# EVALUATION OF MODELS FOR PREDICTING ASCOSPORE MATURATION OF *Venturia inaequalis* AND REFINEMENT OF MOISTURE VARIABLES IN THE MODELS

A Thesis

by

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ABSTRACT

EVALUATION OF MODELS FOR PREDICTING ASCOSPORE MATURATION OF *Venturia inaequalis* AND REFINEMENT OF MOISTURE VARIABLES IN THE MODELS (May 1993)

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Rate of ascospore maturation of the causal fungus of apple scab, *Venturia inaequalis*, is correlated with certain temperature and moisture conditions. Models formulated to predict time of ascospore maturity and release use temperature and moisture parameters in their equations. The models predict spore maturity in areas with a particular climate but are not as reliable in other parts of the country.

Two models were investigated. Neither of these were reliable for both locations used in this study. Both models were more precise when refined by using a biofix date but determination of a biofix date requires procedures and instruments not readily accessible to apple growers.

This study showed that models could be improved by using relative humidity of 60% or higher instead of 100%

and periods of 12 hours or more of wetness instead of the 24 hour periods.

Numbers of mature, discharged spores were determined by using spore traps. This data correlated well with data acquired through studies of crushed pseudothecia to determine stage of maturity of ascospores. This information allowed for an accurate determination of spore release.

Leaf moisture content is an important factor in the development of pseudothecia but is complicated to accurately determine. It was found that relative humidity was correlated to leaf wetness and that relative humidity values could be substituted for leaf wetness for an easily obtainable moisture variable.

## ACKNOWLEDGMENTS

While no thesis is ever truly one individual's work, the acknowledgment section is reserved to mentioned those who incorporated their ideas and talents into helping to complete a particular task. It would take a book the size of the one you are holding in your hands to mention the people who I am indebted to for their contributions. The following are just a few of the many to whom I owe thanks.

I wish to express my sincere appreciation to Dr. John J. Bond for his invaluable assistance and counsel throughout this study. His ever-present good humor and dedication to the project was one of the few enjoyable constants throughout this undertaking. It is impossible for anyone other than myself to realize what an impact Dr. Bond had on my graduate career mainly due to the fact that if it had not been for him, odds are I would not have given graduate school the first thought.

Appreciation is also extended to other members of the Advisory Committee; Dr. E. D. Greene for his statistical knowledge and constant harassment, in good humor; and Dr. G. L. Walker for his sanity-saving social get-togethers and for always helping me keep the

administrative gurus at bay.

I would also like to thank Dr. T. B. Sutton of North Carolina State University for his guidance, use of equipment, and constant input and assistance. Without his help this study never would have gotten off the ground. Also, sincere thanks to Mr. Lindsay Deal and Mr. Bill Hanlin whose help made this study possible to the degree of which you are about to see.

And, of course, thanks must be given to Betsy Harris for always letting me borrow, beg, and steal her pieces of scientific equipment for use in my research.

DEDICATION

To my mother, without whose support this study would have been impossible. Also, to the rest of my family who instilled a desire for me to further my education. Read into the last statement what you will.

To my professors at Appalachian State University who guided, tolerated, and frustrated me for all those years. Now that it is over, I thank you.

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# INTRODUCTION

Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is considered the most important apple (*Malus sylvestris* Mill.) disease in the United States. The fungus infects and damages the leaves and fruit and is found in most areas of the world where apples are grown. It is less severe in semi-arid regions than in cool, humid areas with frequent rainfall. Severe infection can cause heavy defoliation which can lead to weakened trees which are susceptible to low temperature damage.

Control of apple scab is based on protectant fungicides designed to inhibit infection by ascospores or eradicant fungicides which are applied within 24 - 96 hours at the beginning of infection. Mature ascospores are forcibly discharged from pseudothecia during rain and initiate the infection cycle in the spring. Knowing the time of spore release is critical in timing fungicide applications. Conidia are produced on lesions formed after primary infection which perpetuate secondary infection through the summer. At this stage control is more difficult. In the fall the diseased leaves fall to the ground and the fungus initiates

ascospore development.

Several models for predicting spore maturity of *V. inaequalis* have been developed but none satisfy the requirements for an accurate model that is simple to apply. Models incorporate environmental conditions such as temperature and moisture to predict the maturity of ascospores (5,6,7,8,11). Because moisture appears to be the limiting factor, more precise measures of leaf wetness are needed for a more accurate prediction of pseudothecial development.

Pseudothecia of *V. inaequalis* develop after the apple leaves have dropped from the trees in the fall. The fungus enters a dormancy stage which lasts about 45 days until around 1 February in N.C. After this date, rapid spore maturation occurs with favorable temperature and moisture. Optimum range of temperature for pseudothecia development is 8-12° C; 16-18° C is optimum for ascospore maturation (7). Collection and observation of pseudothecia are required to determine the developmental stages in order to correlate them to environmental conditions.

The purposes of this study are: (i) to compare ascospore maturation with meteorological data, such as temperature, relative humidity, and precipitation, to determine which parameters influence development; (ii) to test models previously formulated for predicting

ascospore maturation; (iii) to trap ejected spores and correlate these data with moisture and time of discharge; and (iv) to determine if there is a relation between leaf wetness and relative humidity so that a practical model can be developed for use by apple growers to determine appropriate timing of fungicide applications.

## LITERATURE REVIEW

Apple scab is a disease of apple fruit and foliage caused by the fungus *Venturia inaequalis*. In the mid-1920's, Keitt and Jones (8,9) performed a study that determined the temperature and minimal hours of continual wetting for infection by ascospores of *V. inaequalis*. From these data Mills (15) compiled a best fit table for temperature and moisture combinations necessary for infection to occur and continually adjusted it as he accumulated data from orchard observations.

The accuracy of Mill's table has been the subject of much research. Some studies supported Mill's work (18,19) but many did not (8,9,10,17,21,22). Most researchers found that the Mill's system had to be modified for temperature and moisture variances to improve its accuracy (21,22). Preece and Smith (20) agreed with the temperature and moisture requirements of Mill's table but found that high relative humidity could be substituted for leaf wetness to help predict infection.

Several other models for predicting ascospore maturation have been developed since Mill's work. Most

noteworthy are models formulated by Massie and Szkolnik (13), James and Sutton (6,7), and MacHardy and Gadoury (4,11). Massie and Szklonik's (13) model held true for the Geneva, N.Y area but was unreliable in other parts of the country. James and Sutton (6,7) developed two models but more specific measures of leaf wetness are needed to refine them. MacHardy and Gadoury's (4,11) models estimate ascospore maturity with degree-day accumulation. However, none of these models are useful and reliable enough to be the sole determiner of fungicide application timing.

Several factors have played key roles in developing models besides moisture and temperature. Wilson (23) indicated that temperature, leaf moisture, time of leaf fall, and time of infection were major factors affecting development of pseudothecia. The winter dormancy period is another factor that results in the need to modify models from one locality to the next (13). Because environmental conditions vary from area to area, the dormancy period may not be the same in cooler climates as that in warmer areas. Miller and Waggoner (14) observed that pseudothecia matured at approximately the same time each year regardless of spring temperatures and suggested that the average temperature during the winter was more important than spring temperature for their development. Also, an extremely high percentage of

spores are released during the daytime (1,2,12) which is a factor that needs to be taken into consideration when utilizing the models.

The time of leaf fall and the conditions associated with it have little effect on the spring spore maturation (3,5). However, most models fail to take the various environmental and phenological conditions into account and are therefore not sufficiently accurate for practical applications.

## Materials and Methods

About 800 apple leaves infected with *Venturia inaequalis* were collected 24 October, 1991 from apple orchards in Wilkes and Alexander Counties, North Carolina. Most of the leaves were secured between two pieces of 30 x 30 centimeter wire mesh (12.5 millimeter) and placed in Alexander County at Lindsey Deal Orchards and Watauga County at a location in Boone, N.C. Each held approximately 20 apple leaves. There were 24 wire mesh apple leaf containers in all; 12 at each location. In addition, there were 12 containers with infected crabapple leaves that were collected from Wake County; six were placed at both sites. These were left outdoors and exposed to natural environmental conditions and collected as needed. A tightly woven nylon mesh cloth was placed under the wire mesh containers to help protect the leaves from soil insects and earthworms.

For observations on pseudothecial maturity, a dissecting scope was used to locate pseudothecia on the leaves and then a fine needle was used to separate the pseudothecia from the leaf. Three to five pseudothecia were collected from each leaf and put on a slide with a drop of cotton blue in lactophenol. A cover slip was

then applied on top of the slide and the blunt end of a wooden handled needle used to crush open the pseudothecia. The slide was then placed under a compound microscope to determine the stage (st) of development of the asci and ascospores.

On each collection date, a minimum of 25 asci/ pseudothecium, two pseudothecia/leaf, 10 leaves/ replication were observed. There were five replications for Alexander County and six replications for Boone. Stages were defined as follows: (st 1) subcuticular stroma, (st 2) pseudothecial initial showing coiling of hyphae, (st 3) formation of the ascogonia from the initial, (st 4) pseudoparaphyses beginning to appear in the lumen of a pseudothecium as the ascogonium disappears, (st 5) lumen of the pseudothecium filled with pseudoparaphyses, (st 6) appearance of asci, (st 7) asci 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, (st 13) ascospores discharged, i.e. asci empty, and (st 14) asci aborted (7). Pseudothecia with asci in different stages of development were given average ratings. Since the early stages of development occur during the fall, only stages

5 and greater were utilized in this study.

Leaves were first collected for observation on 21 March, 1992 from Alexander County and 9 April, 1992 from Boone. Collection and observation continued weekly at both sites until most of the ascospores had matured and discharged. Recordings were made of the observation date, leaf number (1-10), number of asci/pseudothecia, and the stage of development.

At both locations the hourly temperature and relative humidity were recorded with a hygrothermograph (Belfort Instrument Company, Baltimore, M.D.) housed in a standard instrument shelter. Leaf wetness was measured with a Dewit leaf wetness meter at Deal Orchards in Alexander County and hourly rainfall was measured with a top weighing rain gauge in Boone. When data were missing because of instrument malfunction, data from the nearest NOAA weather station were utilized. For Alexander County, the NOAA station was located 10 km away, for the Boone site, 2 km away.

Leaf discs (1.27 centimeters in diameter) were punched from infected leaves on 26 October, 1991 and sewn between two pieces of 10 centimeter diameter round nylon mesh. A total of eight mesh screens were made for spore collection; four at each location. Four holes were dug at each site for a 1 liter plastic container so that the top of the containers were level with the ground.

Plastic pots were placed in the holes to allow for easy removal and replacement of the spore traps. Spore traps were constructed from 1 liter containers and a PVC funnel-shaped top with an 8 centimeter tall brim and a wire mesh base inside the top. The mesh bags containing the leaf discs were put into the tops to collect the spores when they discharged from the leaves during rain. Ten milliliters of copper sulfate solution (5 grams $CuSO_4$/100 milliliters distilled water) were put into each container in order to prevent spore germination until they could be counted. The 1 liter bottles of the spore traps were replaced once a week with identical containers.

At time of observation, the 1 liter containers were shaken to suspend the spores. A 10 milliliter sample from each container was vacuum filtered and the spores collected on grided filter paper. The spores were then counted under a microscope using 500X. The date of collection, container number (1-4), the total water amount in milliliters, and the number of spores/grid were recorded. Grids to be counted were picked randomly from the filter paper with a total of three grids/container, four containers/sample date for a total of 12 samples for each sampling date.

Water Saturation Deficit (WSD) is the ratio of the amount of actual water in leaf tissue to the potential

amount of water the tissue can absorb. Leaf water content was measured by using the following equation (7):

$$WSD = \frac{\text{(fully turgid weight - fresh weight)}}{\text{(fully turgid weight - oven dry weight)}} \times 100\%$$

where fully turgid weight equals the weight of the leaves after being soaked in distilled water for 24 hours; fresh weight equals the weight of the leaves when they were collected; and oven dry weight equals the weight of the leaves after drying for 24 hours at 65° Celsius. Sample times were correlated with various degrees of moisture as well as fair days and consisted of five leaves/reading. Sampling began 30 January, 1992 and ended 25 April, 1992 with a total of 38 different sample days. Leaves were obtained from underneath an apple tree neighboring the Appalachian State University campus. A psychrometer was used to measure the relative humidity and the temperature at the time of sampling. Water Saturation Deficit was compared to relative humidity values and analyzed with exponential regression analysis.

The models tested to predict ascospore maturation were James and Sutton's (6) and Gadoury and MacHardy's (4). The James and Sutton model tested was:

$$Y = \Sigma\hat{y} + st\ 5$$

where $\hat{y}$ = the daily predicted change in pseudothecial development and st 5 is the overwintering stage. The value for $\hat{y}$ is calculated using the following equations:

(1) $\hat{y} = 0.0031 + 0.0546(T) - 0.00175(T)^2$

or

(2) $\hat{y} = 0.0370 + 0.0599(T) - 0.00255(T)^2$

in which $\hat{y}$ = predicted daily change in pseudothecial stage and T = degrees Celsius. Only days that met the moisture conditions; 12 or more hours of 0.25 millimeters of rain or 100% relative humidity; were used in the equations (6,7). Gadoury and MacHardy's model is:

$$\hat{y} = 2.51 + 0.01\ X$$

in which $\hat{y}$ = probit of proportion of matured ascospores and $X$ = accumulated Celsius degree days from the first appearance of mature spores. Both models use degree days which is the average of the highest and lowest temperature in a 24 hour day. Environmental variables obtained from meteorological instruments at the experiment sites were used for testing the models.

The James and Sutton model used the logit transformation; $\ln(x/1-x)$, where $x$ = percentage of mature spores at each sample date; to relate mean stage of pseudothecial development to the percentage of mature ascospores. Gadoury and MacHardy's model used probit transformations to relate degree day accumulation to the percentage of mature ascospores. Logit and probit transformations are considered interchangeable but this paper will use each researcher's respective terminology.

Degree days were compared to temperatures averaged at 2 hour intervals for 24 hours on days that met the moisture conditions necessary to advance ascospore maturity in the James and Sutton model. This was done to determine if there was a significant difference in the average temperatures obtained in order to acquire a more accurate indication of the true temperature. Temperatures were calculated for 10 days that met the criteria for moisture and statistical analyses were performed on the data.

A biofix is a date in which field observations are used to determine actual development. This study used a biofix to determine the stage of development of the pseudothecia. This technique was used weekly to verify the accuracy of the models and used as a reference point in the models for their initiation.

## Results

Model Evaluation. Using the James and Sutton model (6):

$$Y = \Sigma\hat{y} + st\ 5$$

the following data were obtained when $\hat{y}$ was calculated with the equation

$$(1) \quad \hat{y} = 0.0031 + 0.0546(T) - 0.0175(T)^2.$$

Alexander County. The model predicted maturation and discharge of spores within 3-5 days of actual field observations when used with a biofix as the point to initiate the model. The model overpredicted spore maturity in the following instances: i) when moisture and temperature conditions were calculated using data from the nearest NOAA weather station only (data not presented); and ii) when 1 February was used as the starting date for maturation to begin from stage 5. The model did not underestimate spore maturity under any circumstances (Figure 1, Tables 1 and 2).

Boone. The model underpredicted spore maturity in the following instances: i) when moisture and

temperature data were from the observation site only (data not presented); ii) when 1 February was used as a starting date for spring spore maturation to begin with the assumption that spores would be in stage 5 at that date; and iii) when supplemental moisture and temperature information was used from the nearest NOAA weather station (Figure 2, Table 3).

The model was accurate when a biofix date was established and the corresponding information used in the model. There were slight discrepancies in the use of different biofix dates but predictions of spore maturity were approximate; ±0.63 stage for Alexander County and ±0.87 stage for Boone on the last observation date (Tables 2 and 3).

The equation:

$$(2) \quad \hat{y} = 0.0370 + 0.0546(T) - 0.00255(T)^2$$

predicted maturation rates similar to equation (1) but at a slightly slower rate and was therefore not further evaluated.

Averages of 2 hour interval temperatures for 24 hour days that met the moisture requirements were compared to degree days to determine if one method was more accurate than the other. Use of a *t*-test indicated no significant difference between degree days and

averages of 2 hour interval temperatures (P = 0.1).

Using the Gadoury and MacHardy model (4):

$$\hat{y} = 2.51 + 0.01\ X$$

the following data were obtained.

Alexander County. The model accurately predicted spore maturation and discharge when 1 April was assumed to be the date of the first appearance of mature spores. On 13 May pseudothecial observation showed 99.93% mature spores present while the equation predicted 100% (Figure 1, Table 4).

Boone. The model slightly underpredicted spore maturity when 1 April was assumed to be the date of the first appearance of mature spores. On 15 May pseudothecial observation showed 100% mature spores while the equation predicted 98% (Figure 2, Table 5).

Spore Traps. Collection and observation of ejected, mature ascospores verified that the time of spore discharge coincided with the date of observation of mature spores within the pseudothecia. Pseudothecial examination on 13 May revealed that the majority of spores were matured and released. Spore trap counts confirmed that the majority of spores were released 11 May and subsequent days (Figure 1, Table 6).

Leaf Moisture Content. Water Saturation Deficit was related to relative humidity ($r^2 = 0.80$, $P = 0.01$, $df = 37$). By use of a best fit graph and assuming pseudothecia do not develop under 0.85 WSD (7), it was concluded that development of pseudothecia would occur when the relative humidity is 60% or greater (Figure 3, Table 7).

## Discussion

The model developed by James and Sutton (6) predicted maturation and discharge of spores within 3-5 days of the actual observed dates when used with a biofix reference date. This degree of accuracy would be useful in aiding growers in making decisions on the timing of fungicide applications. Using 1 February as a starting date reduced the accuracy.

The James and Sutton (6) model consistently underpredicted maturation compared to field observations. One reason may have been the manner in which the field checks were performed. The field study results may have been biased toward the more mature pseudothecia which were more conspicuous. However, when compared with spore collection counts, the field observations agreed favorably.

It has been demonstrated that the James and Sutton model underpredicts spore maturity in Boone (6) and the conclusions of this study support those findings when the 1 February starting date was used. The model seems to be a better predictor in other areas of North Carolina. James, et al. (6) hypothesized that the large amounts of snow cover during their study in 1979 and

1980 affected the maturation rates so that the model underpredicted spore maturity. While 1979 had a high amount of snow cover (90.17 centimeters from February to April), 1980 had a substantially lower amount of snow cover on the ground (29.21 centimeters for March and April). Data for February was incomplete but accompanying data showed little precipitation for the month of February. Weather records for 1992 showed that over 28 centimeters of snow were on the ground from February to April. It was noted that 1979 and 1980 experienced above normal levels of precipitation with the exception of February, 1980 (16). This is insufficient information upon which to draw conclusions on whether or not snow cover affects the dormancy period or pseudothecial development. Snow cover would provide the fungus with insulation and a steady supply of moisture which could have some undetermined effect on the development of the pseudothecia.

Pseudothecia begin to mature at an earlier date than the 1 February starting point in Boone (6). This might explain why the model was not accurate when used with the 1 February starting date but was accurate when used with a biofix starting date. Boone is located at a relatively high altitude, approximately 1 kilometer, with cooler temperatures than other locations in the state where the model has been tested. This could

possibly have some impact on the winter dormancy period of the pseudothecia. The time frame and conditions associated with the end of the dormancy period appear to differ the farther north one proceeds (7). Latitude may not have as much of an influence in this occurrence as does the cooler temperatures. Constantly cooler temperatures in the winter will retard pseudothecial development. Models used in primarily northern areas often overlook the dormancy period because of constantly low temperatures where there is little or no development (7). Models formulated for the Piedmont region of the Carolinas take this dormancy period into account and the models are more accurate, except in Boone. The reason for this may be that Boone experiences greater variation with the winter weather. Because of its higher altitude temperatures are cooler but there are also days when it is warm enough for pseudothecial development to occur. These warm and cold spells occur frequently enough during the winter to cause the models to underpredict spore maturity.

Mature ascospores were observed in pseudothecia on the first collection date, 9 April. This indicated spores had matured before this date but no definite time could be assigned as to when the spores actually started to mature after the winter dormancy period. Because of this, the 1 February starting date was used as a

reference point with the assumption that the spores were in stage 5. The fact that the model underpredicted spore maturation using this method could indicate that 1 February might not be a reliable starting date to initiate the model. This is because the pseudothecia may be at a later stage of development than stage 5, at least in the Boone area, and that a biofix starting point should be used for a more dependable model prediction. This would make the model more difficult to use for growers because they would have to determine the stage of maturity. It is possible that county extension agents could make this determination for growers on a regional or county level.

Gadoury and MacHardy's model accurately predicted spore maturation and discharge when 1 April was used as the assumed date of the first appearance of mature spores. 1 April was used as a starting date because extrapolation of the data showed that this would be in close proximity to the first appearance of mature spores. The use of a calendar starting date would be most beneficial to apple growers to simplify application of the models. Having to use microscopy for a biofix complicates effective use by growers but appears to be necessary for accuracy. When 1 April was used as a starting date for Boone, the Gadoury and MacHardy model was not as accurate.

The data obtained with the spore traps reflect several aspects of the epidemiology of *V. inaequalis*. Collection of spores lasted for 1 month. During this time it was demonstrated that spores were constantly maturing and being released during rainfall. The larger amounts of rainfall corresponded to greater numbers of trapped ascospores. The larger percentage of released ascospores on 11 May coincided with the pseudothecial observation of a large percentage of mature spores and a high amount of rainfall. The previous week had the lowest amount of rainfall and the lowest percentage of released spores (Table 7). This suggests that if apple growers want to provide suitable protection against infection they need to apply fungicide on a 5 to 7 day interval during the maturing phase of the ascospores. It also suggests that timing fungicide applications according to when the majority of spores are mature and moisture conditions are favorable would prevent infection from occurring and reduce the amount of fungicides needed to control infection.

During some periods of this study, the weather stations at the experimental sites were inoperative. Supplemental moisture and temperature data were obtained from nearby NOAA weather stations and the Appalachian State University library. The NOAA stations recorded more days with precipitation than the weather stations

at the research sites. This could have affected the accuracy of the models and led to an overprediction of maturation. Additional time periods in which moisture conditions were met would increase the rate at which predicted maturity would occur. These problems could be overcome by the use of more sensitive and reliable weather collecting equipment at the experiment sites.

One of the most important attributes of a predictive model is its ease of use. The Water Saturation Deficit (WSD) study was an attempt to determine if relative humidity could be used as an indicator of leaf wetness so that the more complicated data gathering techniques and computations of the WSD could be avoided. Statistical analyses indicated a significant relation to WSD and relative humidity ($r^2$ = 0.80, P = 0.01). The comparison determined that at 0.85 WSD, the lowest point at which spores show maturation (7), relative humidity values of 60% or higher could be incorporated into the predictive equations instead of using relative humidity values of 100%. This would increase the predictive maturation rates since the model developed by James and Sutton was derived with 100% relative humidity values. The 60% relative humidity values would not work in this model but further evaluation of this information may produce a more accurate model, especially since moisture conditions

play such a vital role in the development of ascospores (7).

Periods of 12 hours or more of acceptable moisture conditions per 24 hour days were one of the variables for calculations in the James and Sutton model (6). It would seem that 12 hour periods of wetness in the field would constitute a more accurate moisture regime than 24 hour periods of degree days which were used in the calculations. Twelve hour periods of acceptable moisture conditions could be left out of a degree day calculation. For example, if a 12 hour period of rain consisted of 6 hours of rain at the end of one day and 6 hours of rain at the beginning of another day, each day would only contain 6 hours of calculable wetness. This would not meet the 12 hour/day moisture criteria for the accumulation of a degree day.

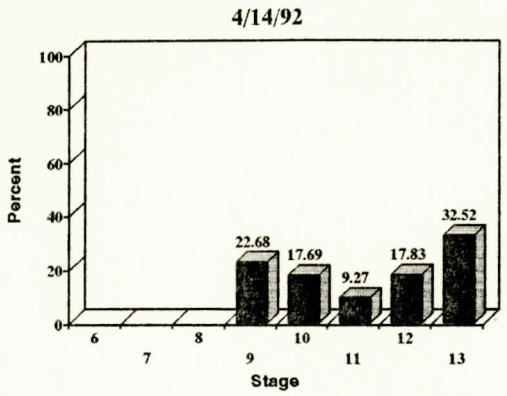
Two conditions seem to hinder the effectiveness of general models that have been studied. First, local environmental conditions influence the development of the fungus. In order for a model to be applicable to various regions of the country it needs to utilize variables that are consistent to the development of the fungus from region to region. Second, problems occur with the application of a calendar date to begin charting maturation progress. There appears to be little consistency from year to year in environmental

conditions associated with the development of the fungus, therefore the use of a calendar date in models does not have a strong biological basis. This technique may satisfactorily predict spore maturation in one part of the country under certain conditions but as this study demonstrated, the use of a calendar date in the models did not accurately predict maturation. The use of a biofix date was needed to verify the actual stage of development.

The factor that appears to limit the use of a calendar date as a starting point for model use is the dormancy period of the fungus. In northern areas where there is no apparent dormancy period, consistently cooler temperatures may retard development until the temperatures are warm enough to initiate maturation. In the mountainous areas of North Carolina, i.e. Boone, the weather plays a vital role in the development of the pseudothecia because the temperature fluxes in the winter may initiate earlier development. The dormancy period of the Piedmont region is longer than that of the mountains. The cooler temperatures of the mountains may slow development of the fungus so that maturity occurs at a later date than in warmer areas such as the Piedmont. The dormancy period of *V. inaequalis* apparently synchronizes timing of the discharge of mature spores with phenology of the apple trees so that

spores are released when the likelihood of infection will be greatest.

Figure 1. Pseudothecial development in Alexander County, 1992. Stage = the stage of pseudothecial development on the corresponding observation date. See text for description of stages. Percent = the percentage of asci in a particular stage.


4/14/92
Percent
100
80
60
40
20
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17.69
9.27
17.83
32.52
6
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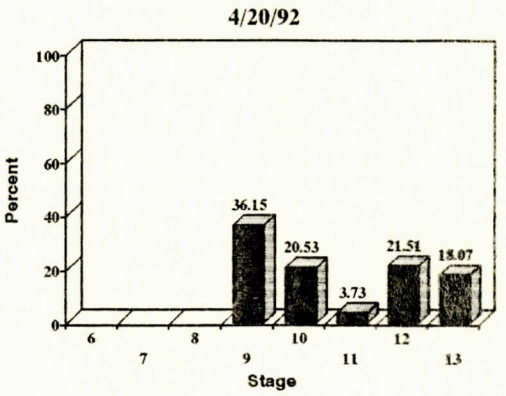
10
11
12
13
Stage


4/20/92
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80
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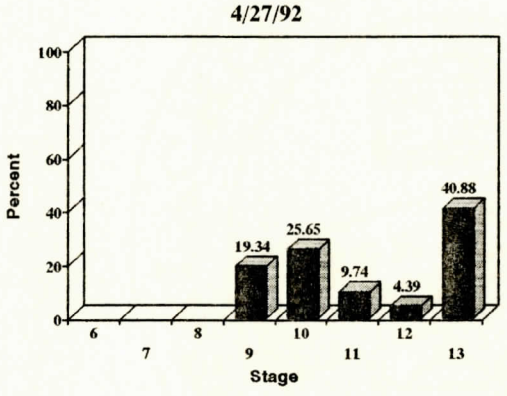
21.51
18.07
6
7
8
9
10
11
12
13
Stage


4/27/92
Percent
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60
40
20
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9.74
4.39
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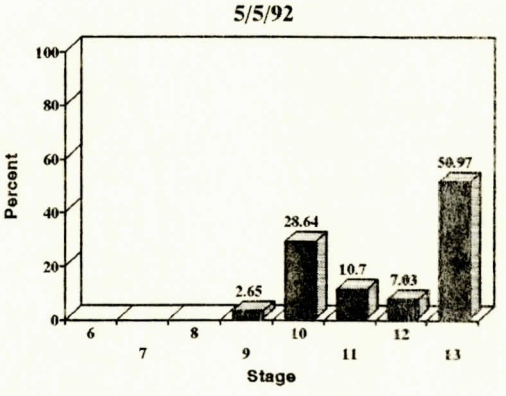
6
7
8
9
10
11
12
13
Stage


5/5/92
Percent
100
80
60
40
20
0
2.65
28.64
10.7
7.03
50.97
6
7
8
9
10
11
12
13
Stage

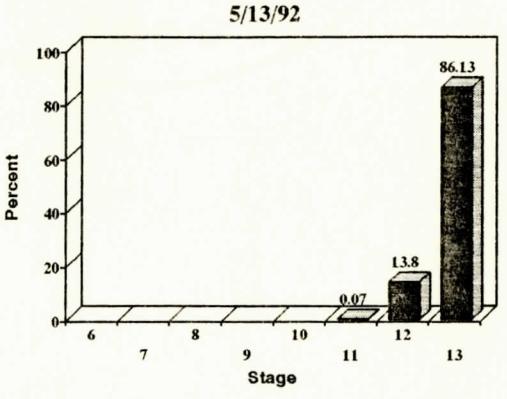

5/13/92
Percent
100
80
60
40
20
0
0.07
13.8
86.13
6
7
8
9
10
11
12
13
Stage

Table 1. Relationship between observed and calculated maturity values of pseudothecial development of *Venturia inaequalis*, 1992. Based on the James and Sutton model.

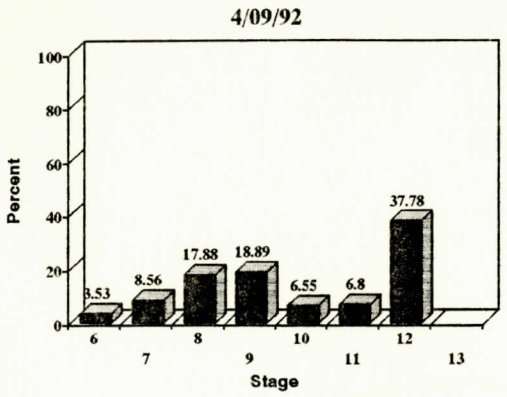
| Date | Average Stage of Maturity at Observation Date | Calculated Percent Mature Spores |
|---|---|---|
| Alexander County | | |
| 14 April | 10.82 | .67 |
| 20 April | 10.64 | .60 |
| 27 April | 11.22 | .79 |
| 5 May | 11.75 | .90 |
| 13 May | 12.86 | .98 |
| Boone | | |
| 9 April | 9.88 | .31 |
| 15 April | 11.72 | .89 |
| 22 April | 12.03 | .93 |
| 3 May | 11.67 | .89 |
| 7 May | 12.09 | .94 |
| 15 May | 12.96 | .98 |

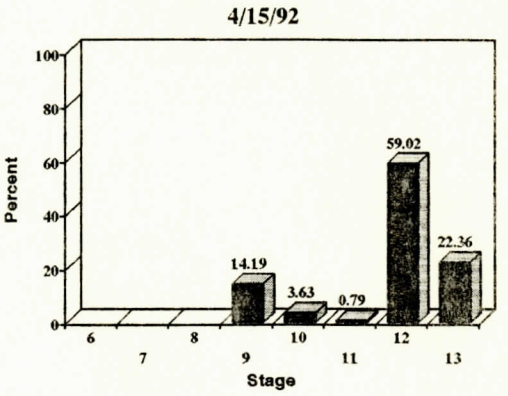
Table 2. Relative stages based on James and Sutton's model in Alexander County, 1992.

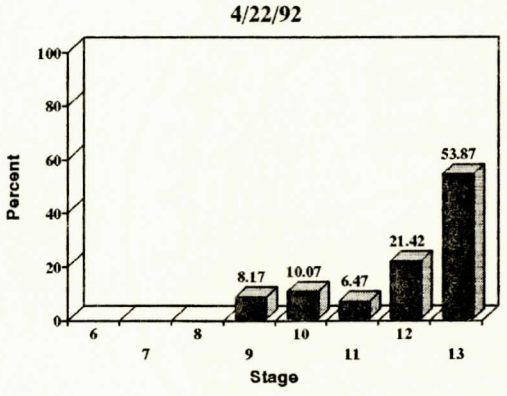
| Date | X | Y | Z | Observed | Plus st. 5 | Biofix 1 | Biofix 2 | Biofix 3 |
|---|---|---|---|---|---|---|---|---|
| 3/25/92 | 6.41 | 0.2810 | | | 5.28 | | | |
| 3/26/92 | 5.49 | 0.2500 | | | 5.53 | | | |
| 3/30/92 | 10.76 | 0.3879 | | | 5.92 | | | |
| 4/7/92 | 7.47 | 0.3132 | | | 6.23 | | | |
| 4/9/92 | 11.51 | 0.3996 | | | 6.63 | | | |
| 4/10/92 | 10.91 | 0.3904 | | | 7.02 | | | |
| 4/11/92 | 15.35 | 0.4289 | | | 7.45 | | | |
| 4/15/92 | 14.31 | 0.4260 | | | 7.88 | | | |
| 4/16/92 | 15.00 | 0.4284 | | | 8.31 | | | |
| 4/17/92 | 17.00 | 0.4256 | | | 8.73 | | | |
| 4/18/92 | 16.83 | 0.4263 | | | 9.16 | | | |
| 4/20/92 | 16.96 | 0.4258 | 60% | | 9.58 | 10.64 | | |
| 4/21/92 | 15.37 | 0.4289 | | | 10.01 | 11.07 | | |
| 4/23/92 | 13.71 | 0.4227 | | | 10.43 | 11.49 | | |
| 4/28/92 | 9.17 | 0.3566 | 79% | | 10.79 | 11.85 | 11.22 | |
| 5/5/92 | 11.94 | 0.4056 | 90% | | 11.20 | 12.25 | 11.63 | 11.75 |
| 5/6/92 | 7.50 | 0.3142 | | | 11.51 | 12.57 | 11.94 | 12.06 |
| 5/7/92 | 7.22 | 0.3062 | | | 11.82 | 12.87 | 12.25 | 12.37 |
| 5/8/92 | 7.78 | 0.3219 | | | 12.14 | 13.20 | 12.57 | 12.69 |
| 5/9/92 | 9.72 | 0.3685 | | | 12.51 | 13.56 | 12.94 | 13.06 |
| 5/10/92 | 15.56 | 0.4290 | | | 12.94 | 13.99 | 13.37 | 13.49 |
| 5/12/92 | - | - | 98% | 12.86 | - | - | - | - |

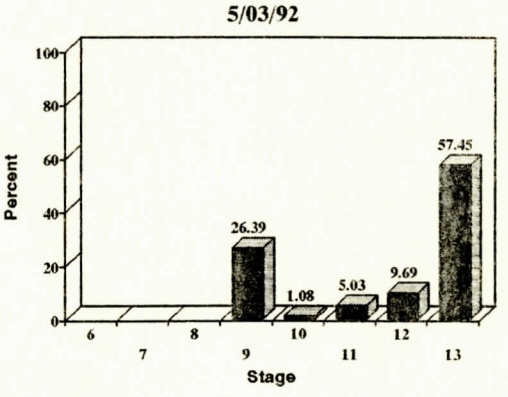
X = the average temperature in Celsius. Y = the predictive change in ascospore maturation. Z = the percentage of mature ascospores calculated by logit transformation of pseudothecial develoopment on that date. Plust st. 5 = stage 5 plus the cumulative predictive change in ascospore maturation.

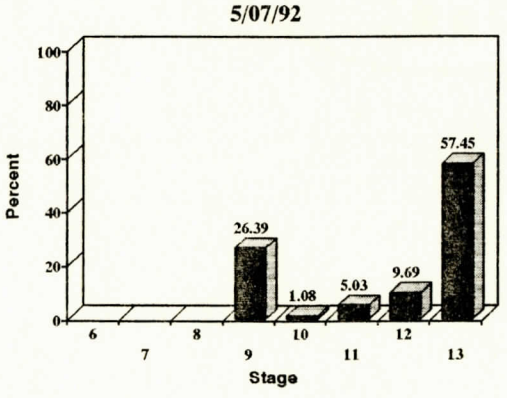
Figure 2. Pseudothecial development in Boone, 1992. Stage = the stage of pseudothecial development on the corresponding observation date. See text for description of stages. Percent = the percentage of asci in a particular stage.


4/09/92
Percent
100
80
60
40
20
0
3.53
8.56
17.88
18.89
6.55
6.8
37.78
6
7
8
9
10
11
12
13
Stage


4/15/92
Percent
100
80
60
40
20
0
14.19
3.63
0.79
59.02
22.36
6
7
8
9
10
11
12
13
Stage


4/22/92
Percent
100
80
60
40
20
0
8.17
10.07
6.47
21.42
53.87
6
7
8
9
10
11
12
13
Stage


5/03/92
Percent
100
80
60
40
20
0
26.39
1.08
5.03
9.69
57.45
6
7
8
9
10
11
12
13
Stage


5/07/92
Percent
100
80
60
40
20
0
26.39
1.08
5.03
9.69
57.45
6
7
8
9
10
11
12
13
Stage

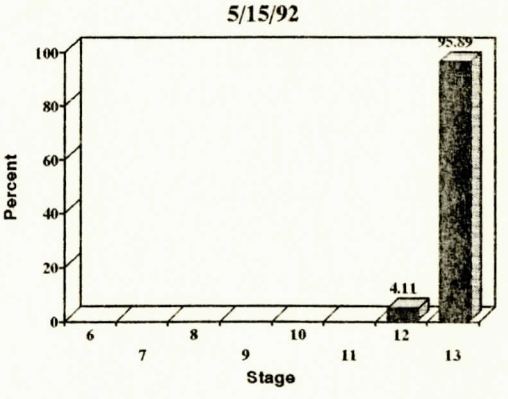

5/15/92
Percent
100
80
60
40
20
0
4.11
95.89
6
7
8
9
10
11
12
13
Stage

Table 3. Relative stages based on James and Sutton's model in Boone, 1992.

| Date | X | Y | Z | Observed | Plus st. 5 | Biofix 1 | Biofix 2 |
|---|---|---|---|---|---|---|---|
| 2/13/92 | 3.61 | 0.17745 | | | 5.18 | | |
| 2/14/92 | 2.50 | 0.1287 | | | 5.31 | | |
| 2/15/92 | 6.11 | 0.2714 | | | 5.58 | | |
| 2/16/92 | 6.67 | 0.2893 | | | 5.87 | | |
| 2/18/92 | 0.28 | 0.0181 | | | 5.88 | | |
| 2/22/92 | 6.11 | 0.2714 | | | 6.16 | | |
| 2/23/92 | 6.67 | 0.2893 | | | 6.45 | | |
| 2/24/92 | 8.33 | 0.3366 | | | 6.78 | | |
| 3/7/92 | 13.33 | 0.4200 | | | 7.20 | | |
| 3/8/92 | 12.78 | 0.4150 | | | 7.62 | | |
| 3/9/92 | 14.44 | 0.4266 | | | 8.04 | | |
| 3/10/92 | 7.22 | 0.3062 | | | 8.35 | | |
| 3/12/92 | 0.00 | 0.0031 | | | 8.35 | | |
| 3/26/92 | 6.67 | 0.2893 | | | 8.64 | 9.88 | |
| 4/20/92 | 15.56 | 0.4290 | | | 9.07 | 10.31 | |
| 4/21/92 | 16.11 | 0.4285 | | | 9.50 | 10.74 | |
| 4/22/92 | 12.78 | 0.4150 | 93% | | 9.92 | 11.15 | 12.03 |
| 4/23/92 | 16.11 | 0.4285 | | | 10.34 | 11.58 | 12.46 |
| 5/8/92 | 6.11 | 0.2714 | 94% | | 10.62 | 11.85 | 12.73 |
| 5/9/92 | 8.33 | 0.3366 | | | 10.95 | 12.19 | 13.07 |
| 5/15/92 | - | - | 98% | 12.96 | - | - | - |

X = the average temperature in Celsius. Y = the predictive change in ascospore maturation. Z = the percentage of mature ascospores calculated by logit transformation of pseudothecial development on that date. Plus st. 5 = stage 5 plus the cumulative predictive change in ascospore maturation.

Table 4. Percent mature ascospores based on Gadoury and MacHardy's model in Alexander County, 1992.

| Date | W | X | Y | Z |
|---|---|---|---|---|
| 01 April | 8.33 | 8.33 | 2.59 | |
| | 5.00 | 13.33 | 2.64 | |
| | 3.06 | 16.39 | 2.67 | |
| | 1.94 | 18.33 | 2.69 | |
| | 6.39 | 24.72 | 2.76 | |
| | 7.50 | 32.22 | 2.83 | |
| | 5.83 | 38.06 | 2.89 | |
| | 12.22 | 50.28 | 3.01 | |
| | 15.28 | 65.56 | 3.17 | |
| | 17.22 | 82.78 | 3.34 | |
| | 16.67 | 99.44 | 3.50 | |
| | 17.50 | 116.94 | 3.68 | |
| | 12.22 | 129.17 | 3.80 | |
| 14 April | 12.78 | 141.94 | 3.93 | 14.00% |
| | 13.89 | 155.83 | 4.07 | |
| | 17.78 | 173.61 | 4.25 | |
| | 19.44 | 193.06 | 4.44 | |
| | 19.17 | 212.22 | 4.63 | |
| | 19.44 | 231.67 | 4.83 | |
| 20 April | 18.33 | 250.00 | 5.01 | 50.00% |
| | 18.33 | 268.33 | 5.19 | |
| | 18.89 | 287.22 | 5.38 | |
| | 16.67 | 303.89 | 5.55 | |
| | 18.33 | 322.22 | 5.73 | |
| | 14.17 | 336.39 | 5.87 | |
| | 10.83 | 347.22 | 5.98 | |
| 27 April | 8.06 | 355.28 | 6.06 | 85.00% |
| | 9.17 | 364.44 | 6.15 | |
| | 10.28 | 374.72 | 6.26 | |
| 30 April | 12.50 | 387.22 | 6.38 | |
| 01 May | 16.11 | 403.33 | 6.54 | 94.00% |
| | 18.89 | 422.22 | 6.73 | |
| | 18.89 | 441.11 | 6.92 | |
| | 16.67 | 457.78 | 7.09 | |
| 05 May | 11.94 | 469.72 | 7.21 | 98.50% |
| | 7.50 | 477.22 | 7.28 | |
| 07 May | 7.22 | 484.44 | 7.35 | 99.00% |
| 08 May | 7.78 | 492.22 | 7.43 | 99.20% |
| 09 May | 9.72 | 501.94 | 7.53 | 99.40% |
| | 15.56 | 517.50 | 7.69 | |
| 11 May | 16.11 | 533.61 | 7.85 | 99.80% |
| | 18.06 | 551.67 | 8.03 | |
| 13 May | 19.72 | 571.39 | 8.22 | 100.00% |

W = average temperature in Celsius. X = accumulated degree days. Y = Probit. Z = the calculated percentage by use of a probit table of mature ascospores at that date.

Table 5. Percent mature ascospores based on Gadoury and MacHardy's model in Boone, 1992.

| Date | W | X | Y | Z |
|---|---|---|---|---|
| 01 April | 2.22 | 2.22 | 2.53 | |
| | -3.06 | | | |
| | -2.22 | | | |
| | -2.22 | | | |
| | 1.94 | 4.17 | 2.55 | |
| | 5.28 | 9.44 | 2.60 | |
| | 4.17 | 13.61 | 2.65 | |
| | 9.17 | 22.78 | 2.74 | 1.00% |
| 09 April | 12.50 | 35.28 | 2.86 | |
| | 14.44 | 49.72 | 3.01 | |
| | 15.00 | 64.72 | 3.16 | |
| | 14.72 | 79.44 | 3.30 | |
| | 9.17 | 88.61 | 3.40 | |
| | 10.83 | 99.44 | 3.50 | 7.00% |
| 15 April | 13.06 | 112.50 | 3.64 | |
| | 15.28 | 127.78 | 3.79 | |
| | 16.39 | 144.17 | 3.95 | |
| | 15.56 | 159.72 | 4.11 | |
| | 12.78 | 172.50 | 4.24 | |
| | 12.78 | 185.28 | 4.36 | |
| | 13.89 | 199.17 | 4.50 | |
| 22 April | 12.22 | 211.39 | 4.62 | |
| | 14.17 | 225.56 | 4.77 | |
| | 15.00 | 240.56 | 4.92 | |
| | 8.89 | 249.44 | 5.00 | 50.00% |
| | 3.33 | 252.78 | 5.04 | |
| | 1.67 | 254.44 | 5.05 | |
| | 3.89 | 258.33 | 5.09 | |
| | 6.67 | 265.00 | 5.16 | |
| 30 April | 6.39 | 271.39 | 5.22 | 59.00% |
| 01 May | 12.78 | 284.17 | 5.35 | |
| | 14.44 | 298.61 | 5.50 | |
| 03 May | 15.56 | 314.17 | 5.65 | |
| | 10.28 | 324.44 | 5.75 | |
| | 6.94 | 331.39 | 5.82 | 79.00% |
| | 2.50 | 333.89 | 5.85 | |
| 07 May | 2.50 | 336.39 | 5.87 | |
| | 2.78 | 339.17 | 5.90 | |
| | 5.28 | 344.44 | 5.95 | |
| | 10.83 | 355.28 | 6.06 | 85.50% |
| | 13.89 | 369.17 | 6.20 | |
| | 14.44 | 383.61 | 6.35 | 91.00% |
| | 15.28 | 398.89 | 6.50 | |
| | 15.28 | 414.17 | 6.65 | 95.00% |
| | 13.89 | 428.06 | 6.79 | 96.00% |
| 15 May | 16.94 | 445.00 | 6.96 | 98.00% |

W = average temperature in Celsius. X = accumulated degree days. Y = Probit. Z = the calculated percentage by use of a probit table of mature ascospores at that date. Temperatures below 0° Celsius were not incorporated.

Table 6. Discharged ascospore count from spore traps in Alexander County.

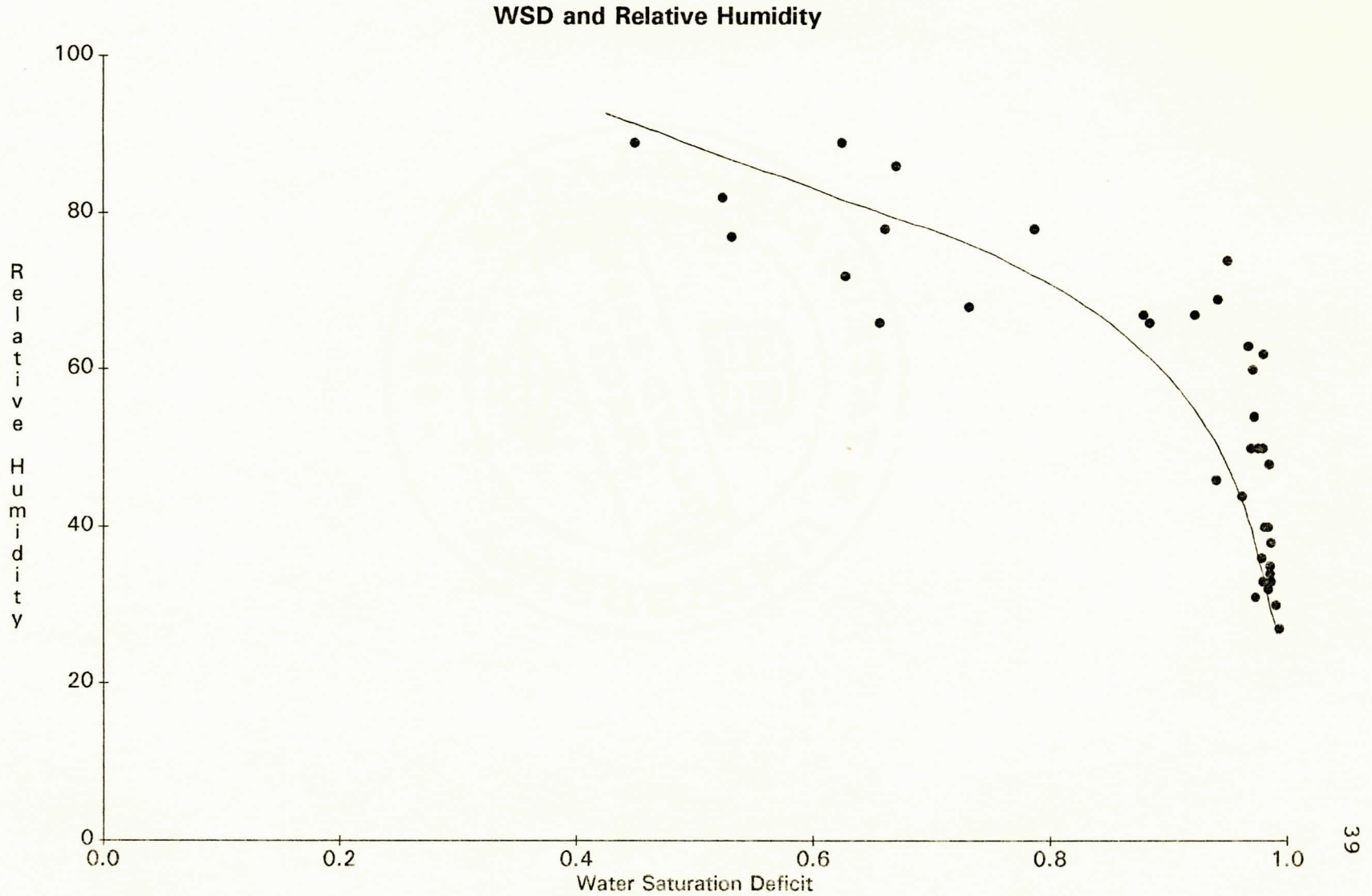
| Date 1992 | Average Rain Collected/ Bottle (ml)* | Calculated Number of Spores/ Bottle** |
|---|---|---|
| 18 April | 1000 | 12,448 |
| 27 April | 814.5 | 7,020 |
| 4 May | 5.7 | 5.5 |
| 11 May | 512.5 | 25,362 |
| 18 May | 56.5 | 558 |

*Average rain collected from four 1 liter bottles.
**Ascospore numbers were calculated from samples taken from four spore traps. All samples with 1000 milliliters of rain water were in excess of the sample bottle's capacity.

Table 7. Observed relative humidity and calculated Water Saturation Deficit (WSD) values. Winter/spring, 1992.

| Relative Humidity (%) | Average WSD | Relative Humidity (%) | Average WSD |
|---|---|---|---|
| 60 | .9716 | 50 | .9798 |
| 48 | .9850 | 72 | .6279 |
| 35 | .9856 | 33 | .9796 |
| 31 | .9732 | 86 | .6705 |
| 40 | .9813 | 68 | .7323 |
| 78 | .6611 | 78 | .7872 |
| 46 | .9405 | 40 | .9837 |
| 78 | .7874 | 66 | .6567 |
| 69 | .9418 | 74 | .9500 |
| 82 | .5242 | 66 | .8844 |
| 36 | .9785 | 38 | .9863 |
| 33 | .9863 | 67 | .8795 |
| 27 | .9933 | 67 | .9224 |
| 30 | .9904 | 54 | .9727 |
| 32 | .9840 | 50 | .9759 |
| 63 | .9676 | 89 | .6249 |
| 34 | .9853 | 89 | .4498 |
| 77 | .5317 | 62 | .9805 |
| 44 | .9620 | 50 | .9699 |

Figure 3. WSD and relative humidity values. Exponential regression was performed on the data ($r^2 = 0.80$).


WSD and Relative Humidity
100
80
60
40
20
0
Relative Humidity
0.0
0.2
0.4
0.6
0.8
1.0
Water Saturation Deficit

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VITA

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He received his Bachelor of Science degree under the Naturalist Program from Appalachian State University in May, 1990 and completed an internship at Puale Bay, Alaska in the summer of 1989. In the fall of 1990 he entered the graduate program at Appalachian State University to begin studies toward a M. S. degree. He currently resides in Todd, North Carolina.