Hampshire, Durham.

We thank John Sondej and Chang-cheng Hu, technicians, Department of Botany and Plant Pathology, University of New Hampshire, Durham; Eric Leadbeater and Russell Cross, NH apple growers; and Robert Seem, associate professor, Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva, for assistance in collecting data.

Accepted for publication 14 January 1982.

ABSTRACT

Gadoury, D. M., and MacHardy, W. E. 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. Phytopathology 72:901-904.

The rate of maturation of ascospores of *Venturia inaequalis* was directly proportional to temperatures ranging from 6 to 20 C. There was a linear relationship between the probit of ascospore maturity and degree-day accumulation (base = 0 C) from the first appearance of mature ascospores. This relationship allowed the formulation of a simple linear statistical model of ascospore maturation. The model can be used to estimate the cumulative percentage of matured ascospores, given the degree-day accumulation since the first appearance of mature ascospores. The model was verified by comparison to its component data sets and was validated with additional field and laboratory observations of ascospore maturity.

Additional key words: apple scab.

Models of pathogen, disease, and host development have become increasingly important in the management of apple scab. The advent of microprocessor (7) and computer (1,6) delivery systems will allow complex integration of models to aid both researchers and growers in disease management. This paper describes the formulation and applications of a pathogen development model suitable for use either in computerized systems or in graphic form.

The causal agent of apple scab, *Venturia inaequalis* (Cke.) Wint., overwinters as immature pseudothecia in the fallen leaves of apple (*Malus* spp.). In spring, the pseudothecia mature and ascospores are forcibly discharged when the leaves are wet by rain. The disease is generally controlled by the repeated application of fungicides, usually directed against the primary inoculum. A common principle shared by various management strategies is that the interval between fungicide applications is increased and/or fungicide rates are decreased once the supply of primary inoculum is thought to be exhausted. The more precisely the length of the primary infection season can be defined, the more efficiently fungicide applications can be scheduled. Estimation of ascospore maturity with a mathematical model was reported (11), but the model did not provide accurate estimates of ascospore maturity in tests performed in Michigan (11), New Hampshire (2), and North Carolina (13). Ascospore maturity can be assessed by examination of crushed pseudothecia (3,14), but such methods are too tedious and time consuming for use on more than a limited basis. Hence, in commercial apple orchards the timing and rate of fungicide applications have not been significantly based upon or equated to the supply of primary inoculum.

Our objective was to develop a mathematical model to accurately estimate ascospore maturity of *V. inaequalis* and to describe the model in forms usable by researchers and commercial apple growers.

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0031-949X/82/07090104/$03.00/0

MATERIALS AND METHODS

Formulation and verification of the model. Infected leaves were collected beneath unsprayed trees (cultivar McIntosh) during the autumns of 1978 and 1979, and were stored in wire-mesh baskets at the Mast Road Research Orchard in Durham, NH. On 24 March 1979 and on 13 April 1980, 2.5-cm squares were cut from the leaves and fastened between two rectangular pieces of fiberglass screen. The screen was then rolled and the ends fastened to form a cylinder. Leaf samples were incubated at 10, 15, and 20 C at 90 ± 5% RH in 1979 and at 6, 10, 15, and 20 C at 90 ± 5% RH in 1980. In 1980, leaf samples were also stored in moist chambers housed in standard U.S. Weather Service instrument shelters in each of three orchards near Contoocook, Londonderry, and Durham, New Hampshire. The Contoocook and Londonderry orchards were about 75 and 50 km, respectively, from Durham. A modified hygrothermograph (10) in each instrument shelter provided a continuous record of temperature, relative humidity, leaf wetness, and rainfall. To determine the cumulative proportion of ascospores matured, leaf samples were immersed in water at approximately weekly intervals as described by Gadoury and MacHardy (4). The matured ascospores were harvested and then counted. All treatments were replicated three times.

Ascospore maturity was also assessed by examination of crushed pseudothecia (3). Entire leaves were stored in wire-mesh baskets on the floor of the research orchard in Durham during the springs of 1979 and 1980. At approximately weekly intervals, 20 pseudothecia were removed from a sample of 10 leaves. The pseudothecia were crushed, examined microscopically, and the cumulative proportion of matured ascospores was calculated.

All data collected on ascospore maturation were combined and plotted against degree-day accumulation using base temperatures from 0 to 6 C, beginning with the first appearance of mature ascospores. Degree-day accumulation was calculated by computing the mean of the maximum and minimum daily temperatures. Linear regression analysis was used to select the base temperature that yielded the highest correlation between degree-day accumulation and ascospore maturation. A model using this base temperature was formulated to estimate ascospore maturity based on the accumulation of degree days from the first appearance of mature ascospores. Ascospore maturation at the various incubation temperatures, and at the field stations, was compared with ascospore maturity as estimated by the model.

Validation of the model. During the spring of 1981, screen-encased leaf samples were prepared as previously described and were stored in incubators at 6 and 12 C at 90 ± 5% RH and on the floor of research orchards in Durham, NH, and at the New York State Agricultural Experiment Station in Geneva. A 46 × 46-cm wooden shield was placed 61 cm above the field-stored leaf samples to prevent loss of ascospores during rain. At weekly intervals the leaf samples were immersed in water to release the matured ascospores (4). The harvested ascospores were fixed in an iodine solution and the spore suspensions were shipped to the University of New Hampshire for examination. All treatments were replicated three times. Ascospore maturity was also assessed at weekly intervals by examination of 20 crushed pseudothecia (3) removed from leaves stored at the research orchard in Durham.

RESULTS

The ascospore maturation rate increased as temperature increased from 6 to 20 C. The time required for the percentage of matured ascospores to increase from 1 to >95% was 68, 40, 27, and 22 days at 6, 10, 15, and 20 C, respectively. Curves of ascospore maturation plotted against degree-day accumulation were sigmoid and approximated cumulative normal distributions. A similar trend was evident when data from the various incubation temperatures and field stations were combined (Fig. 1). The highest correlation between ascospore maturation and degree-day accumulation (R^2 = 0.93) was obtained when the probit of ascospore maturity was plotted against degree-day accumulation calculated by using a base temperature of 0 C (Fig. 2). The model is defined as follows:

$$\hat{Y} = 2.51 + 0.01\ X$$
$$\hat{Y}' = \hat{Y} + 0.814$$
$$\hat{Y}'' = \hat{Y} - 0.814$$

in which $\hat{Y}$ = probit of proportion of matured ascospores, X = accumulated Celsius degree days from the first appearance of mature ascospores, $\hat{Y}'$ and $\hat{Y}''$ = upper and lower limits, respectively, of the 90% confidence bands (Bonferroni procedure) for estimated maturity of ascospores (12).

The model was analyzed in the linear form (Fig. 2), but data represented on the Y-axis were converted to the original units for the purpose of display (Fig. 3). In the linear form, the confidence

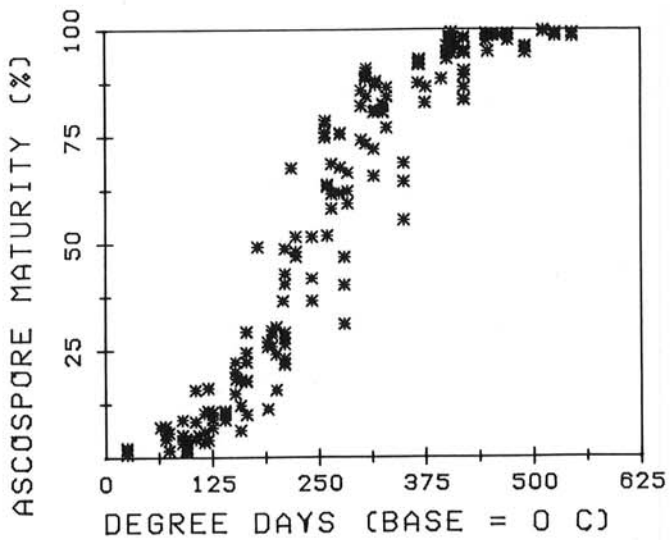

Fig. 1. Effect of degree-day accumulation on *Venturia inaequalis* ascospore development during 1979–1980 field and laboratory studies. The cumulative percentage of matured ascospores harvested from each leaf sample was plotted against degree days accumulated from the first appearance of mature ascospores.

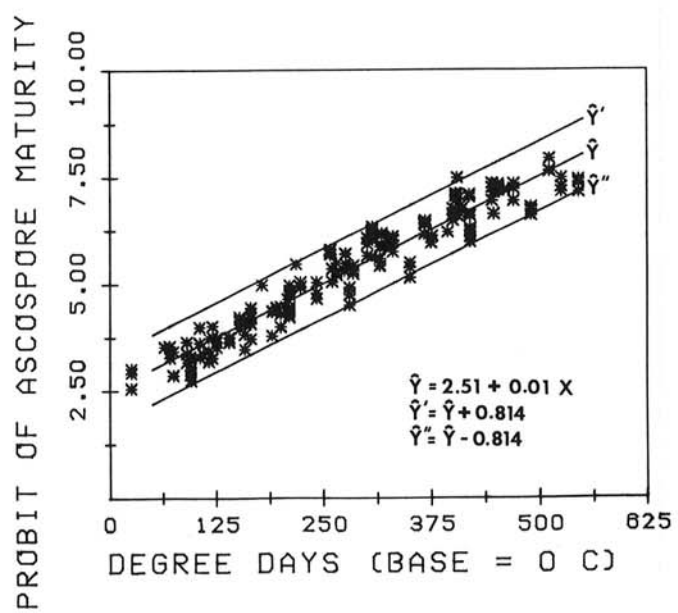

Fig. 2. Linear model of *Venturia inaequalis* ascospore maturation developed from data collected in 1979–1980 field and laboratory studies. Degree-day accumulation begins with the first appearance of mature ascospores. $\hat{Y}'$ and $\hat{Y}''$ are the upper and lower limits, respectively, of the 90% confidence bands (Bonferroni procedure). R^2 = 0.93.

bands are parallel to the regression line. However, deviation from estimated maturity, as indicated by the width of the confidence interval, actually decreases at the beginning and end of ascospore maturation (Fig. 3).

The data set upon which the model is based is composed of 12 subsets. Each subset describes ascospore maturation, as assessed by ascospore discharge or examination of crushed pseudothecia, either at a constant temperature between 6 and 20 C, or at one of the orchards used in the field studies. Ascospore maturity as described in the model subsets was compared to values estimated by the model. There was no significant deviation from estimated maturity at $P = 0.10$.

When the model estimations were compared to the 1981 field and laboratory observations of ascospore maturity, there was no significant deviation ($P = 0.10$) from estimated maturity in 43 of 49 observations (Fig. 4A and B). For the six observations in which deviation from estimated maturity was statistically significant, the difference between estimated and observed maturity never exceeded 5%.

DISCUSSION

To estimate the value of a parameter (eg, maturity) from units of an environmental factor, it was necessary to select a stage in pseudothecial development as the starting point for data accumulation; ie, a biofix. The environmental factor of concern in our study was temperature, expressed as degree-days. We chose the first appearance of mature ascospores as the biofix of our model for the following reasons. The optimum temperature for maturation of pseudothecia changes during winter from 10 C for early diameter increase to 20 C for ascospore maturation (4), indicating that the selection of a biofix that occurred prior to this change would complicate the formulation and application of a model based on degree-day accumulation with no guarantee of a commensurate increase in accuracy. We found, as have others (8,9,15), that temperature was directly proportional to the rate of ascospore maturation over the range of temperature commonly observed under natural conditions. Finally, the biofix could be accurately detected by simply inducing ascospore discharge (4) or by examination of crushed pseudothecia (3,14).

The linear statistical model of ascospore maturation is a regression equation that describes the relationship between degree-day accumulation and ascospore maturation. The 90% confidence bands were retained as a part of the model to allow estimation of deviation from expected maturity at various times during the primary infection season. Ascospore maturity was expressed as an estimate with upper and lower limits of confidence at $P = 0.10$. The confidence limits were quite wide during the middle of the primary infection season (Fig. 3). For example, 300 degree-days after the biofix the estimated maturity was 69.5% and the upper and lower limits were 90.5 and 38.1%, respectively. However, the model was more precise at the beginning and end of ascospore development. At degree-day 477, the estimated maturity was 98.9% while the upper and lower limits were 99.9 and 92.9%, respectively.

The mathematical description of the model will prove useful in research, but apple growers may find it difficult to transform estimated maturity values expressed in probits into more meaningful percentage values. However, growers could estimate ascospore maturity from degree-day accumulation values from their orchards if provided with the graph shown in Fig. 5 along with a set of guidelines on the use of the model, the date of the biofix, and an inexpensive maximum-minimum thermometer. The model can also be incorporated in a computer program for use in microprocessor and small computer-based pest management systems. Other applications include forecasting of ascospore maturity based on historical degree-day accumulation from the

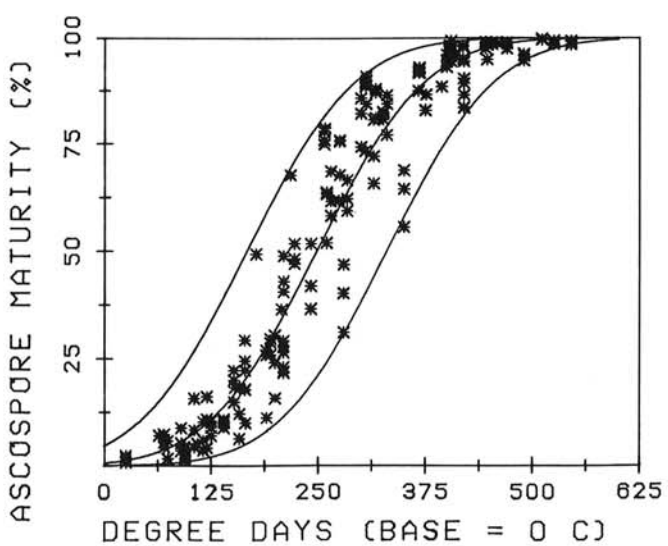

Fig. 3. Transformation of linear model of *Venturia inaequalis* ascospore maturation. The central curve indicates ascospore maturity as estimated by the model. The outer curves are the limits of the 90% confidence bands.

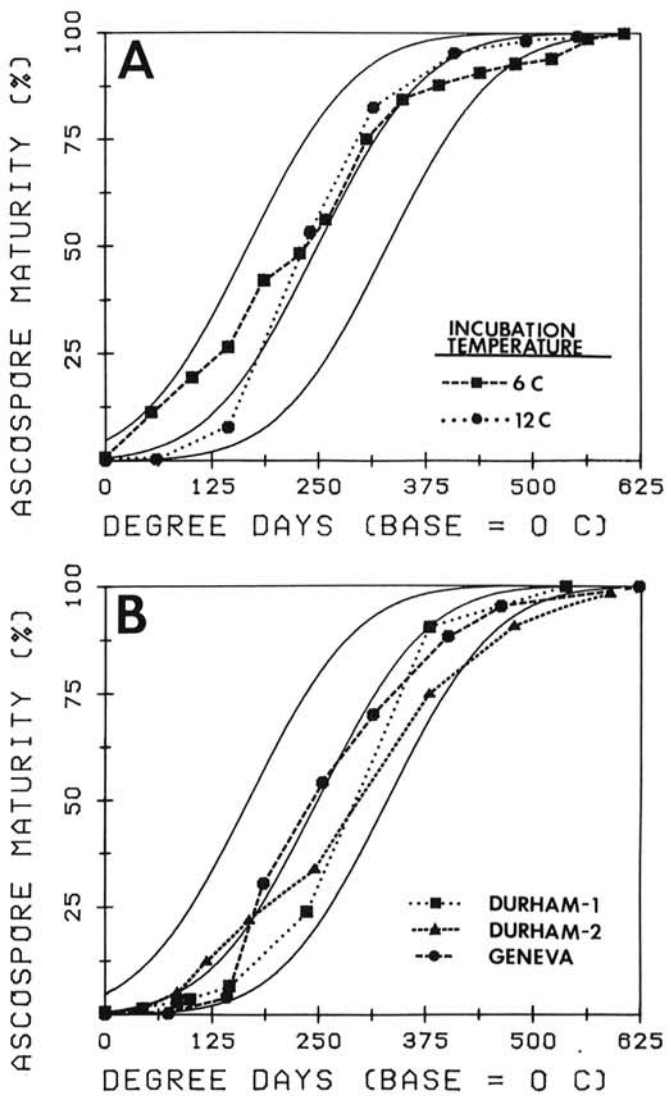

Fig. 4. *Venturia inaequalis* ascospore maturity model validation. In both graphs the smooth sigmoid curves represent estimated values of *Y* and the upper and lower limits of the 90% confidence bands. **A**, 1981 controlled environment studies. Leaf samples were incubated at 6 and 12 C at 90 ± 5% RH. **B**, 1981 orchard studies. Leaf samples Durham-1 and Durham-2 were both stored at the Mast Road Research Orchard in Durham, NH. The Geneva leaf samples were stored at the New York State Agricultural Experiment Station in Geneva. Ascospore maturity was assessed by measuring ascospore discharge from the Durham-1 and Geneva leaf samples and by examining crushed pseudothecia from the Durham-2 leaf sample.

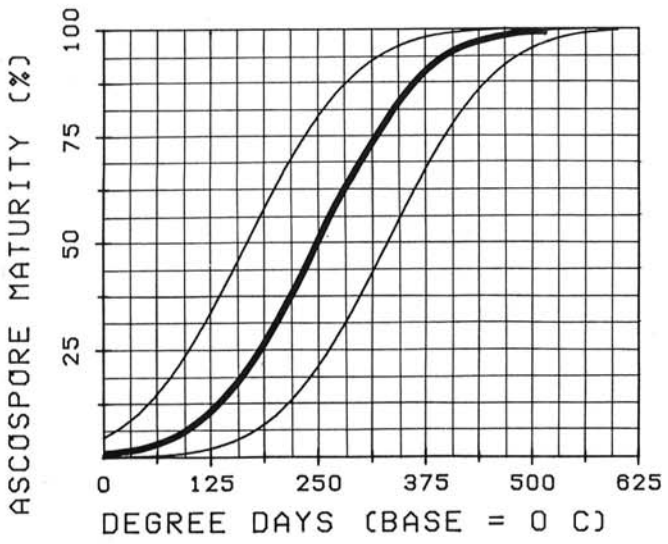

Fig. 5. Transformation of linear model of *Venturia inaequalis* ascospore maturation. This form of the model can easily be used to estimate ascospore maturity, without complex probit transformations.

date of the biofix. We are investigating the use of apple fruit bud phenophases as alternative biofixes. The use of tree phenology for determining the biofix would eliminate the need for early spring assessments of ascospore maturity, thus making the model usable in situations where more sophisticated assessments are not possible.

We propose this model as a means of estimating relative inoculum levels within apple orchards, ie, the percentage of the population that has matured. At the present time the model is useful in the estimation of the beginning, peak, and end of ascospore development; events that significantly affect the use of fungicides to control apple scab. The effects of absolute inoculum levels or ascospore dose (5) upon disease development are poorly understood, and further research on this subject is necessary before the full potential of the model can be realized.

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