An Epidemiological Study of Mummy Berry Disease of Highbush Blueberry

D. C. Ramsdell, J. W. Nelson, and R. Myers

Assistant Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824; Director of Research, Michigan Blueberry Growers Association, Grand Junction 49056; and Laboratory Technician, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

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ABSTRACT

A Burkard recording volumetric spore trap was operated in a highbush blueberry field in Michigan from 19 April to 15 June 1972. Ascospores of *Monilinia vaccinii-corymbosi* (incitant of mummy berry disease) were trapped from 28 April to 11 May, a period during which the bushes progressed from green tip to pink bud prebloom stage. Ascospore discharge occurred mostly during daylight when relative humidity (RH) was <100%; very few spores were trapped at night when RH was 100%. Ascospore discharge was inversely correlated with RH and wind speed ($P = <0.001$ and <0.01, respectively). Nocturnal periods of continuous leaf wetness ranged from 5.5 - 12.0 h which, according to germination studies on glass slides, could allow primary infection to occur. Bushes had a mean of 92 visible leaf and shoot infections by 23 May. Triarimol sprays applied 72 h after the first major ascospore discharge on 4 May, while bushes were still at the green tip stage, and again on 15 May, were much more effective in reducing primary infection than sprays applied only on 15 May. Trapping of conidia commenced 19 May, when bushes were at ca. 20% pink bud prebloom stage, and ended 3 June at petal fall. Large numbers of conidia were trapped both during day and night. Release of conidia was inversely correlated with leaf wetness ($P = <0.1$) and directly correlated with wind speed ($P = <0.1$). Continuous leaf wetness at night ranged from 1.5 - 12.0 h during the period conidia were trapped.

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Mummy berry disease of highbush blueberry *(Vaccinium corymbosum* L.) is incited by the fungus *Monilinia vaccinii-corymbosi* (Reade) Honey. A description of the fungus and disease cycle was given by Honey (8). This disease is of major economic importance in Canada and the northern United States (15). Pepin and Toms (12) calculated a loss of 8.14% of the highbush blueberry crop in British Columbia resulting from this disease in 1969. Commercial control of the disease consists of chemical eradication of the apothecial stage in the spring (4). There is a low degree of resistance in some commercially grown cultivars (13), but additional control measures are necessary. Nelson and Bittenbender (11) showed that honeybees vector conidia from ascosporic infections of leaves and shoots to the blossoms and thereby increase the incidence of the mummy berry phase of the disease. Therefore, foliar fungicide sprays are needed during the blossom period. There has been no report to date of an epidemiological study involving spore trapping and measurement of the influence of climatological parameters on spore release, relative to the phenology of the host. The purpose of this work was twofold: (i) to determine the environmental conditions favorable for dispersal of both ascospores and conidia and (ii) to relate the timing of spore release to stages of host plant development in order to improve the timing of protectant fungicidal control of mummy berry disease.

MATERIALS AND METHODS.—*Trapping of ascospore and conidia relative to certain climatological factors.*—The studies were conducted in a highbush blueberry field containing mature bushes of the cultivars 'Jersey' and 'Rubel', which comprise about 75% of Michigan's acreage, near Pullman, in southwestern Michigan, during the 1972 season. The field was 45 × 120 meters (49.2 × 131.2 yds) in size.

A Burkard 7-day recording volumetric spore trap (2) (Burkard Scientific Sales Ltd., Rickmansworth, Hertfordshire, England) was placed in the center of the narrow dimension of the field and about two-thirds of the way downwind on the long dimension of the field. Prevailing winds are from the west. The spore trap orifice was located 0.5 m (19.7 in) above ground level. Bush height ranged from 1.5 - 2.0 m (5.0 - 6.7 ft). The trap, situated midway between four bushes and 1.0 m (39.4 in) equidistant from them, was operated continuously from 19 April 1972, when both cultivars were at bud swell stage until 15 June 1972, when the bushes were at 100% petal fall stage and spore liberation was completed. The air pump which served the trap delivered 10 liters/min (10.6 qt/min) airflow through the trap orifice. Hourly counts of ascospores and conidia collected on the trap tape surface were made after staining the tape surface with 0.5% aqueous safranin. Counts were made by traversing across the width of the tape with a light microscope objective using ×187.5 magnification. Ascospores were collected on tape, stained, and used for a reference. The distinctive football shape and average size (5.3 × 9.7 μm) of ascospores made their identification relatively simple. A reference tape was also made for conidia. Conidia of *M. vaccinii-corymbosi* bear one or two disjunctors; thus, their identification was relatively easy and accurate. Their average size was 15.2 × 20.8 μm. Actual numbers of spores trapped/m^3/h are reported. No attempt was made to correct for trap efficiency, which is rated at 70% ± 20% for spores of this size range (10).

A sheltered 7-day recording hygrothermograph

(Bendix Corp., Baltimore, Md.) ca. 0.5 meter (19.7 in) above ground, and a 7-day recording leaf wetness meter (M. DeWit, Hengelo, Holland) were located 5 m (16.4 ft) from the spore trap. The hygrothermograph was calibrated weekly with a sling psychrometer. All times reported are Eastern Standard Time (EST). Amount and duration of rainfall were determined from data recorded at a U.S. Weather Bureau station about 6.4 km (4 mi) from the test field. Wind speeds were determined every 3 h by a U.S. Weather Bureau station at Muskegon, Michigan, 56.5 km (35 mi) north of Pullman. According to U.S. Weather Bureau personnel, wind intensity and duration are essentially identical at the two locations. Both locations have flat terrain and are located approximately 11.3 km (7 mi) east of Lake Michigan. Prevailing winds in that region are usually from the west across the lake.

Measurement of stage of development of bushes relative to spore release.—The stage of vegetative and flower development of the blueberry bushes was measured at intervals during the spore trapping period. The mean length of 50 leaves located just below terminal blossom buds or flowers was used as an index of vegetative growth. Stage of blossom development was also recorded. Numbers of apothecia/m^2 (ft^2) on the ground under the bushes were counted at intervals and related to spore counts.

Ascospore germination studies.—Ascospores were discharged from mature apothecia onto clean glass slides in the laboratory and suspended in glass-distilled water for germination studies in free water. In addition, germination of ascospores was investigated at 98 and 100% relative humidity (RH). Ascospores were discharged onto slivers of cover slip glass, which were then held in place by inserting them into a clay ball situated on the center well of a sealed Warburg flask.

TABLE 1. Summary of highbush blueberry growth/development relative to *Monilinia vaccinii-corymbosi* spore trapping, infection, and protective fungicide spraying at Pullman, Michigan, 1972

Developmental event	Date (1972)	Cultivar	
		'Jersey'	'Rubel'
Vegetative development			
leaf bud swell	4/19	100%	100%
early green tip stage (length)	5/2	5.1 mm (0.20 in)	6.1 mm (0.24 in)
late green tip stage (length)	5/11	8.8 mm (0.35 in)	11.3 mm (0.44 in)
young leaf stage (length)	5/15	13.9 mm (0.55 in)	18.0 mm (0.71 in)
young leaf stage (length)	5/23	40.0 mm (1.68 in)	44.0 mm (1.85 in)
young leaf stage (length)	6/1	70.0 mm (2.94 in)	79.0 mm (3.32 in)
Blossom development			
blossom bud swell	4/19	100%	100%
blossom truss buds open	4/28	100%	100%
blossom buds protruding from truss buds	5/2	56%	51%
pink bud prebloom stage	5/11	100%	100%
percent bloom	5/23	20%	20%
percent petal fall	6/1	14%	1.5%
percent petal fall	6/15	100%	100%
Avg. no. leaf and shoot infections/bush (bushes surrounding spore trap)	5/23	94.0	90%
Avg. no. leaf and shoot infections/bush (fungicide timing test bushes)[a]			
control	5/23	68.4	---
triarimol, 40 mg/liter (0.53 oz/100 gal), sprayed 5/15	5/23	60.9	---
triarimol, 40 mg/liter (0.53 oz/100 gal), sprayed 5/4 and 5/15	5/23	30.0	---
triarimol, 80 mg/liter (1.06 oz/100 gal), sprayed 5/4 and 5/15	5/23	11.6	---

[a]LSD ($P = 0.01$) = 28.9.

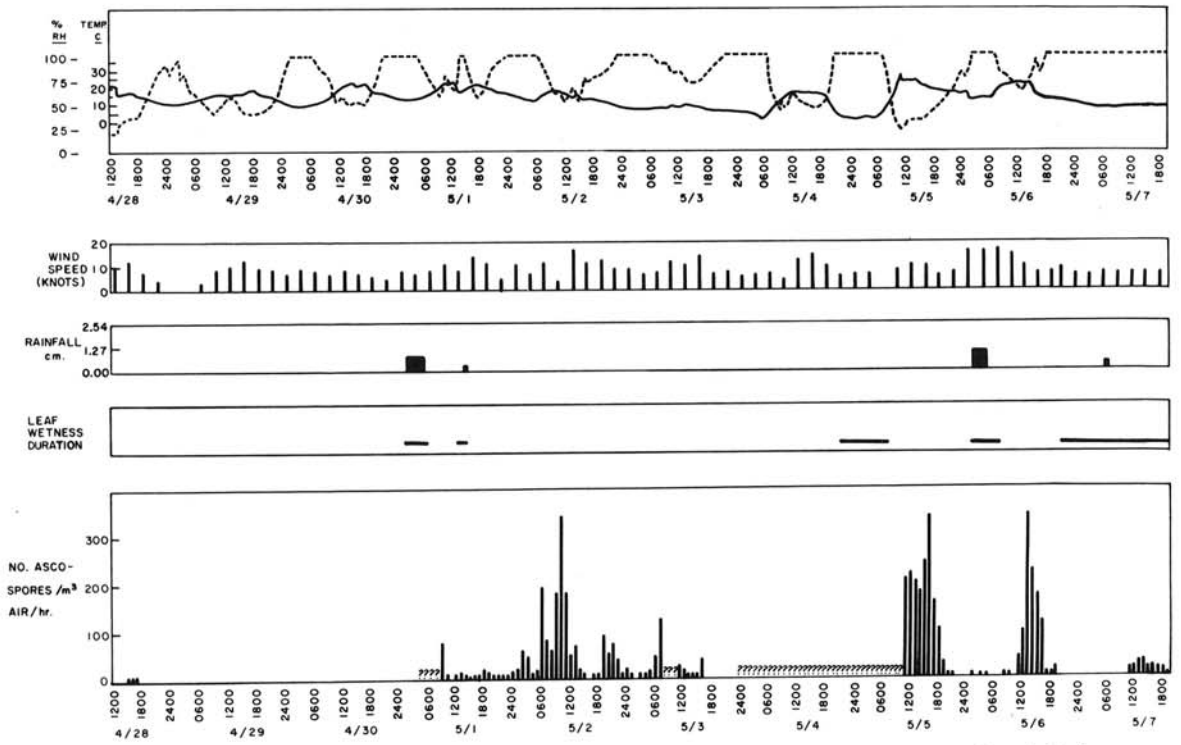

Fig. 1. Hourly *Monilinia vaccinii-corymbosi* ascospore trap counts and climatological data for the peak period of ascospore discharge in a highbush blueberry field at Pullman, Michigan, 1972. Time intervals marked (?) indicate periods of trap malfunction. The dotted line indicates percent relative humidity and the solid line indicates temperature.

Glass-distilled water and a saturated K_2SO_4 solution were placed in the bottoms of the flasks for 100% and 98% RH, respectively. The slides and flasks were placed in constant temperature boxes at 5, 15, and 20 C ± 1 C (41, 59, and 68 F ± 1.8 F). Percent germination of 100 spores/replication and germ tube lengths of 10 spores/replication were recorded at 6, 24, and 48 h. Each treatment was replicated three times.

Conidial germination studies were impossible due to a very low rate of germination.

Effect of protectant fungicide spray timing relative to counts of trapped ascospores, host development, and reduction of primary infection.—A randomized complete block design field plot was established in the vicinity of the spore trap. Treatments consisted of: (i) an untreated control; (ii) triarimol [α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol] at 40 mg (active ingredient)/liter (0.53 oz/100 gal) water applied on 15 May only; (iii) triarimol applied at the same rate on 4 May and on 15 May; (iv) triarimol applied at 80 mg/liter (1.06 oz/100 gal) on both dates. Seven single-bush replications of Jersey were sprayed with a knapsack sprayer at 1 liter/bush (250 gal water/acre). On 4 May, 5-6 mm (0.2 - 0.24 in) of green leaf tissue was protruding from the leaf buds. On 15 May, leaves were about 14 mm (0.56 in) long. Leaf and shoot infection counts made on the spray plot bushes after primary infection was completed, were used as an index of control given by the various treatments. In addition, leaf and shoot infection counts were made on four additional untreated bushes of both Jersey and Rubel immediately around the spore trap to relate disease severity adjacent to the trap to spore counts. An unseasonable frost on 10 to 11 June almost eliminated the berry crop and precluded determining incidence of mummy berry as an index of secondary infection.

Computer analysis of spore trapping and climatological data.—The climatological and spore trapping data, for both spore types, on an hourly basis were subjected to complete multiple regression analysis. A Model 3600 Computer (Control Data Corp.) was used for data anlaysis. The Michigan State University Agricultural Experiment Station STAT Series least squares regression program (10) was employed.

RESULTS.—*Spore trap counts relative to blueberry bush development.*—(See Table 1 for summary of bush phenology relative to spore trapping and weather data.) The first ascospores, $<10/m^3/h$ ($<0.4/ft^3/h$) were trapped from 1200 to 1800 h on 28 April (Fig. 1). At this time blossom truss buds of both cultivars were open, blossom buds were becoming exposed, and leaf buds were beginning to show green tips (Fig. 2-A). The first continuous catch of ascospores commenced at 0900 h or earlier on 1 May, but spore counts were low. On 2 May, a major discharge of ascospores began at 0600 and subsided by 1600 h, and a peak of 338 spores/m^3 (13.2/ft^3) air was trapped between 1000 and 1100 h. A second smaller release occurred from 1900 to 2300 h. New leaves of Jersey and Rubel were an average of 5.1 mm (0.20 in) and 6.1 mm (0.24 in) long, respectively, and were still tightly rolled. Spore discharge on 3 May was low with a peak

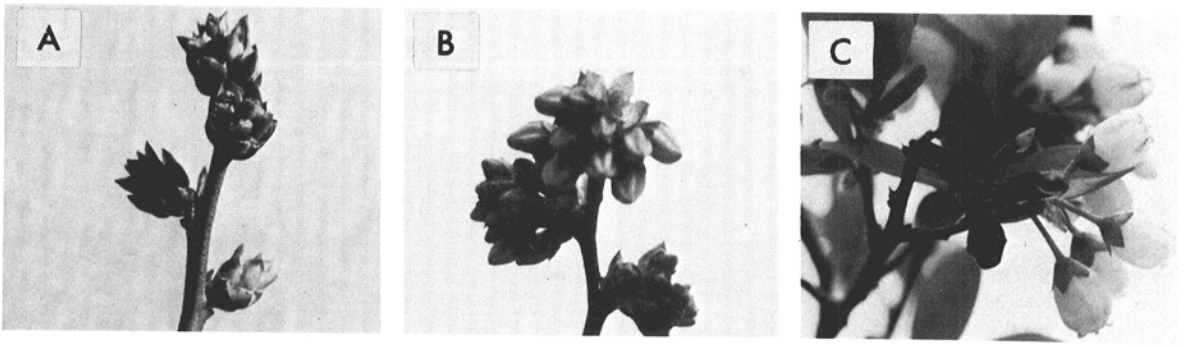

Fig. 2-(A to C). Prebloom stages and ascosporic infection of 'Jersey' highbush blueberry by *Monilinia vaccinii-corymbosi.* **A)** blossom truss buds opened, exposing tightly closed individual blossom buds. **B)** blossom buds shown are at the early pink bud prebloom stage. **C)** an ascosporic leaf and shoot infection, which gives rise to conidia, which infect blossoms, causing mummy berries.

trap count of 124 spores/m^3 (4.8/ft^3) air between 0700 and 0800 h. On 4 May there was an average of 37 apothecia/m^2 (2.4/ft^2) under the bushes. Because of spore trap malfunction, the next major release of ascospores was not detected until 1200 h on 5 May. A peak catch of 340 spores/m^3 (13.3/ft^3) air occurred between 1700 and 1800 h and subsided by 2000 h. The last major catch, similar in most aspects to that of 5 May, occurred on 6 May. On this day, the seasonal peak ascospore catch of 342/m^3 (13.3/ft^3) air occurred between 1300 and 1400 h.

Daily catches of diminishing magnitude occurred through 10 May, beginning from 0800 to 1100 h and reaching a peak of from 42 to 75 spores/m^3 (1.6 to 2.9/ft^2) air/h between 1200 and 1600 h. The last ascospores were caught at 1500 h on 11 May. On this date both cultivars were at 100% pink bud prebloom stage as shown in Fig. 2-B, and new leaves averaged 8.8 mm (0.35 in) and 11.3 mm (0.44 in) in length, respectively, and were becoming flattened out. By 15 May, the density of apothecia was reduced to 4.5/m^2 (0.5/ft^2) under the bushes and they

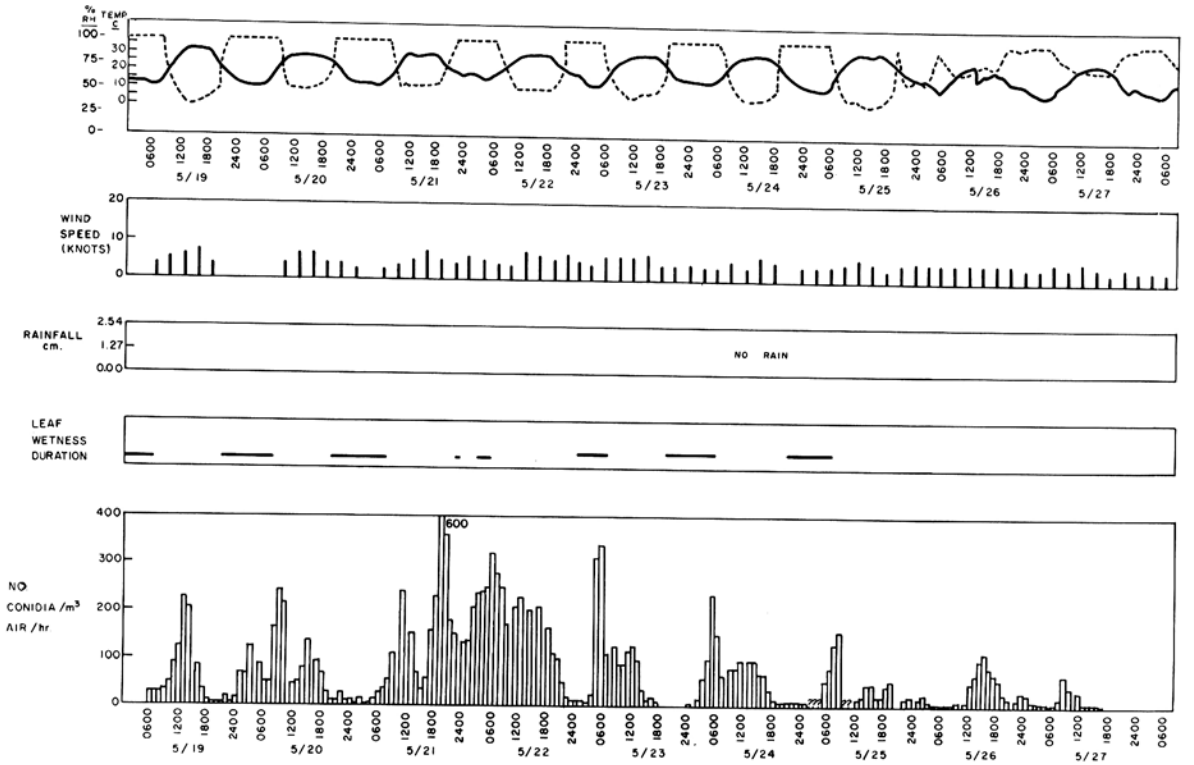

Fig. 3. Hourly *Monilinia vaccinii-corymbosi* conidial trap counts and climatological data for the peak period of conidial release in a highbush blueberry field at Pullman, Michigan, 1972. The dotted line indicates percent relative humidity and the solid line indicates temperature.

appeared dried. On this date, prebloom development had not progressed appreciably since 11 May, but average leaf lengths were 13.9 mm (0.55 in) and 18.0 mm (0.71 in), respectively, for cultivars Jersey and Rubel.

The first ascosporic leaf and shoot infections appeared on 21 May, 23 days after the initial ascospore discharge. Infected leaves and shoots were turning dark brown (Fig. 2-C) and conidial sporulation on infected tissue was apparent.

The main ascospore liberation period lasted 10 days. No more spores were caught until 0600 h on 19 May, when the first conidia were trapped (Fig. 3). Peak numbers of conidia trapped on this date were 230/m^3 (8.9/ft^3) air, occurring from 1300 to 1400 h, and subsided to low levels by 1800 h. On 20 May a major peak of conidia, 214/m^3 (8.4/ft^3), occurred at 0900-1000 h and two minor peaks at 0300-0400 and 1500-1600 h. Two major conidial peaks occurred on 21 May; one from 1000-1100 h and a very large one from 0700-0800 h, 600 conidia/m^3 (23.4/ft^3), which was the seasonal high. Counts of trapped conidia continued at a fairly high level through the night. Large numbers of conidia were trapped on 22 May through the day and night until about 2100 h. Conidia were trapped daily from 22 May according to the previously described pattern, and decreased in total numbers daily until 3 June when the last conidia of the season were trapped from 1400-1500 h. On 23 May, no apothecia were found beneath the bushes.

Primary infection relative to climatological factors.—Disease incidence was surprisingly high by 23 May in spite of low rainfall during the primary infection period. A total of 2.79 cm (1.1 in) was measured from the onset of ascospore trapping until primary infection symptoms appeared. Leaf and shoot infections averaged 90 and 94 per bush on Jersey and Rubel, respectively, immediately surrounding the spore trap. The longest continuous period of rainfall was 5 h. Only 0.38 cm (0.15 in) was measured during the period of conidial trapping.

Ascospores germinated readily in glass-distilled water (Table 2) at all three temperatures within 6 h. Fifteen C (59 F) was the most favorable temperature for ascospore germination and germ tube growth under laboratory conditions. No ascospores germinated under conditions of 98% or 100% RH at any of the temperatures. Although the amount and duration of rainfall at the field site was slight, there were frequent periods of continuous leaf wetness caused mainly by dew during the ascospore release period, which extended into early morning and lasted from 5.5 - 12.0 h. One extended period of continuous leaf wetness lasted 32 h.

Although conidial germination studies were not possible, there were similar continuous periods of free moisture at night and early morning during the conidial trapping period. The temperatures during this period were 10-15 C (50-59 F). Temperatures were higher during conidial trapping compared to those found during the ascospore trapping period.

Ascospore and conidial release as measured by spore trap counts relative to climatological factors.—Results of the computer analysis of spore trap and weather data are expressed as both simple and partial correlation coefficients (Tables 3, 4). Partial correlation coefficients between each climatological parameter (independent variable) and numbers of spores trapped (dependent variable) are determined by analyzing the effect of a single independent variable upon the dependent variable, while all other independent variables are held constant. Therefore, a partial correlation coefficient gives a sharper indication of the "real" effect of a given climatological parameter upon spore release than does a simple correlation coefficient.

The effect of rain and free moisture (leaf wetness) upon the release of ascospores was essentially nil (Table 3); although significant negative simple correlations exist, the partial correlation coefficients are nonsignificant.

TABLE 2. Germination of *Monilinia vaccinii-corymbosi* ascospores in water at various temperatures

	Germination (%)[a] and germ tube length (μm)[b]					
	6 h		24 h		48 h	
Temperature C	(%)	(μ)	(%)	(μ)	(%)	(μ)
5	38	14	43	115	45	186
15	39	84	74	215	79	451
20	38	19	44	92	46	393

[a]One hundred spores were counted/replication; each treatment was replicated three times.

[b]The germ tube lengths of ten spores/replication were measured.

Relative humidity had a pronounced inverse effect upon ascospore release. Both simple and partial correlation coefficients were highly significant. ($P = <0.001$ for both coefficients). As relative humidity dropped below 100%, ascospore trap counts increased sharply (Fig. 1). Temperature showed a highly significant direct simple correlation with ascospore discharge, but the partial correlation coefficient was nonsignificant. The seemingly important direct effect of temperature upon ascospore release is rather an indirect one, e.g., an effect upon relative humidity. Figures 1 and 3 show the inverse relationship between temperature and relative humidity. There is a highly significant inverse simple correlation coefficient of −0.650 between temperature and relative humidity (not shown in Table 3). Wind showed a significant inverse partial correlation with ascospore trap counts ($P = <0.01$).

Because there was so little rain during the period of conidial release, the effect of rain was not included in the computer analysis of these data. Temperature and RH had no effect upon conidial release (Table 4). The number of conidia trapped (Table 4) was inversely correlated with leaf wetness ($P = <0.1$) and directly correlated with wind speed ($P = <0.1$).

Effect of protectant fungicide spray timing relative to ascospore trap counts, host development, and reduction of primary infection.—Nontreated control bushes on 23 May had a mean of 68.4 leaf and shoot infections per bush (see Table 1 for summary). The triarimol spray at 40 mg active ingredient/liter (0.53 oz/100 gal) water applied on 15 May (although unknown at that date, ascospore discharge was finished) resulted in a mean of 60.9 leaf and

TABLE 3. Complete multiple regression analysis of *Monilinia vaccinii-corymbosi* ascospore trap counts (dependent variable) as affected by climatological factors, Pullman, Michigan, 1972[a]

Independent variables	Regression coefficient	Standard error of regression coefficient	Level of significance of regression coefficient	Correlation coefficients	
				Simple[b]	Partial[c]
Leaf wetness	10.783	38.617	0.038	−0.190*	0.030
Rain	−0.909	45.415	0.984	−0.128*	−0.002
Wind	−8.468	2.494	0.001	−0.191*	−0.346*
Temperature	0.925	0.857	0.283	0.420**	0.116
Relative humidity	−1.970	0.485	<0.0005	−0.554**	−0.403**
				R^{2}[d] = 0.441**	
				= 0.664**	

[a] Data input are on an hourly basis and for the peak period of ascospore trap counts.
[b] ** indicates significant difference, $P = <0.001$; * $P = <0.1$.
[c] ** indicates significant difference, $P = <0.001$; * $P = <0.01$. For numbers not followed by an asterisk, the probability of significant difference, $P = >0.1$.
[d] R = Multiple correlation coefficient; R^2 = coefficient of determination; ** $P = <0.001$.

shoot infections/bush. The same spray concentration applied on 4 May (about 72 h after the onset of the first sizeable ascospore shower and bushes at green tip stage) and again on 15 May resulted in a mean of 30 infections/bush. Triarimol at 80 mg/liter (1.06 oz/100 gal) water applied on 4 May and on 15 May resulted in a mean of 11.6 infections/bush.

DISCUSSION.—Although our data show a strong inverse relationship between relative humidity and ascospore release, they are at variance with those of Skilling (14), who found no correlation between ascospore discharge and RH in field studies involving *Scleroderris lagerbergii.* However, Hadley (5), working with *Claviceps purpurea,* observed that ascospore discharge was restricted in a saturated atmosphere, but greatly increased as RH was lowered. Austin (1) observed an immediate increase in ascospore discharge by *Sordaria fimicola* as the RH dropped below 95%.

Relative humidity seems to be responsible for the circadianlike rythym of ascospore discharge observed in the field; alternate wetting and drying of the apothecium could rupture the inoperculate asci and thereby cause ascospore discharge to occur.

The inverse effect of rain and free moisture upon ascospore release shown by our data contrasts with findings involving similar studies with *Venturia inaequalis* (6, 7) and *Guignardia citricarpa* (9), both pyrenomycetes, and *Scleroderris lagerbergii* (14), a discomycete.

The fact that there was a significant inverse partial correlation coefficient between ascospore catch and wind speed at first seems odd. This phenomenon is probably due to the dilution of ascospores in the air by the action of the wind. In other words, the trap could catch a higher concentration of ascospores during calm periods, since they are released in clouds near the ground, than during windy periods when they are dispersed in a larger volume of air.

The phenomenon of maximum ascospore discharge occurring during dry periods during the day, followed by

TABLE 4. Complete multiple regression analysis of *Monilinia vaccinii-corymbosi* conidial trap counts (dependent variable) as affected by climatological factors at Pullman, Michigan, 1972[a]

Independent variables	Regression coefficient	Standard error of regression coefficient	Level of significance of regression coefficient	Correlation coefficients	
				Simple[b]	Partial[c]
Leaf wetness	−69.349	28.389	0.016	−0.226*	−0.213*
Rain (none)					
Wind	10.786	4.359	0.015	0.217*	0.215*
Temperature	− 2.553	1.862	0.173	0.073	−0.121
Relative humidity	− 0.181	0.917	0.844	−0.138	−0.018
				R^{2}[d] = 0.155**	
				= 0.294**	

[a] Data input are on an hourly basis and for the peak period of conidial trap counts.
[b] * indicates significant difference, $P = <0.05$; for numbers not followed by an asterisk, the probability of significant difference is $P = >0.1$.
[c] * indicates significant difference, $P = <0.1$; for numbers not followed by an asterisk, the probability of significant difference is $P = >0.1$.
[d] R = multiple correlation coefficient; R^2 = coefficient of determination; ** $P = 0.001$.

extended wet, nocturnal periods gives a probable explanation for the existence of moderate to severe ascosporic infections even during relatively dry seasons. Nocturnal temperatures during the primary infection period ranged from 0-15 C (32-59 F). Since ascospores germinated well at 5-20 C (41-68 F) within 6 h, it is probable that primary infection occurred at night following daytime dispersal of inoculum.

It is interesting that RH had no effect upon the release of conidia; there was no definite circadian-like rythym as shown with ascospore release. Free moisture in the form of dew did, however, inhibit conidial release. The strong direct correlation between conidial release and wind speed supports Honey's statement (8) regarding the function of the disjunctor present between the conidia in chains, "It appears to be a special adaptation for wind dissemination." Also, Corbin et al. (3) found a direct correlation between wind speed and *Monilinia laxa* conidia trapped in an apricot orchard infected with brown rot.

It is unfortunate that the berry crop was markedly reduced by the late frost, thus precluding a measurement of mummy berries on the bush. The steady barrage of conidia over the 13-day period during prebloom and bloom, coupled with long nocturnal wet periods should have resulted in considerable secondary infection.

Our ascospore trapping relative to host phenology has shown that primary inoculum can be present at the time of bud breaking, when a few mm of green tissue is present. Protectant fungicide timing data presented show that initial sprays at this early stage are critical in achieving control of ascosporic leaf and shoot infections, shown by Pepin and Toms (12) to be responsible for the greatest amount of crop loss caused by this disease.

Further work is in progress to develop more precise timing of fungicide application relative to spore release, primary and secondary infection, and host phenology.

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