Production and Dispersal of Ascospores and Conidia by *Physalospora obtusa* and *Botryosphaeria dothidea* in Apple Orchards

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Appreciation is extended to Ray Pope for technical assistance and to Robert Healy and Sandy Donaghy for programming assistance.
Journal Series Paper 6491 of the North Carolina Agricultural Research Service, Raleigh.
The use of trade names in this article does not imply endorsement by the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.
Accepted for publication 4 November 1980.

ABSTRACT

Sutton, T. B. 1981. Production and dispersal of ascospores and conidia by *Physalospora obtusa* and *Botryosphaeria dothidea* in apple orchards. Phytopathology 71:584-589.

Ascospores and conidia of *Physalospora obtusa* and *Botryosphaeria dothidea* were produced from naturally colonized apple prunings throughout the apple growing season. Conidia of both fungi were primarily dispersed in rainwater; ascospores were airborne and waterborne. Ascospores of *P. obtusa* were detected in greatest numbers during April and May. Conidia of *P. obtusa* and ascospores and conidia of *B. dothidea* were most abundant in May and June. Amount and duration of rainfall were the most important factors in waterborne dispersal of ascospores and conidia. Current-season prunings were rapidly colonized by *B. dothidea* and *P. obtusa*. Ascospores and conidia produced in fruiting bodies in these prunings could have caused fruit infections during the same growing season. Management programs for *B. dothidea* and *P. obtusa* may be developed based on sanitation to reduce inoculum levels, a knowledge of factors favoring infection, and fruit susceptibility during the season.

Additional key words: black rot, *Malus sylvestris*, white rot.

Disease management programs have been developed for only a few apple diseases. An apple scab management program based on the Mills system (6) led to a reduction in early-season fungicide application during dry years. Management programs for powdery mildew and fire blight are currently being refined and evaluated (1,10). The development of management programs for many other apple diseases depends on determining the factors that influence inoculum availability and dispersal, as well as those that favor infection.

White rot (or Bot rot), caused by *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. et de Not. (imperfect stage *Dothiorella* sp.), and black rot caused by *Physalospora obtusa* (Schw.) (= *Botryosphaeria querquum* (Schw.) Sacc.), imperfect stage *Sphaeropsis malorum* Pk., are two of the most important apple diseases in the southeastern United States. In the orchard, dead wood and mummified fruit are sources of *B. dothidea* and *P. obtusa* ascospores and conidia (2–4, 9). Spores of the two fungi were produced for at least 6 yr in dead wood tied in the trees (2). *P. obtusa* spores are most commonly detected early in the season, often just prior to bloom (3,5,9). Eid (3) reported that *B. dothidea* spore production in apple wood was greatest from the latter part of May to mid-June, and Weaver (11) found that *B. dothidea* conidia were produced abundantly in infected peach wood from mid-March to mid-December.

Holmes and Rich (5) found that splashing rain is the most important mechanism for dispersing *P. obtusa* spores and that spores were rarely caught in windblown rain. The amount of rain was not correlated with the number of spores trapped, but 13 times more spores were caught during night rains than during daytime rains. In addition, spores were most commonly released at temperatures from 6 to 16 C with a small daily temperature range (1.1–4.9 C). Their study further showed that the number of hours of 100% relative humidity within a 24-hr period is significantly correlated with the number of spores trapped during that period. Spores were also dispersed within the orchard by ladybird beetles. Holmes and Rich (5) did not differentiate between ascospores and conidia in their study.

The dispersal of *B. dothidea* in apple orchards has not been studied, but Weaver (11) found that *B. dothidea* conidia were abundant in rainwater in peach orchards and postulated that windblown rain was important for spore dispersal.

This study was undertaken to determine the factors influencing the availability and dispersal of ascospores and conidia of *B. dothidea* and *P. obtusa*.

MATERIALS AND METHODS

Trapping sites and techniques. Dispersal of ascospores and conidia of *B. dothidea* and *P. obtusa* was studied in the air and rainwater from 3 March to 30 August 1976, 22 March to 1 September 1977, and 21 March to 21 August 1978 at the Central Crops Research Station, Clayton, NC (CC) and from 12 March to 30 August 1976, 27 April to 5 September 1977 and 24 March to 21 August 1978 at the Mountain Horticultural Crops Research Station, Fletcher, NC (MHCRS). Airborne ascospores and conidia were trapped with a Burkard 7-day recording volumetric spore trap (Burkard Scientific [Sales] Ltd., Rickmansworth, Hertfordshire, England).

Each Burkard trap was surrounded by four cages containing naturally infected apple prunings. The wood-framed cages measured 1.2 × 0.6 × 0.6 m, were covered with 5.08-cm mesh poultry wire, and were supported on 30-cm legs. The cages were placed 3 m from the spore trap and equally spaced around it at 90-degree intervals. The Burkard trap was supported so that its orifice was about 45 cm from the ground.

In 1976 and 1977, prunings were obtained from piles of naturally infected 1-yr-old prunings from orchards at CC and MHCRS the previous year. Prunings selected were 1–5 cm in diameter and numerous fruiting bodies of *B. dothidea* and *P. obtusa* were evident. Prunings were placed in each cage and were loosely arranged to allow air movement among them. In 1978, 1-yr-old prunings, colonized primarily by *P. obtusa*, were obtained from an orchard near Bakersville, NC, and placed in the cages at both sites. Before each growing season, all previous year's prunings were removed from the cages and replaced with 1-yr-old prunings.

Burkard traps were adjusted to sample 10 L of air per minute. Hourly spore counts were made by treating the Melinex tape from the trap with cotton blue in lactophenol and scanning across the width of the tape at 2-mm intervals by using a microscope. Counts

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were corrected to compensate for the tape area sampled but not for trap efficiency and were recorded as the number of spores per cubic meter of air sampled per hour.

Water dispersal of ascospores and conidia was studied with a funnel trap by collecting rainwater beneath each cage of prunings with a 10.5-cm-diameter funnel inserted in a 400-ml plastic bottle. Ten milliliters of a 5% copper sulfate solution was placed in each bottle to prevent spore germination. Bottles were changed weekly. The number of spores in each bottle was determined by filtering a 1- or 5-ml sample through a 25-mm-diameter (1.2-μm pore size) gridded filter and counting three grids selected at random.

Environmental monitoring. At CC, temperature at 1.5 m above ground level and wind speeds 4 m above ground level were measured with an automatic weather station in the orchard. At MHCRS, in all years weather data were obtained from the Asheville Airport, which is located about 2 km from the orchard.

Analysis of water trap catches. To determine some of the meteorological factors that affect spore production and dispersal, correlation analyses were run between the $\log_e (n + 1)$ (hereafter referred to as $\log_e$) of waterborne spore counts (n) and selected meteorological factors measured during rain periods at CC and MHCRS. Rain periods were defined as beginning with the first measurable rainfall and ending on the hour of the last measurable rainfall. Thus, during periods with intermittent rain, hours in which rain did not occur were included in the periods. Because traps were changed weekly, more than one rain period often contributed to the rainwater catch. Factors used in the analyses were average temperature during the period (AVGTEMP), maximum temperature during the period (MAXTEMP), total rain (TOTRAIN), maximum hourly rainfall (MAXRAIN), hours of rainfall (HOURSR), the average rate of rainfall per hour (AVRAINHR), the duration of the period (DURATION), and the rate of rain per hour per period (AVGRAIN).

A stepwise multiple linear regression was performed on the $\log_e$ of the waterborne spore catch of each spore type, and the variables listed above. With the stepwise procedure used, any variable not producing a partial F-statistic significant at $P = 0.05$ was not retained in the model. Year and location were included as categorical variables to account for certain location-to-location and year-to-year variability in the data.

The data from CC and MHCRS for the 3 yr of this study were combined for the correlation and regression analysis of *B. dothidea* and *P. obtusa* conidia catch.

Colonization of the current season's prunings. Natural colonization and spore production by *P. obtusa* and *B. dothidea* on apparently healthy current season prunings were studied at CC. Branches 1–4 cm in diameter from Golden Delicious apple trees were cut at monthly intervals in the late winter and spring and placed in 30.5 × 30.5-cm wire mesh cages similar to those around the Burkard traps. Prunings were taken on 9 February, 10 March, 7 April, and 5 May in 1976, and 14 February and 8 March in 1977. Prunings were inoculated by spores produced in a nearby orchard (about 50 m away). Spore dispersal in rainwater was monitored weekly with funnel traps until 28 November 1977 for the 1976 prunings and 5 September 1978 for the 1977 prunings. A funnel trap placed in the open about 20 m away from the cages was used to determine ambient spore concentrations in rainwater.

RESULTS

Airborne dispersal of ascospores and conidia of *P. obtusa*. During the 3 yr of this study, ascospores of *P. obtusa* were most abundant in the air during rainy periods from mid-March through May except in 1976 at MHCRS when airborne ascospores were trapped throughout the summer. During that year, the largest catch occurred during a rainy period on 19 and 20 June. In 1976 at CC, most airborne ascospores were trapped from mid-March to early May. In 1977 at MHCRS, ascospore concentrations were low; the largest discharge occurred during the first week in May. At CC in 1977, airborne ascospores were most abundant during rain in the last week in April and the first 3 wk in May. Discharge was highest during a 33-hr wetting period during 24 and 25 May. In 1978, more airborne ascospores were caught than in the previous 2 yr. At MHCRS (Fig. 1) spore concentrations were highest during a 31-hr wetting period during 4 and 5 May, during a 21-hr wetting period during 23 and 24 May, and during a 23-hr wetting period during 30 and 31 May. During 1978 at CC, ascospores were most frequent during the latter part of April (Fig. 1). Spore concentrations were greatest during a 13-hr wetting period on 13 April.

Airborne conidia of *P. obtusa* were detected during most rain periods but their concentration was much less than the corresponding ascospore catch.

Airborne dispersal of ascospores and conidia of *B. dothidea*. Airborne ascospores of *B. dothidea* were trapped during and just after rainfall at both locations throughout the summer months. In 1976, ascospore catches at both sites were low although spores were detected throughout the summer. During 1977 at MHCRS, the greatest number of spores was recorded on 24 May (Fig. 2). At CC

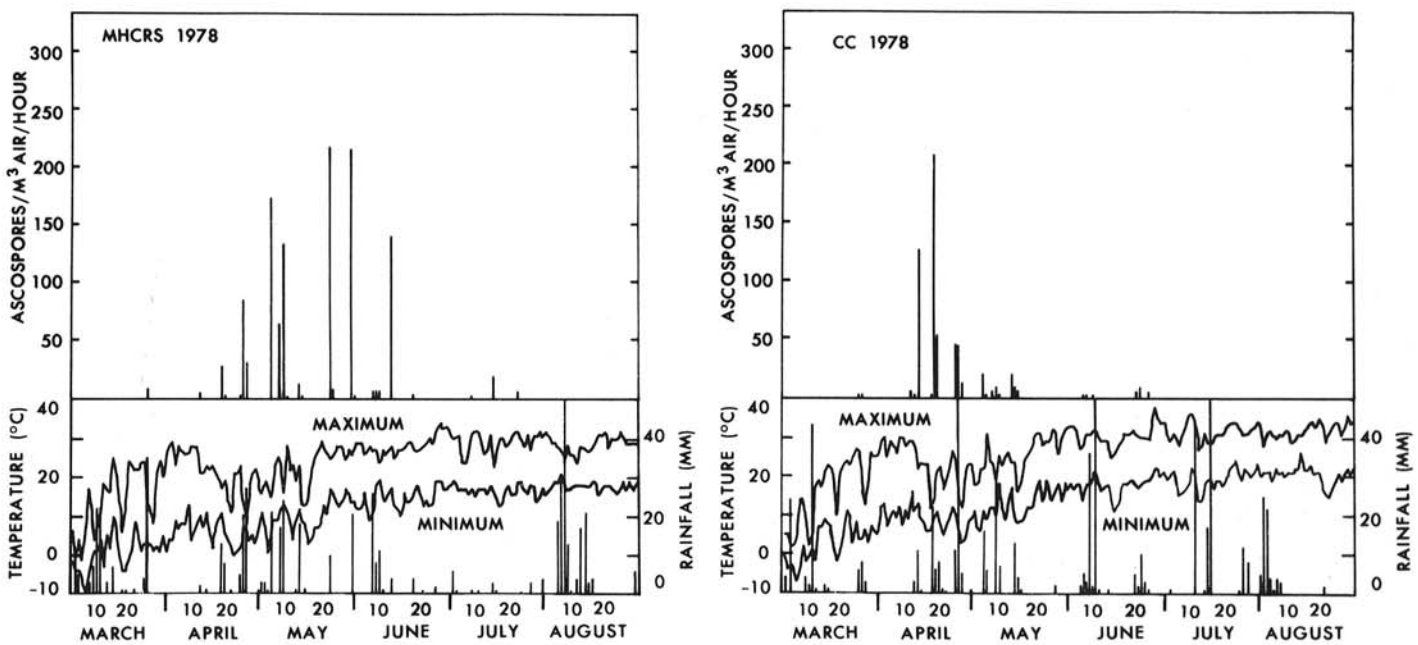

Fig. 1. Mean hourly concentration of airborne ascospores of *Physalospora obtusa* from 24 March to 21 August 1978 at the Mountain Horticultural Crops Research Station, Fletcher, NC (MHCRS) and from 21 March to 21 August 1978 at the Central Crops Research Station, Clayton, NC (CC).

in 1977, airborne concentrations were highest on 3 August; spore concentrations were also high during rains on 19 May, 6 June, and 1 July. In 1978, ascospores of *B. dothidea* were abundant during rainfall throughout the summer at CC but hourly counts were not made. Only a few airborne ascospores were trapped at MHCRS in 1978.

Airborne conidia of *B. dothidea* were detected infrequently; a few conidia were usually detected during each rain period but catches were much less than corresponding ascospore catches.

Characteristics of ascospore dispersal during rain periods. During the 3 yr of this study, airborne ascospores of *B. dothidea* and *P. obtusa* were trapped only during and immediately after periods in which rainfall occurred. As little as 0.25 mm of rainfall was sufficient to trigger spore discharge. Ascospores of both fungi were usually detected during the first hour of rainfall; spore concentration often increased as the rain continued; and spores were often detected for several hours after the rainfall ended (Figs. 3 and 4). Ascospore discharge by *B. dothidea* usually peaked earlier in a rain period (Fig. 3) than did that by *P. obtusa*. Ascospores of *P. obtusa* were often more abundant at the end of a rain period or after the rainfall ceased (Fig. 4). Airborne ascospores of both fungi were most abundant when rainfall was less than 5–6 mm/hr.

TABLE 1. Correlation of the number of waterborne spores of *Botryosphaeria dothidea* and *Physalospora obtusa* trapped during rain periods and meteorological variables in the 1976–1978 growing seasons at the Mountain Horticultural Crops Research Station and the Central Crops Research Station in North Carolina

	Simple correlation coefficients[a]			
	B. dothidea		*P. obtusa*	
Variables[d]	Conidia	Ascospores[b]	Conidia	Ascospores[c]
AVGTEMP	0.2281*[e]	0.1787	−0.1545	−0.3224*
MAXTEMP	0.3109**	0.2196	0.0792	0.0014
TOTRAIN	0.5109**	0.6155**	0.6489**	0.6562**
MAXRAIN	0.5163**	0.5751**	0.5465**	0.4730**
HOURSR	0.3283**	0.4546**	0.5468**	0.6639**
AVGRAINHR	0.4056**	0.3862**	0.4069**	0.3717**
DURATION	0.3156**	0.4362**	0.5204**	0.5929**
AVGRAIN	0.3505**	0.3252**	0.3421**	0.3125*

[a] Calculations based on data transformed $\log_e$ (number of waterborne spores + 1).

[b] AVGTEMP = average temperature; MAXTEMP = maximum temperature; TOTRAIN = total rainfall; MAXRAIN = maximum hourly rainfall rate; HOURSR = hours of rainfall; AVGRAINHR = average rainfall rate during hours of rain; DURATION = length (hr) of the period; AVGRAIN = average rainfall rate per hour per period.

[c] Correlations based on 1976 and 1977 seasons only.

[d] Correlations based on 1977 and 1978 seasons only.

[e] *, $P = 0.05$; **, $P = 0.01$.

Spore dispersal in rainwater. Ascospores and conidia of both fungi were abundant during the growing season (approximately 15 March to 31 August) in rainwater collected in funnel traps beneath cages containing 1-yr-old prunings, but no ascospores of *B. dothidea* were observed in rainwater collections in 1978 at MHCRS. Although spores of both fungi were trapped throughout the growing season, ascospores and conidia of *P. obtusa* were often more abundant earlier in the season than were *B. dothidea* ascospores.

The total number of spores collected and their concentrations varied greatly from one sampling period to another. For example, during 1977 the concentration of *B. dothidea* conidia ranged from 0 during a 19.05-mm rainfall on 20 March to 13,187/ml during a 14.22-mm rainfall on 19 and 20 May. During the same season, total trap catch of *B. dothidea* conidia ranged from 0 during the 20 March period to about 4.65×10^6 conidia during the rain on 19 and 20 May.

Correlation and multiple regression analysis. All variables relating to the amount of rainfall or the duration of rain periods were positively correlated with the number of spores of either fungus caught in rainwater (Table 1). AVGTEMP was positively correlated with *B. dothidea* conidia catch and negatively correlated with *P. obtusa* ascospore catch.

The stepwise regression procedure of the $\log_e$ of the counts of each spore type over the environmental variables yielded the following equations:

$$\log_e BC = -3.013 + 2.269\,Y2 + 1.701\,LOC + 0.013\,TOTRAIN + 0.077\,DURATION + 0.099\,AVGTEMP + 0.109\,AVRAINHR \quad (1)$$

$$\log_e BA = 2.217 + 1.744\,LOC + 0.210\,TOTRAIN + 0.0941\,AVRAINHR \quad (2)$$

$$\log_e PC = 7.476 + 0.167\,HOURSR + 0.118\,AVRAINHR \quad (3)$$

$$\log_e PA = 9.178 + 0.156\,HOURSR - 0.399\,AVGTEMP + 0.281\,MAXTEMP + 0.199\,AVRAINHR \quad (4)$$

in which PC and PA are the rainwater catches of *P. obtusa* conidia

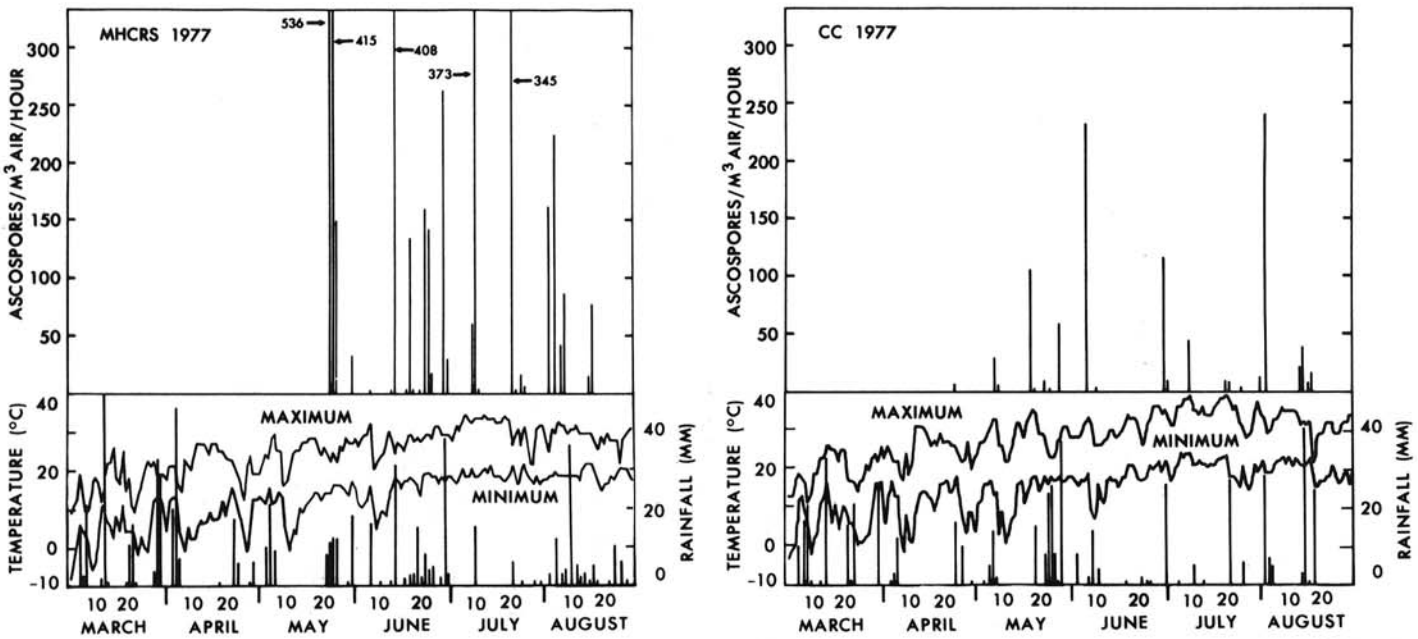

Fig. 2. Mean hourly concentration of airborne ascospores of *Botryosphaeria dothidea* from 27 April to 5 September 1977 at the Mountain Horticultural Crops Research Station, Fletcher, NC (MHCRS) and from 22 March to 1 September 1977 at the Central Crops Research Station, Clayton, NC (CC).

and ascospores, BC and BA are the rainwater catches of *B. dothidea* conidia and ascospores, Y2 and LOC are categorical variables for year and location, and HOURSR, AVGTEMP, MAXTEMP, AVRAINHR, DURATION, and TOTRAIN represent the environmental variables previously described. Coefficients of determination for equations 1–4 were 55.1, 50.8, 47.8, and 69.9, respectively.

Spore production in current-season and 1-yr-old prunings. The time of first detection of spores in rainwater collected from current season's prunings was related to the time the prunings were cut in the spring. Generally, the later the prunings were taken, the later spores were first detected.

Spores of *B. dothidea* were usually detected before those of *P. obtusa* in rainwater traps beneath current season's prunings. In 1977 at CC, ascospores of *B. dothidea* were detected in water traps beneath February and March prunings as early as from naturally infected 1-yr-old prunings; however, ascospores were not trapped from the April and May prunings until 6–8 wk later (Fig. 5). *B. dothidea* conidia were detected from February and March prunings about the same time as from 1-yr-old prunings, but they were not detected from the other prunings for 2–3 mo. Ascospores of *P. obtusa* were present in rainwater from February and March prunings 2–4 wk after they had been detected from 1-yr-old prunings; however, none were detected from the April and May prunings until 3–4 mo later (Fig. 5). *P. obtusa* conidia were not detected in water traps beneath current season's prunings until 3–4 mo after they had been detected in 1-yr-old prunings. Results were similar from prunings cut in February, March, and April 1977.

More spores were usually produced in 1-yr-old prunings than in those colonized during the current season. From 1 April 1976 to 1 November 1977, about 25% of the spores trapped from prunings taken 9 February 1976 were trapped during the first season; about 75% were trapped during the second season (Fig. 6). Few spores of either fungus were trapped from 15 November to 1 March.

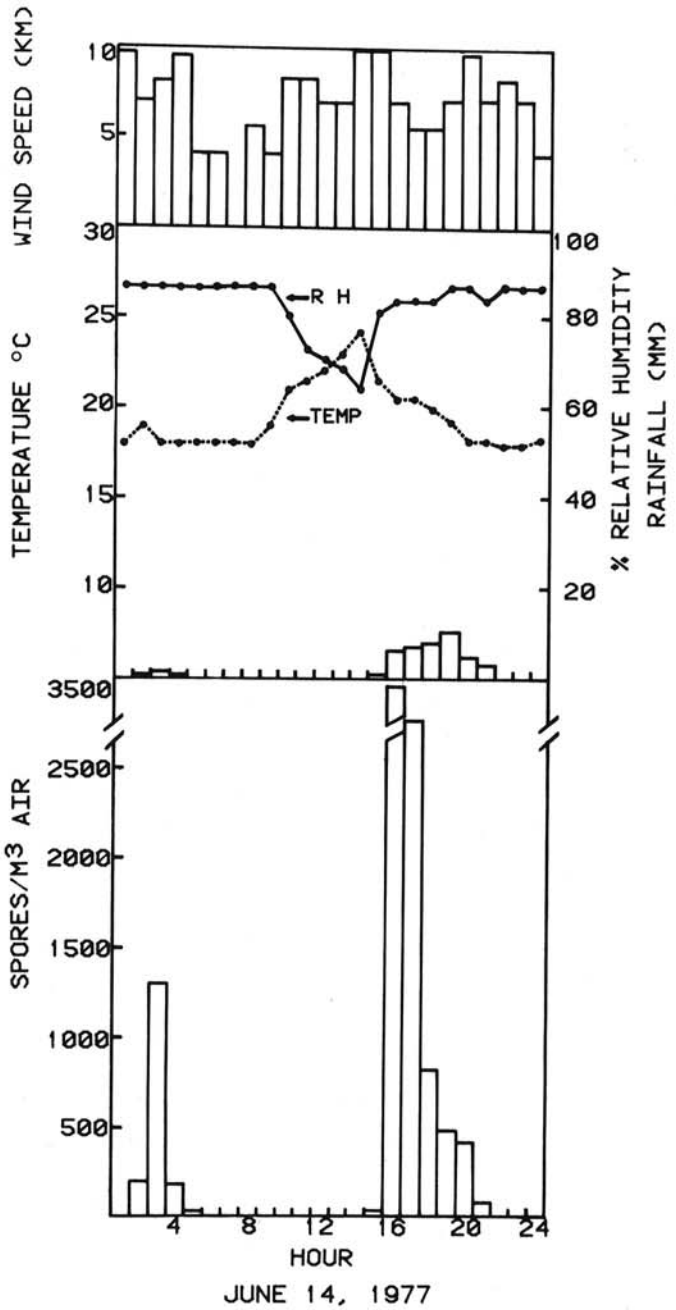

Fig. 3. Concentration of airborne ascospores of *Botryosphaeria dothidea* in relation to wind velocity, temperature, relative humidity, and rainfall on 14 June 1977 at the Mountain Horticultural Crops Research Station, Fletcher, NC.

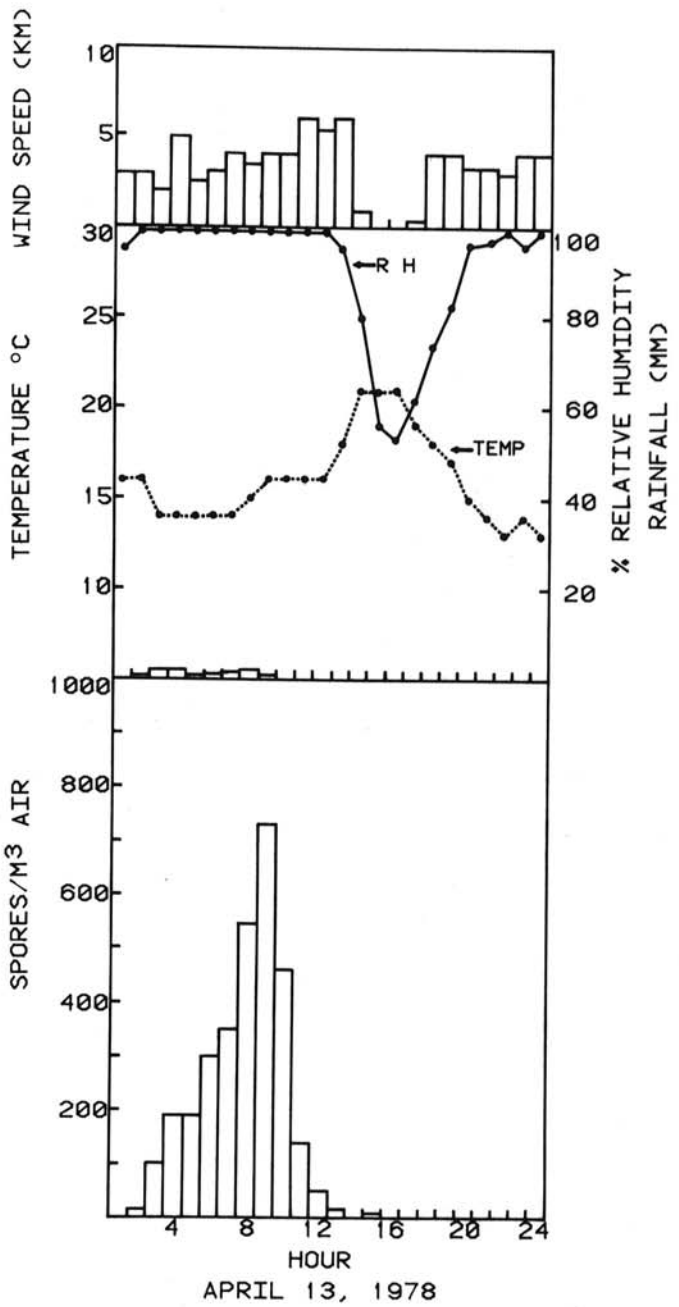

Fig. 4. Concentration of airborne ascospores of *Physalospora obtusa* ascospores in relation to wind velocity, temperature, relative humidity, and rainfall on 13 April 1978 at the Central Crops Research Station, Clayton, NC.

DISCUSSION

In this study, ascospores and conidia of *B. dothidea* and *P. obtusa* were produced in colonized 1-yr-old apple prunings throughout the apple growing season. The spore supply in the orchard is also influenced by current season colonization of dead wood within the tree. Spore production by fruiting bodies in this wood largely depends on when colonization occurs. Colonized mummified apples from the previous season or the current season may also serve as an inoculum source in the orchard (4). Mummies were not considered in this study because, quantitatively, dead wood is much more important as a source of inoculum in North Carolina. Spore production from mummies may differ from that from dead wood.

Rainfall triggered discharge of ascospores and conidia of both fungi and was the most important mechanism for conidia dispersal. Because conidia are primarily waterborne, they may be more important in intratree spread of these pathogens. Airborne ascospores, on the other hand, have the potential for intertree spread or spread from nearby piles of prunings.

The number of ascospores and conidia of both fungi trapped during a rain period was positively correlated to measures of rainfall amount and duration. These results differ from those of Holmes and Rich (5) who found no relationship between rainfall amount and *P. obtusa* spore catch. However, their study primarily involved airborne spores whose concentrations in the air may be more closely related to rainfall rate than amount.

Low mean temperature apparently suppressed spore production during the winter months, but temperature did not appear to be limiting during the apple growing season. Drake (2) implied that temperatures during June and July are not as favorable for spore release as those earlier in the season or near harvest. In this study, *P. obtusa* ascospores were not as abundant during the summer as during the spring, but *P. obtusa* conidia and *B. dothidea* ascospores and conidia were.

The multiple regression models selected in the stepwise regression procedure accounted for only 50–60% of the variation among the $\log_e$ of the numbers of ascospores and conidia of *B. dothidea* and *P. obtusa* and the environmental parameters monitored. Sampling error and variation in the spore supply from one sampling period to another may account for some of the unexplained variation. Meteorological variables that were not measured also may have influenced the catch of ascospores and conidia.

There appeared to be a difference in ascospore release during

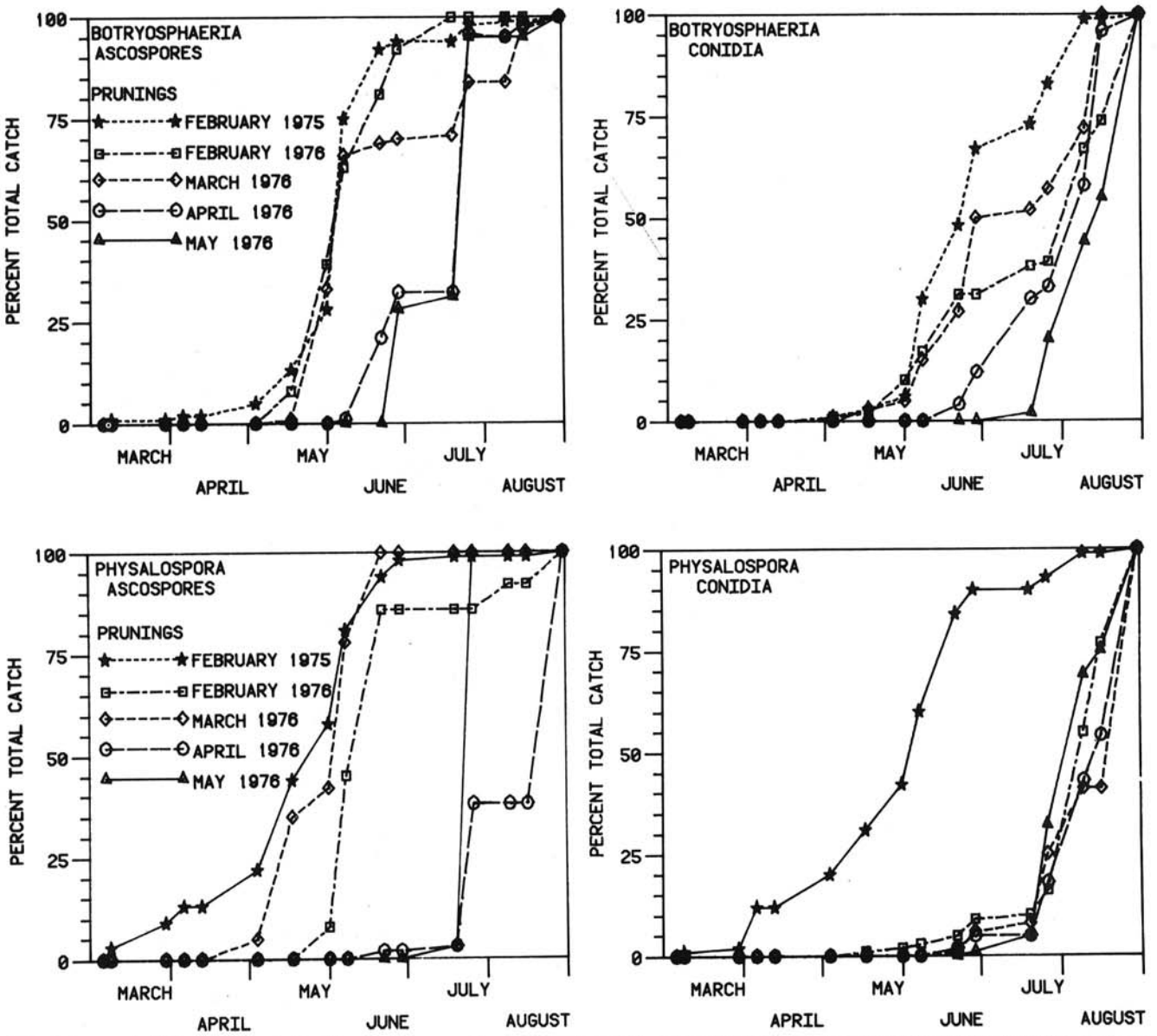

Fig. 5. Relative catch of *Botryosphaeria dothidea* ascospores and conidia (top) and *Physalospora obtusa* ascospores and conidia (bottom) from 15 March to 30 August 1976 in rainwater collected from funnel traps placed beneath apple prunings taken in February 1975 and on 9 Feburary 1976, 10 March 1976, 7 April 1976, and 5 May 1976, and infected naturally.

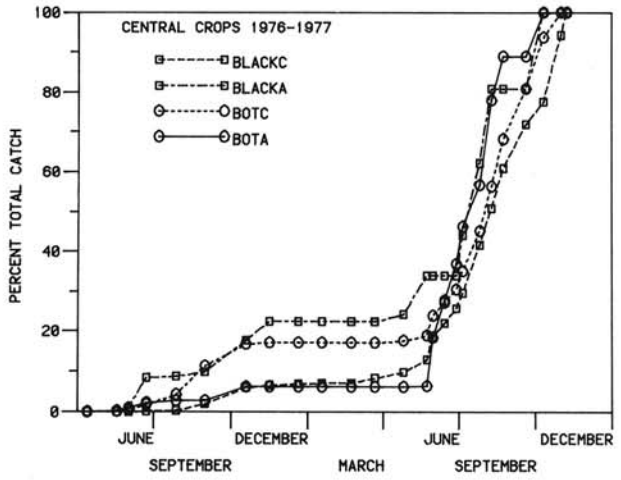

Fig. 6. Relative catch of *Physalospora obtusa* and *Botryosphaeria dothidea* ascospores and conidia in rainwater traps from 9 February 1976 to 28 November 1977 at the Central Crops Research Station, Clayton, NC. Prunings for the study were naturally infected.

rainfall between *B. dothidea* and *P. obtusa*. *B. dothidea* ascospores were usually abundant soon after rain began; *P. obtusa* ascospores were usually more abundant toward the end of a rain period. This may be due to differences in the discharge mechanism of the two fungi or may reflect the inability of the larger *P. obtusa* ascospores to become airborne during rainfall. The dispersal of *P. obtusa* ascospores during the latter portion of rain periods raises the question of their epidemiological significance. It is possible that ascospores deposited on fruit can remain viable and germinate and cause infection during ensuing dew or rain periods. It was observed that *P. obtusa* ascospores were much thicker walled than *B. dothidea* ascospores.

In this study, prunings were quickly colonized by *B. dothidea* and *P. obtusa*. Ascospores and conidia produced in these colonized prunings in an orchard could serve as inoculum for infection during the summer growing season. This reemphasizes the concept that careful pruning and disposal of dead wood should be an important component of both current-season and long-range management programs for these two diseases.

The availability of inoculum of *B. dothidea* and *P. obtusa* throughout the growing season and the apparent susceptibility of fruit of all ages to infection by the two fungi (2,3,10) seem to indicate that the opportunities for reducing the dependence on fungicides for the control of these two diseases are limited. However, orchard data suggest that some reduction in fungicide use is possible. Growers commonly use less fungicide than is recommended and have little white rot or black rot problem (T. B. Sutton and G. C. Rock, *unpublished*). Similarly, small-plot tests have shown no increase in white rot or black rot incidence at harvest if fungicides in early-season sprays are reduced 25–50% (7,8, and *unpublished*). This indicates that there may be opportunities for developing disease management programs for white rot and black rot based on reduced fungicide use and sanitation (to lower the inoculum). However, before reliable management programs can be developed, a practical and economical method for assessing inoculum density must be developed and more must be learned about factors favoring fruit susceptibility and infection.

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