Ecology and Epidemiology

Prediction of Weather-Mediated Release of Conidia of *Botrytis squamosa* from Onion Leaves in the Field

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Journal Series Article 10430 of the Michigan Agricultural Experiment Station.
Accepted for publication 23 November 1982.

ABSTRACT

Lacy, M. L., and Pontius, G. A. 1983. Prediction of weather-mediated release of conidia of *Botrytis squamosa* from onion leaves in the field. Phytopathology 73:670-676.

Large catches of *Botrytis squamosa* conidia with a Burkard recording spore trap in an onion field plot were almost always preceded by a 2- to 3-day period of fairly constant moderate (12–20 C) temperatures and low (0–5 mb) vapor pressure deficits (E_{def}). The more common weather pattern yielding smaller spore catches consisted of greater fluctuations in temperatures (15–30 C) and E_{def} (0–15 mb) values. Frequent rain showers usually occurred during periods when large numbers of spores were trapped, but did not directly trigger spore release. Multiple regression analysis identified temperature and E_{def} as the weather variables most strongly associated with spore release. A sporulation index (based on average temperature and E_{def} values for the preceding 72-hr period) was devised that accurately predicted the initial large release of conidia by *B. squamosa* about 85% of the time. Subsequent conidial releases were predicted with somewhat lower accuracy.

Serious outbreaks of onion leaf blight occur frequently in the United States (9,14), Canada (11), Great Britain (4), and France (19). Although there was some early confusion regarding which *Botrytis* species caused leaf blight (14), it has been satisfactorily proven to be *Botrytis squamosa* (3,4). Germinating conidia on onion leaves cause small (1–2 mm) necrotic spots surrounded by a light green halo. The halo disappears within a few days, and the lesion may expand slightly. If sufficient lesions are present, leaf tip dieback and collapse of entire leaves may occur (4,14), resulting in large yield reductions (9).

Since there are no sources of resistance to onion leaf blight in *Allium cepa* (1), control is usually achieved by 8–12 fungicidal sprays beginning from late June to early July and continuing from late August to late September, depending on the earliness of planting (15,18). Attempts have been made (7,15,16) to reduce the number of sprays by using a biological monitoring technique; sprays were delayed until an average of one lesion on 10 leaves could be found on a sample of 20 plants in a nonsprayed area of the field (16). Because young lesions are inconspicuous, we found that method to be tedious and highly dependent upon the training and experience of the observer. However, our field observations suggested that onion leaf blight epiphytotics usually did not begin until late July or early August, which supported the conclusions of others (7,15,16) that unnecessary sprays probably were being applied. A research group at the University of Guelph examined the relationship of weather variables to conidia release by *B. squamosa* (18) and concluded that leaf wetness duration, high temperatures, and leaf dieback were important factors affecting production and release of conidia, but did not propose a method of prediction.

The experiments described here were designed to determine which weather parameters were associated with production and release of conidia by *B. squamosa*, and whether spore release in the field could be predicted accurately from weather data, which would enable the application of fungicidal sprays only when actually needed.

MATERIALS AND METHODS

Yellow-skinned, long-day, storage-type onions were seeded in early May in a 15.4 × 30.8-m block at the Michigan State University Muck Experiment Farm, Bath, MI, in single rows spaced 53 cm apart. Thiram (tetramethylthiuram disulfide) (0.5 kg active ingredient [a.i.] per hectare) and fonofos (*O*-ethyl-*S*-phenylethylphosphonodithioate) (1 kg a.i. per hectare) were applied in the furrow along with the seed for damping-off and onion maggot (*Hylemya antiqua*) control, respectively. Weeds were controlled with chlorpropham (isopropyl-*m*-chlorocarbanilate, 3 kg a.i. per hectare) and allidochlor (*N,N*-diallyl-2-chloroacetimide, 3 kg a.i. per hectare) applied within 3 days of seeding, and with three to four applications of nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether, 1 kg a.i. per hectare) at 14-day intervals after the onion plants were in the four-leaf stage. Weeds not killed by the herbicides were removed by hand. The plot was irrigated when lack of water threatened to reduce plant growth.

A Burkard 7-day recording spore trap (Burkard Mfg. Co. Ltd., Rickmanswirth, Hertfordshire, England), powered by a 12-V rechargeable battery, was placed several meters to the east of the center of the plot so that it would be downwind of the prevailing west-to-east winds. The sampling orifice was ~42 cm above ground level. A

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recording hygrothermograph, rain gauge, recording anemometer (Weathermeasure Division, Systron Donner Co., Sacramento, CA 94520), and a DeWit leaf wetness recorder (Valley Stream Farm, Orono, Ontario, Canada L0B 1M0) were located nearby. The recording anemometer, which measured wind speed and direction, was placed about 200 m from the plot in an open area away from nearby windbreaks and other obstacles and within 25 m of a small building (the source of the necessary 110-V AC power supply). The anemometer sensor was placed ~6 m above ground.

Spore-trap tapes coated with an adhesive mixture of petroleum jelly:paraffin (9:1, w/w), dissolved in sufficient toluene to give a thick liquid consistency, were removed weekly, cut into 48-mm lengths that represented 24-hr periods, stained with aniline blue in lactic acid (28 mg aniline blue, 20 ml distilled water, 10 mg glycerol, and 10 ml 85% lactic acid), and mounted on glass slides beneath 22 × 50-mm coverslips. Before being mounted, each piece of tape was marked at 2-mm (=1 hr) intervals with a razor blade. These marks were used during spore counting to determine the beginning and end of each hour's spore catches.

Spore counts (*B. squamosa*), hourly temperature, relative humidity, rainfall, leaf wetness, and wind speed and direction data were recorded on coding sheets. The data were then entered on punched cards and stored on magnetic tape until used. Weather variables and spore catches were analyzed by using a stepwise multiple regression analysis program (10) on the Michigan State University's Cyber 750 computer. Spore count data were transformed to natural logarithms before they were analyzed by multiple regression analysis so that the relationship with independent variables was linear and the variance was stabilized. The independent (weather) variables that did not contribute significantly to the coefficient of multiple determination (R^2) were discarded, and the two variables (vapor pressure deficit [E_{def}] and air temperature) that did contribute significantly to R^2 were retained and used to predict spore release. Data were plotted on a Tektronix model 4014-1 graphics terminal connected via modem and telephone with the main computer or on the Cal-Comp 936 Incremental Pen Plotter.

Vapor pressure deficit (E_{def}) was used rather than percent relative humidity (RH) because E_{def} is less temperature-dependent than RH. RH is the ratio of vapor pressure of air saturated with water (E_{sat}) to ambient vapor pressure (E_{amb}):

$$\%\,\mathrm{RH} = (E_{amb}/E_{sat})100 \qquad (1)$$

whereas vapor pressure deficit at temperature T ($E_{def(T)}$) is the difference between saturation and ambient vapor pressure at temperature T:

$$E_{def(T)} = E_{sat(T)} - E_{amb(T)} \qquad (2)$$

$E_{sat(T)}$ changes with temperature, whereas $E_{amb(T)}$ may be the same at different temperatures. Both RH and vapor pressure deficit will vary as temperature changes even though ambient vapor pressure ($E_{amb(T)}$) may remain the same, but the variation in vapor pressure deficit is lower so that this error is reduced by using $E_{def(T)}$. If RH is plotted at various temperatures against corresponding E_{def} values, the relationship is nonlinear (Fig. 1), which illustrates the differences in variation using RH rather than E_{def}.

$E_{sat(T)}$ can be obtained from meteorological tables (6), or can be estimated at any temperature T by using Lowe's (8) algorithm:

$$E_{sat(T)} = a_0 + T(a_1 + T(a_2 + T(a_3 + T(a_4 + T(a_5 + a_6 T))))) \quad (3)$$

in which $a_1, a_2, \ldots a_6$ are the numerical coefficients for each term of the polynomial. This algorithm can be stored in a computer and used to generate $E_{sat(T)}$ values as needed.

Once $E_{sat(T)}$ is known, $E_{amb(T)}$ can be calculated from RH by rearranging equation (1):

$$E_{amb(T)} = \%\mathrm{RH}(E_{sat(T)})/100 \qquad (4)$$

and then $E_{def(T)}$ is calculated by using equation 2. We prepared written tables for converting RH to $E_{def(T)}$ so that $E_{def(T)}$ can be determined quickly and easily when RH and temperature are known. A copy will be furnished free of charge upon request.

Partial correlation coefficients from multiple regression analyses, and predictions from these analyses, were improved when spore counts and weather variables were analyzed by using "days" that ran from 0700 hours one day to 0659 hours the next day, rather than from 0001 to 2400 hours on the same calendar day. This is probably because the rapid diurnal change in E_{def} and temperature occurred between 0700 and 1000 hours, and between 1800 and 2000 hours. E_{def} and temperature values during other periods of time were changing much more slowly, and if the days were broken at midnight, parts of these periods of relatively constant low temperatures, low E_{def}, and dew periods were placed into different days, so that continuity of these periods was lost. This may be especially important where spore catches on a given day depend on weather during the previous 2 or 3 days, as it does in this system.

RESULTS

When numbers of spores caught during various hours of the day were averaged over 60-day periods in both 1977 and 1978, numbers of spores caught showed a strong diurnal periodicity (Fig. 2) confirming previous reports (7,18). Most spores were caught between 0700 and 1400 hours, although deviations from this pattern could be found on individual days, usually when spore release seemed to be delayed by similarity of conditions between dark and daylight hours (dark, cloudy, cool days with low E_{def} with or without rainfall). Release of spores seemed to coincide with

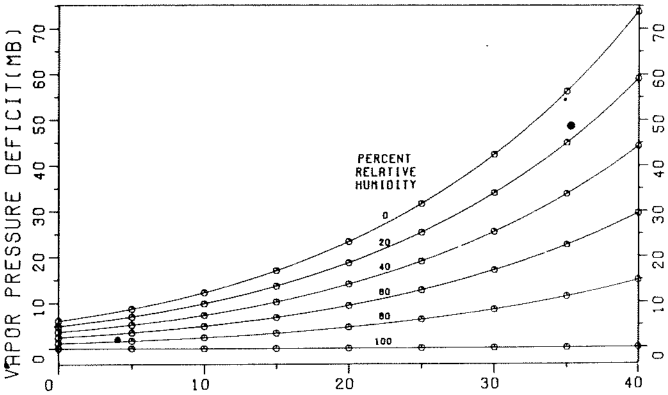

Fig. 1. Nonlinearity of relative humidity with vapor pressure deficit (E_{def}) at temperatures of 0–40 C.

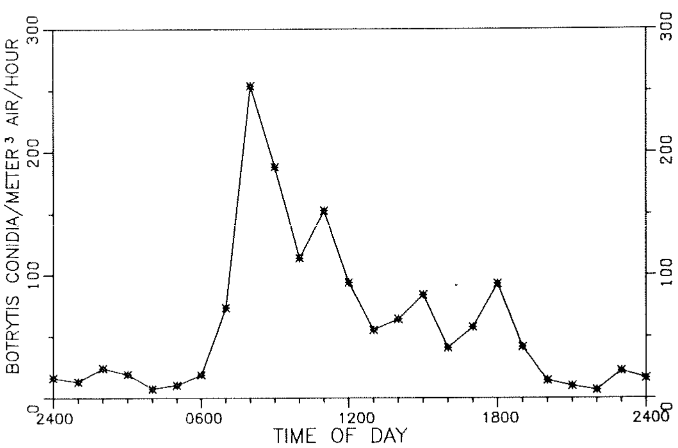

Fig. 2. Diurnal periodicity of *Botrytis squamosa* conidia trapped during a 60-day period.

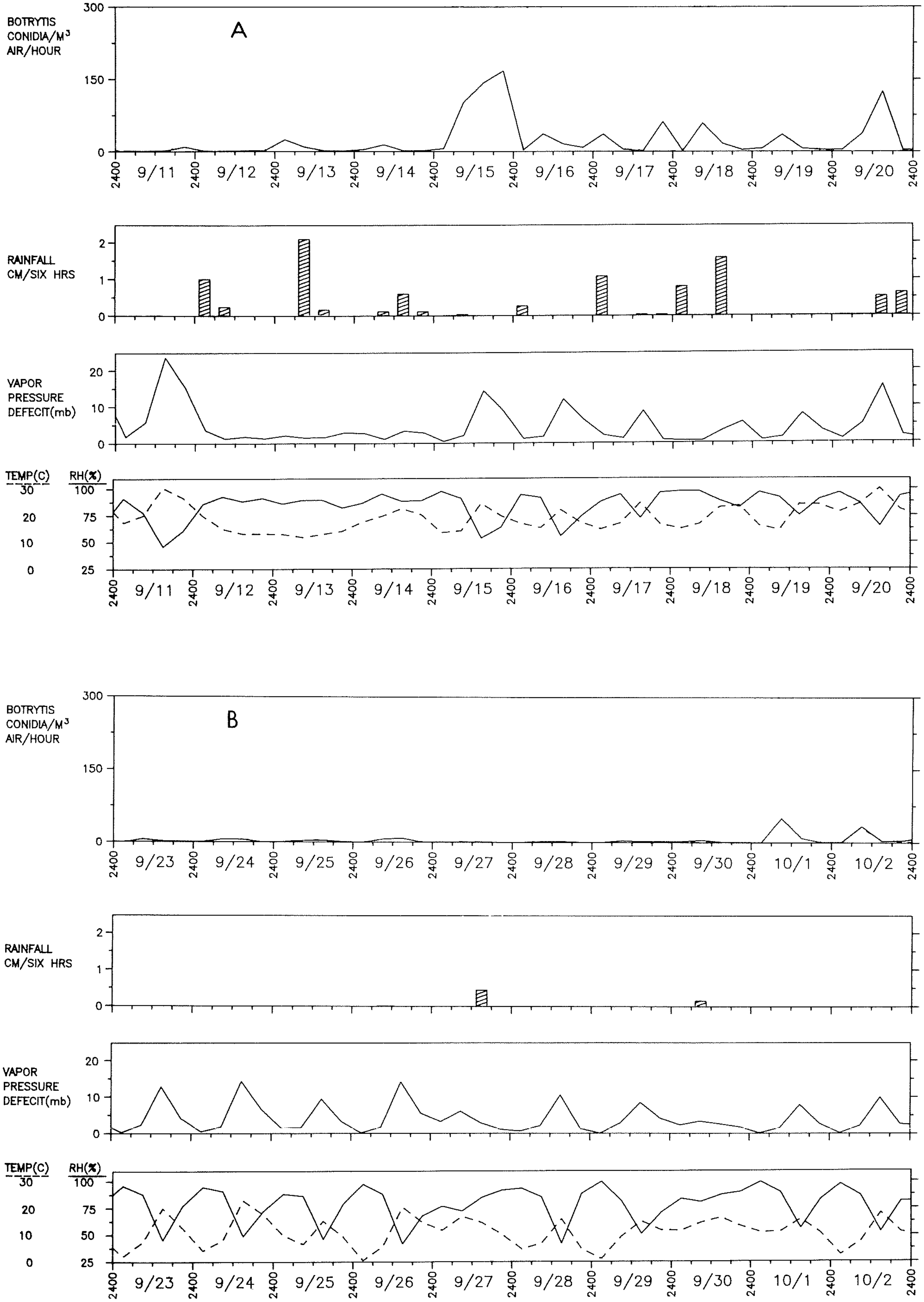

Fig. 3A and **B.** *Botrytis squamosa* conidia trapped and weather variables measured during two 10-day periods in 1977.

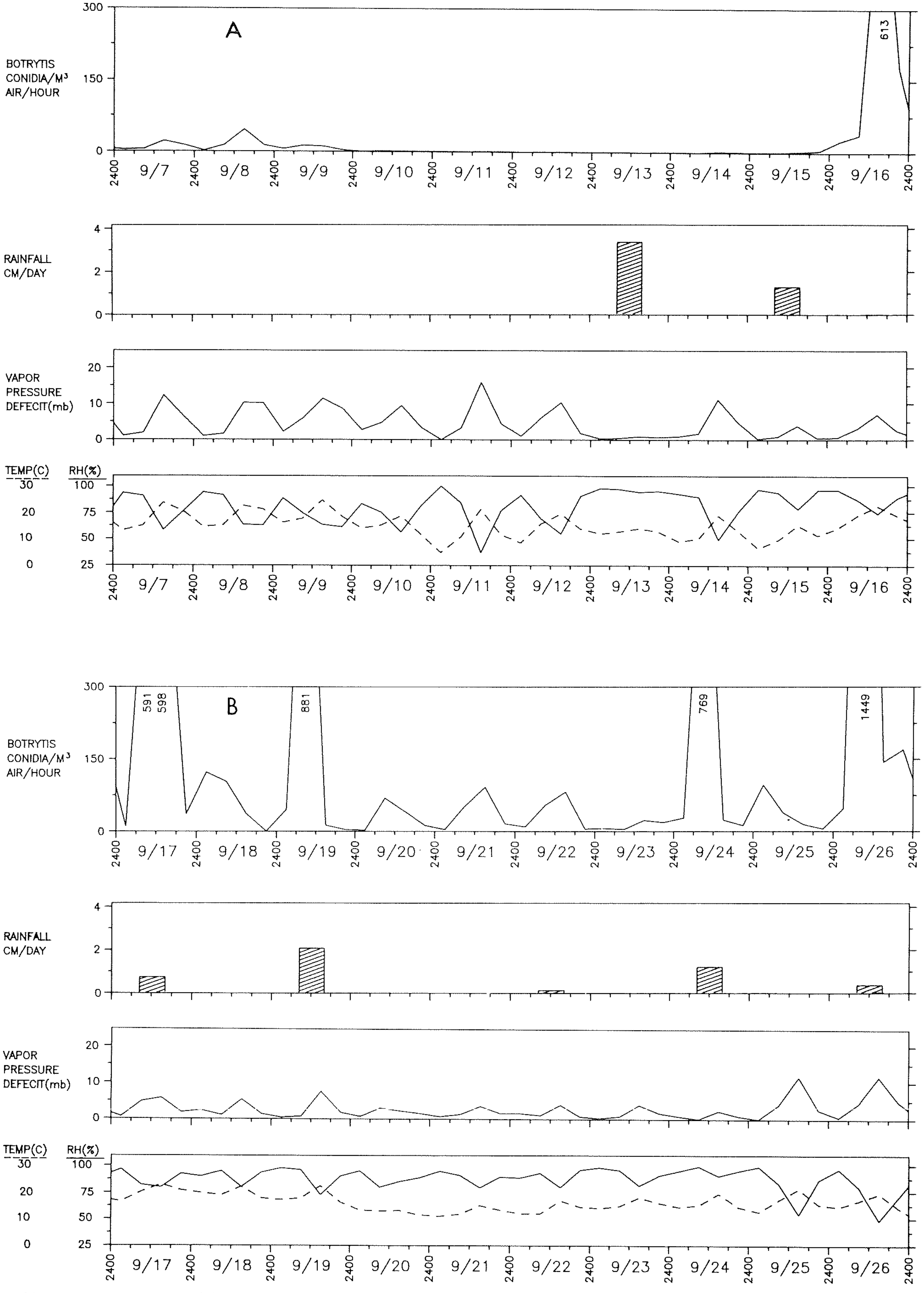

Fig. 4A and **B.** *Botrytis squamosa* conidia trapped and weather variables measured during two 10-day periods in 1978.

changes in air temperature and moisture content.

The initial large spore catch of the season was usually preceded by a two- or three-day period of rather constant cool temperatures (12–20 C) and low E_{def} values (<5 mb), usually accompanied by rain showers (Figs. 3A and 4A). Weather preceding these large spore catches was typically that of 1) fluctuating temperatures (15–30 C) and E_{def} values (0–15 mb), 2) a two- or three-day period of relatively constant cool temperatures and low E_{def}, and 3) a resumption of fluctuating temperatures and E_{def} at the time of the large spore catch (Fig. 4A). Rain showers usually accompanied but did not directly trigger release of spores. Laboratory experiments indicate that *B. squamosa* sporulates more profusely under conditions near saturation than in a fully saturated atmosphere (*unpublished*).

Spore releases in 1977–1978 may have occurred somewhat later than normal because the plots were planted about 2 wk later than usual for Michigan. Also, August weather was not as favorable as September weather in either year for leaf blight development. However, periods favorable to *Botrytis* evidently occur earlier in the season in some years, as evidenced by epiphytotics observed in August.

Total numbers of conidia of *B. squamosa* trapped were larger in 1978 than in 1977, although rainfall was more frequent in 1977 (Fig. 3) than in 1978 (Fig. 4). However, the pattern of spore release was very similar. The data in Figs. 3 and 4 are representative. Other data collected earlier in the season are not shown in the interest of brevity.

Stepwise multiple regression analyses (10,17) revealed that temperature and E_{def} were the two variables most strongly associated with spore release. In a given period of time (10–14 days) being analyzed, spore releases on any given day during that period could be predicted with 85% accuracy (ie, 85% of the variation in numbers of spores trapped could be accounted for using the regression equation) using only average temperature and E_{def} values for the previous 72 hr. A regression equation of the general form

$$\hat{y} = b_0 + b_1 \text{ Temp.} + b_2 E_{def} \tag{5}$$

was used, in which $\hat{y}$ = estimate of number of spores trapped on a given day, b_0 is a constant and b_1 and b_2 are partial regression coefficients.

The longer the period of time that were analyzed greater than 14 days, the lower was the percentage of variation in spore catches that could be explained with weather variables. Further, when temperature and E_{def} values were used to predict spore catches using coefficients (equation 5) obtained from analysis of a given time period, daily spore catches could be predicted accurately for the time period used in the multiple regression analysis, but when this same equation was used to attempt to predict daily spore catches from a different time period, predictive accuracy usually fell off significantly. The loss in predictive accuracy in a time sequence removed from the time period used in generating the multiple regression equation is a well-known phenomenon (2). To avoid this problem, an empirical sporulation index (Table 1) for *B. squamosa* was constructed. The natural logarithm (ln) of average total numbers of spores caught during 1977–1978 in 24-hr periods during 16- to 25-day periods with more than one large spore catch was plotted against average E_{def} values at various temperatures (Fig. 5). Maximum number of spores caught was about e^{10}, so a

TABLE 1. Sporulation index for prediction of release of *Botrytis squamosa* conidia

Vapor press. deficit (mb)	Average temperature (C)																
	46.4[a]	48.2	50.0	51.8	53.6	55.4	57.2	59.0	60.8	62.6	64.4	66.2	68.0	69.8	71.6	73.4	75.2
	8[a]	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0.00[a]	90[b]	92	94	96	97	97	98	98	98	99	99	99	99	99	100	100	100
0.25	88	91	93	95	96	97	97	98	98	99	99	99	99	99	99	100	100
0.50	86	88	91	94	95	96	97	97	97	98	98	98	99	99	99	99	99
0.75	83	86	89	92	94	95	96	96	97	97	98	98	98	99	99	99	99
1.00	79	83	87	90	92	94	95	96	96	97	97	98	98	98	99	99	99
1.25	75	80	84	87	90	92	94	95	96	96	97	97	98	98	98	99	99
1.50	67	78	81	85	87	90	92	94	95	96	96	97	97	98	98	98	98
1.75	60	69	77	82	85	87	90	92	94	95	96	96	97	97	97	98	98
2.00	51	62	71	78	82	85	88	90	93	94	95	96	96	97	97	97	98
2.25	42	55	65	74	79	82	85	88	91	93	94	95	95	96	96	97	97
2.50	31	46	59	68	75	79	83	85	89	91	93	94	94	95	95	96	97
2.75	23	36	50	62	70	76	80	83	86	89	91	93	93	94	95	95	96
3.00	17	28	40	53	64	71	77	80	84	86	89	91	92	93	94	95	95
3.25	12	20	32	44	56	65	72	77	81	84	86	89	91	92	93	94	94
3.50	7	15	25	37	48	58	66	72	77	81	84	86	89	90	92	92	93
3.75	4	10	20	29	39	50	60	67	73	78	81	84	86	88	89	91	92
4.00	1	6	14	22	32	43	52	62	68	74	77	81	83	85	87	88	90
4.25	0	4	9	17	25	36	45	54	62	68	74	76	80	82	84	85	87
4.50	0	2	7	12	20	28	38	46	54	61	67	72	75	78	80	82	83
4.75	0	1	4	9	15	22	31	39	47	53	59	64	71	72	75	78	80
5.00	0	0	2	6	11	17	25	32	39	44	51	56	60	68	68	71	74
5.25	0	0	0	3	8	13	19	25	32	38	44	48	52	56	61	64	66
5.50	0	0	0	1	5	9	15	20	25	31	37	40	45	49	52	55	58
5.75	0	0	0	0	3	6	10	16	20	25	29	33	37	41	45	48	50
6.00	0	0	0	0	1	4	7	11	15	19	23	26	30	33	37	40	42
6.25	0	0	0	0	0	2	4	7	11	14	17	20	24	27	30	33	35
6.50	0	0	0	0	0	0	2	4	7	10	12	15	18	20	23	25	28
6.75	0	0	0	0	0	0	0	1	4	6	8	10	12	14	18	19	22
7.00	0	0	0	0	0	0	0	0	1	3	5	6	8	9	13	14	17
7.25	0	0	0	0	0	0	0	0	0	1	2	3	4	5	9	10	12
7.50	0	0	0	0	0	0	0	0	0	0	0	1	2	3	5	7	8
7.75	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	4
8.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

[a] Average temperatures (Fahrenheit above and Celsius below) and vapor pressure deficits (E_{def}) calculated from hourly observations recorded during the previous 72 hr.

[b] The higher the value of the index number, the more likely that a large release of *B. squamosa* conidia will occur on the following day.

logarithmic scale from 0–10 was used to correspond with spore numbers, and each logarithm was multiplied by 10 to create a scale from 0–100. Data points of ln (daily spore catch) × 10 were plotted against various E_{def} values at a given temperature and a curve was fitted to the data points by hand, with extrapolation to extreme values. Temperatures of 8–24 C and E_{def} values of 0–8 mb were used to fit the curves. Values were then read from the curves and inserted in the predictive index table (Table 1). The table is entered with average temperature and E_{def} values calculated from data collected hourly during the previous 72 hr, and the value in the table occurring at the intersection of the coordinates indicates the likelihood of a spore release of significant magnitude. The larger the number (0–100), the higher the probability of significant spore release. A value $\geqslant$50 was empirically selected as a sufficiently high index value to warrant a spray application. This threshold value may have to be adjusted upward or downward, depending on local conditions.

The predictive index has been tested on spore and weather data from 1977, 1978, and 1980. In cases where a spore-conducive period was preceded by a period $\leqslant$5 days of fluctuating temperature and E_{def} values with low numbers of spores caught, a large spore catch could be predicted about 85% of the time. Once a large spore catch occurred, it tended to be followed with several more days of relatively large spore catches. Accuracy of predicting these later peaks was somewhat lower than for the initial peaks.

DISCUSSION

Temperatures below 12 C or above 25 C seemed to have a direct limiting effect, exclusive of the effect of E_{def}, on conidial formation and release in these studies. In controlled laboratory studies by others, temperatures either lower or higher than optimum had a direct limiting effect on sporulation of other fungi, but may also have interacted with other environmental factors such as light or air humidity in affecting amount of sporulation (13). In *Rhynchosporium secalis* on barley, for example, the optimum temperature for sporulation depended on leaf wetting duration, since temperature and leaf wetting period evidently affected the development of antagonistic bacteria (12).

Generally most fungi sporulate best under low (<5 millibars) E_{def} conditions, although they can sporulate to some extent under a wide range of air humidities (5,13). Sporulation of some fungi has been reported to be enhanced or even triggered by leaf wetness (13,18). It is difficult to study the effects of E_{def} and leaf wetness separately under conditions of high air humidities because of transient water condensation on leaves. In our studies, leaf wetness was evidently not necessary for spore formation, and long periods of leaf wetness seemed to inhibit conidial production.

Multiple regression analysis was found to be a very valuable tool for identifying the weather variables most closely associated with production and release of significant numbers of conidia of *B. squamosa*. However, the longer the period of time analyzed greater than 14 days, the lower the coefficient of multiple determination (R^2) obtained, and the lower the partial regression coefficients. This was probably due in part to the fact that many weather variables (eg, temperature) vary intermittently, and the value of each observation is partly determined by its position in the time series (2). This introduces systematic error, and the more observations that are included, the more systematic error is introduced into the analysis, which detracts from precision. Where multiple regression is used for prediction, Butt and Royle (2) advocate the generation of new equations each year or even more frequently. There is always the danger of extrapolation, where the equation estimates erroneously new values, which lay outside the range of the independent variables used in calculating the equation. There is also the danger of some new factor(s), which either are not or cannot be measured, influencing the results. Regression equations are empirical in that they predict from a limited number of observed responses, and cannot be expected to allow for unexpected changes in weather patterns, cultural practices, or other factors.

One of the reasons that we were able to use only two variables in our predictor table is that there was some dependency between our so-called independent variables. Interrelationship between independent variables does not necessarily prevent the formulation of a good predictor equation with a high R^2, but the accuracy and statistical significance of the partial regression coefficients may be low (2). A weather variable such as rainfall will obviously have some effect on E_{def}, and even though rainfall may be eliminated from the MRA equation, the influence of rainfall on E_{def} still exists. Thus, when E_{def} is measured, the effects of rainfall are being measured indirectly to some extent.

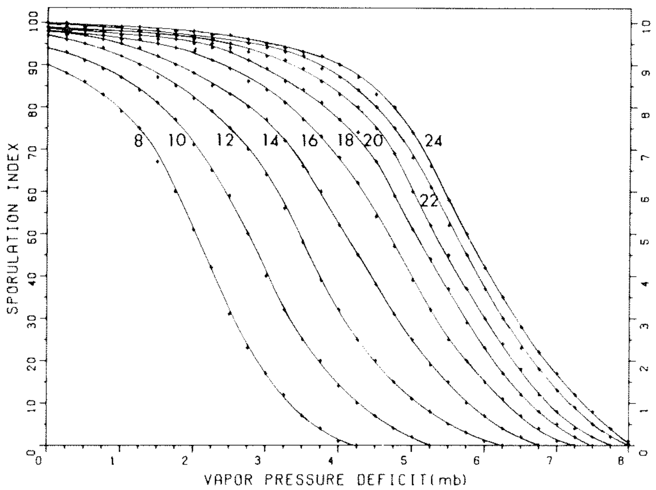

Fig. 5. Relationship of ln spore catches to vapor pressure deficit at various temperatures.

Scatter diagrams of the variation in the dependent variable not explained by the independent variables were used to ensure homoscedasticity, which is an important assumption when making inferences about data using multiple regression.

It is possible that other nonmeasured variables such as light intensity may have some effect on spore release. Such effect may or may not be indirectly reflected in one of the measured variables, such as temperature.

We found that the empirical sporulation index table was much more accurate in predicting spore release than a regression equation from a particular data set. Although this approach is unconventional, we feel it has potential value in prediction of plant disease. Further field testing of this system is underway.

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