Availability and Dispersal of Ascospores and Conidia of *Botryosphaeria* in Peach Orchards

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ABSTRACT

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Spore dispersal of three *Botryosphaeria* spp. from peach prunings and from diseased trees was studied from 1984 to 1985. Airborne ascospores of *B. dothidea* from prunings were at peak levels in the spring. Ascospores of *B. obtusa* and *B. rhodina* were indistinguishable and were detected at relatively high levels in most months, depending on the year. Discharge of ascospores occurred during or soon after periods of wetness. Waterborne ascospores and conidia were detected in rainwater runoff from prunings and diseased scaffold limbs of trees throughout most of the year. Trap catches for spores of all three species were generally low during December and January and from August to October. Waterborne spores of *B. dothidea* were never detected in January and February. Conidia of *B. dothidea* made up the greatest proportion of the total waterborne spores collected from diseased trees and conidia of *B. obtusa* made up the greatest proportion of the total trapped from dead prunings.

Botryosphaeria dothidea (Moug. ex Fr.) Ces. & de Not. (= *B. ribis* Gross & Dugg.) invades peach (*Prunus persica* (L.) Batsch) trees through wounds and lenticels (6,10,15,17). Invasion of lenticels frequently leads to the development of sunken necrotic lesions and gum exudation on the trunk and scaffold limbs. Areas surrounding lenticels on young branches often become swollen. Because of the characteristic swelling of bark, the disease is known in Japan as peach blister canker (1,9). In the United States (11,15,17) and China (6), the disease is generally referred to as a gummosis disease of peach trees.

B. obtusa (Schw.) Shoem. and *B. rhodina* (Berk. & Curt.) Arx invade peach trees primarily through wounds (3) or areas already infected by *B. dothidea* (5). A seasonal succession of the fungi in cankers has been reported (4). Based on isolation frequencies from cankered sites that were surface sterilized but did not have outer bark removed, *B. dothidea* predominated in summer and fall and *B. obtusa* predominated in winter and spring.

A major source of *Botryosphaeria* inoculum infecting fruit trees is dead wood left in or near orchards. *B. dothidea* and *B. obtusa* rapidly colonize and sporulate on current-season prunings of apple (14). Numerous pycnidia of *B. dothidea* appear on prunings of peach on the orchard floor and on dead branches left in the tree, as well as on diseased bark (15,16). Because of the abundance of conidia of *B. dothidea* in rainwater, windblown rain was considered to be important for spore dispersal (17). The sexual stage of *B. dothidea* has also been observed on peach (15); however, ascospore dispersal has not been studied. The objective of this study was to determine peak periods of conidia and ascospore release by *Botryosphaeria* in peach orchards and to examine factors influencing inoculum availability and dispersal.

MATERIALS AND METHODS

Airborne dispersal. Airborne spores of *Botryosphaeria* were trapped from 1 January 1984 to 31 December 1985 at Byron, GA, with a Burkard 7-day recording volumetric spore trap (Burkard Scientific Sales, Ltd., Rickmansworth, Hertfordshire, England). The Burkard trap was surrounded by peach prunings that were 10-mo-old at the beginning of each year. The prunings were gathered in March from a 9-yr-old planting of the cultivar Redglobe and kept in a pile until the following January. They were then placed in four wood-framed cages (14) that were spaced equally around the Burkard trap at 90° intervals. The trap orifice was 71 cm from the cages and 46 cm from the ground. Air intake was adjusted to 10 L per minute.

The Melinex spore trap tapes were prepared by using the procedure described by Gottwald and Bertrand (8). Exposed tapes were cut into daily segments (48 mm) and mounted in acid-fuchsin-lactophenol containing 1% polyvinyl alcohol to increase the life of the mounts. Spores were counted at ×200. Hourly counts were made for peak periods of airborne spore release for *B. dothidea* during 15–23 April 1984, and for ascospore of *B. obtusa*/*B. rhodina* (the two were indistinguishable) during 14–19 June 1985.

Waterborne dispersal. Conidia and ascospores of *Botryosphaeria* were collected in rainwater runoff below caged prunings surrounding the Burkard trap. Water dripped from the prunings into a 10.5-cm-diameter funnel inserted in a 1-L glass bottle. In 1984, one trap was used under a single cage, and in 1985, one trap was used under each of four cages.

During the same period, spores were collected in rainwater runoff from diseased limbs of peach trees in the orchard from which the prunings were collected. Traps consisted of a 15-cm-diameter funnel attached with wire to a scaffold limb (2). Florist clay was applied to help channel water into the funnel. The lower end of the funnel was connected by a 25–30 cm long polyurethane tube to a 3.8-L bottle at the base of the tree. One trap was placed at each of four trees at widely separated locations within the orchard.

Water trap bottles were changed weekly when it rained. Ten milliliters of 5.0% copper sulfate solution was added to each clean bottle (14) to prevent spore germination. The number of spores collected in water was determined by filtering a 10-ml sample through a 25-mm-diameter gridded filter (1.2-μm pore size). The filter membrane was mounted in acid-fuchsin-lactophenol containing 1% polyvinyl alcohol. Spores were counted at $\times200$ in three 9-mm^2 grids selected at random and the number was adjusted for sample size and water volume.

Environmental monitoring. The Burkard trap and surrounding cages were approximately 5 m from the U. S. Weather Bureau substation at Byron, GA. Orchard trees under study were approximately 1.2 km from the substation. Temperature and relative humidity were recorded with a 7-day recording hygrothermograph (Belfort Instrument Co., Baltimore, MD) located in a weather shelter 1.5 m above ground. Rainfall was measured with a 7-day universal rain gauge (Belfort Instrument Co.). Vegetative wetness was recorded on a strip chart recorder connected to a series of five wetness sensing elements, each consisting of a chemically treated printed circuit whose surface resistance changes with hydration (7). The sensors were located at various angles in a bush 0.5 m above ground. Any change in surface resistance from the base line was considered as evidence of vegetative wetness. Wind speed was recorded on a similar strip-chart recorder connected to a three-cup anemometer with four-digit odometer (Model 53498, Belfort Instrument Co.) located approximately 3 m above ground.

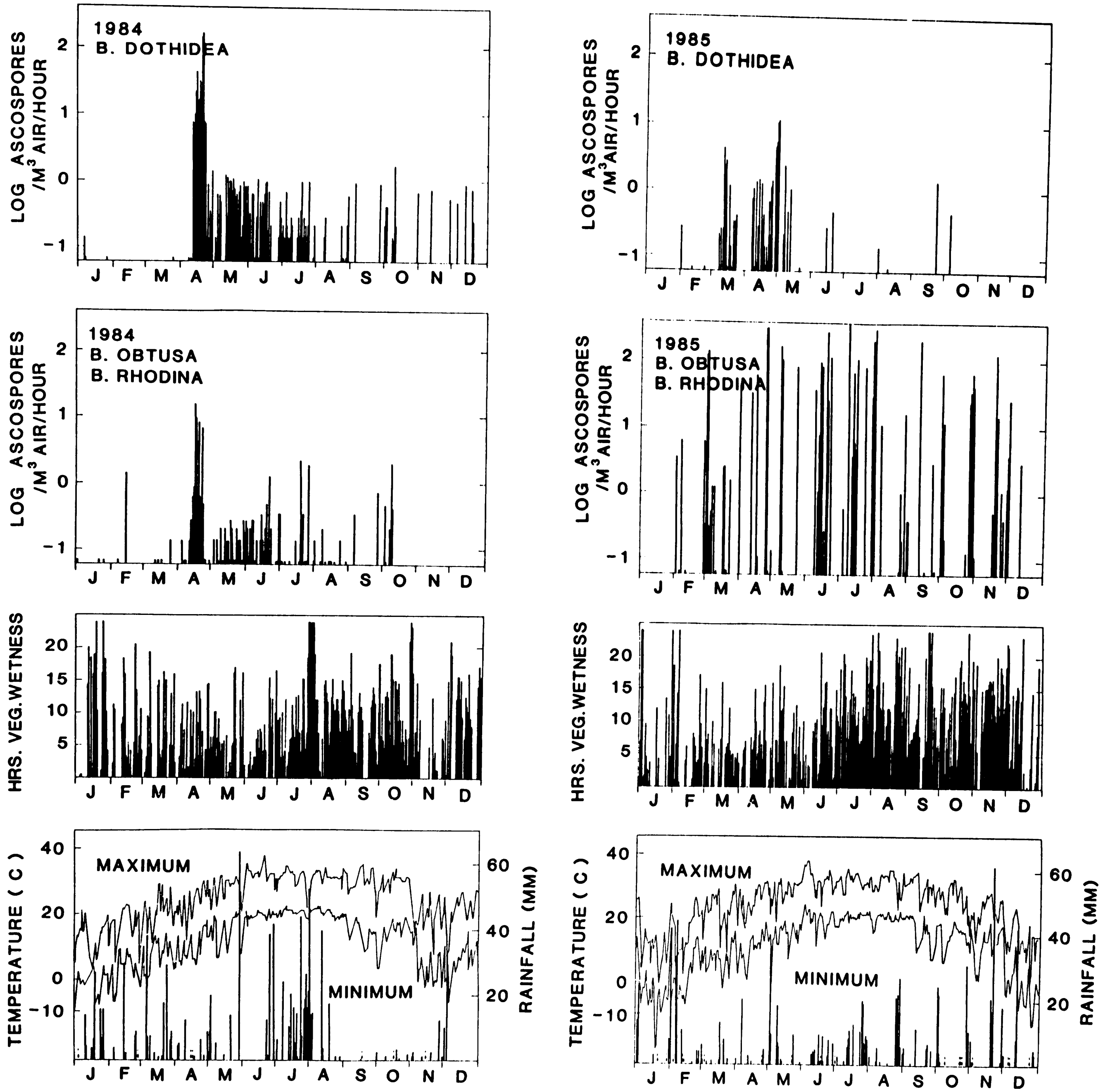

Fig. 1. Mean hourly concentration of airborne ascospores of *Botryosphaeria* in relation to temperature, rainfall, and vegetative wetness in 1984 and 1985. Trace amounts of rain are indicated with arrows. Ascospores of *B. dothidea* and of *B. obtusa* or *B. rhodina* were monitored with a Burkard trap.

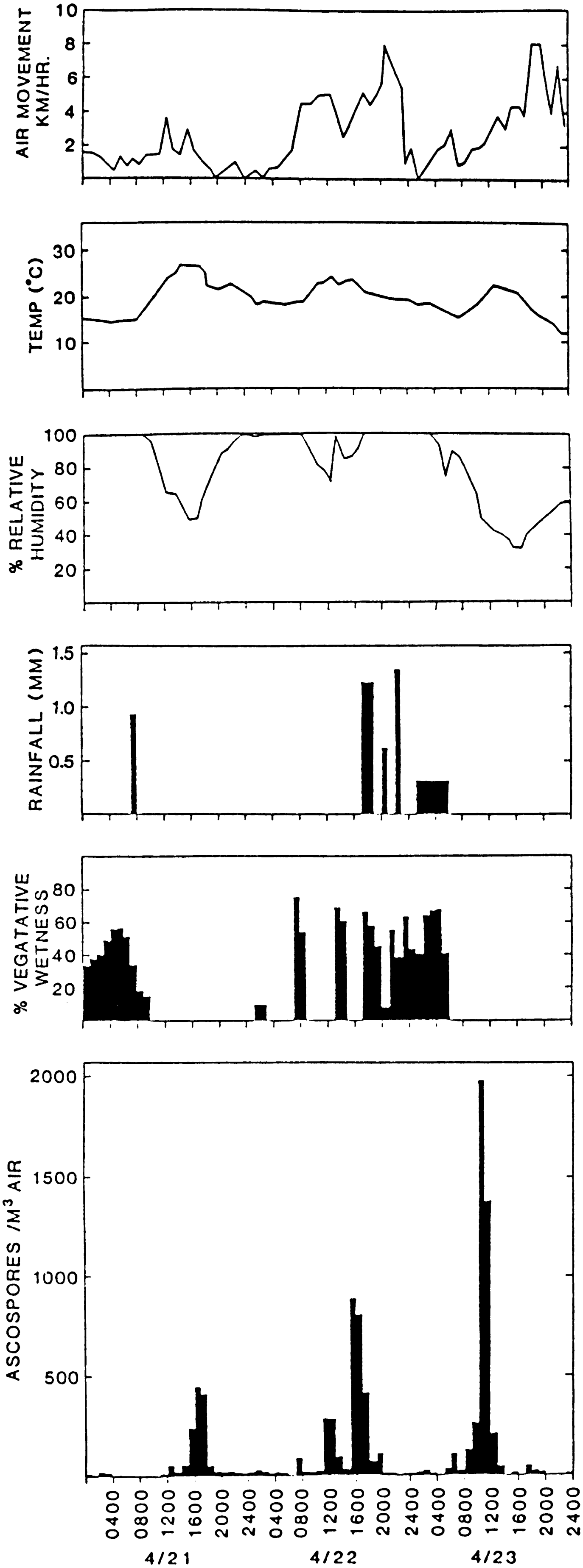

Fig. 2. Concentration of airborne ascospores of *Botryosphaeria dothidea* in relation to air movement, temperature, relative humidity, rainfall, and vegetative wetness on 21–23 April 1984.

RESULTS

Airborne dispersal. Spores of *Botryosphaeria* collected in the Burkard trap were almost exclusively ascospores and rarely conidia. Ascospores of *B. dothidea* were separated from ascospores of *B. obtusa* and *B. rhodina* based on staining characteristics, size, and shape. Ascospores of *B. obtusa* and *B. rhodina* were indistinguishable, and no attempt was made to separate the ascospores of these two species.

In 1984, airborne ascospores of *B. dothidea* and of *B. obtusa*/*B. rhodina* were most abundant in April (Fig. 1). In 1985, airborne ascospores of *B. dothidea* were most frequently detected at high levels from mid-March to mid-May and those of *B. obtusa*/*B. rhodina* were detected at high levels during all months except January.

The number of airborne ascospores of *Botryosphaeria* was generally lower in winter than in other seasons (Fig. 1). Possibly this was related to temperature. Ascospores were frequently released on days when rainfall was recorded; however, they were also detected on days when wet periods were not caused by measurable rainfall. A comparison of hourly weather data and counts of airborne ascospores of *B. dothidea* showed that spore discharge occurred soon after periods of surface wetness. On 22 April 1984, a day with no detectable rainfall, peak periods of spore discharge coincided with intermittent periods when vegetative wetness was low. On 5 of 9 days monitored in April, ascospore discharge by *B. dothidea* was triggered by wetness not attributable to rainfall. Peak periods of discharge of ascospores of *B. obtusa* or *B. rhodina* occurred during periods of wetness on 14–19 June; all but one discharge was triggered by rainfall.

Based on hourly counts, peak periods of airborne dispersal of *Botryosphaeria* ascospores occurred during the daylight hours when, in general, relative humidity was lower, temperature was higher, and air movement was greater, relative to nighttime conditions (Fig. 2).

Waterborne dispersal. Conidia of *Botryosphaeria* were detected in rainwater runoff from scaffold limbs of diseased trees and from prunings held in cages. Conidia of the three species were separated based on staining characteristics, size, shape, and the presence or absence of a septum. Ascospores of *B. dothidea* and of *B. obtusa*/*B. rhodina* were also detected.

When the percent of total waterborne conidia trapped during each year was plotted, results for diseased trees and caged prunings were very similar (Fig. 3). In 1984, conidia of *B. obtusa* were detected beginning in January; conidia of *B. dothidea* were not detected until March or April. All conidia of *B. rhodina* were trapped between May and August. Few collections were made between August and November because of a lack of rainfall (Fig. 1). In December, conidia of both *B. obtusa* and *B. dothidea* were detected in rainwater runoff from trees, but only conidia of *B. obtusa* were detected in runoff from prunings.

The observations in 1985 were very different from those in 1984. Eighty percent or more of the conidia of *B. obtusa* trapped in 1985 was collected in February; in 1984, 80% was not reached until August. Nearly 70% of the conidia of *B. dothidea* trapped in 1984 were collected by April; in 1985, only 15% of the conidia were collected from January to October. Data for conidia of *B. rhodina* from diseased trees in 1985 did not correspond to the same type of data for prunings in the same year. However, detection of *B. rhodina* in 1985 was low (Table 1), particularly with diseased trees.

Although the pattern of conidia dispersal, when plotted as percent of total catch for a given year and for a given *Botryosphaeria* species, was generally similar for water runoff from trees and prunings (Fig. 3), the proportions of the spores of the different species in water from trees and prunings were not the same (Table 1). Conidia of *B. dothidea* and *B. obtusa* accounted for 43 and 31%, respectively, of the total number of waterborne spores of *Botryosphaeria* from trees in 1984. However, conidia of *B. dothidea* and *B. obtusa* made up <1 and 67%, respectively, of the total waterborne spores from prunings in the same year. Ascospores of *B. obtusa*/*B. rhodina* accounted for

24% of the total waterborne spores from prunings. In 1985, 97% of waterborne spores from trees were conidia of *B. dothidea* while 96% of the spores from prunings were conidia of *B. obtusa*.

DISCUSSION

Airborne ascospores of *Botryosphaeria* could be a major cause of primary infections. Numbers of ascospores of *B. dothidea* in the air were highest in the spring. Concentrations of airborne ascospores of *B. obtusa*/*B. rhodina* were also at peak levels in the spring of 1984, but were relatively high during most months in 1985.

Sutton (13) reported that the release of airborne ascospores of *B. dothidea* and *B. obtusa* from dead apple prunings in the mountains and Piedmont of North Carolina was triggered primarily by rainfall. The results of my study conducted at Byron,

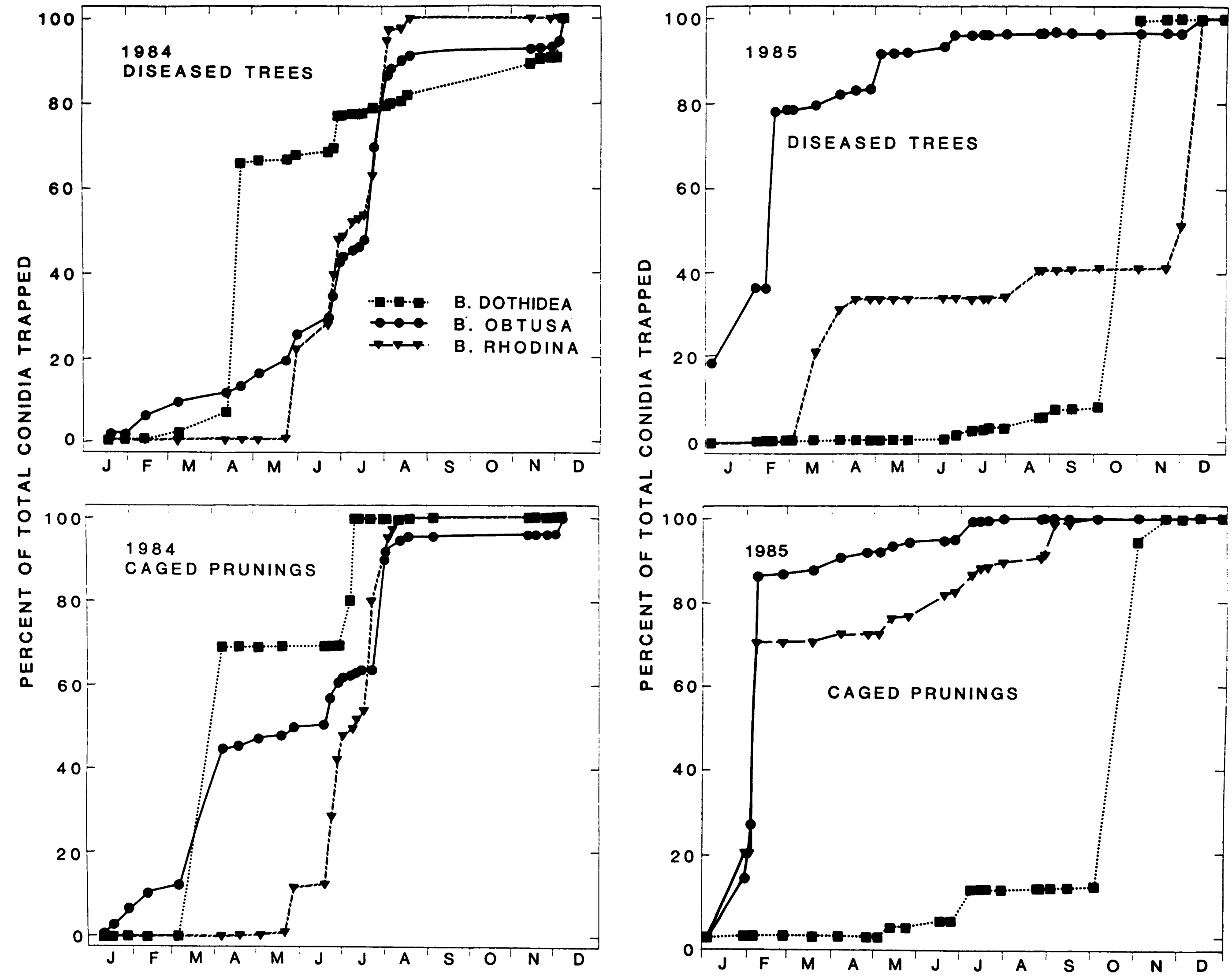

Fig. 3. Percentage of total yearly catch of conidia for each of three *Botryosphaeria* spp. collected in rainwater runoff from diseased trees and caged prunings during 1984 and 1985.

TABLE 1. Numbers and proportions of ascospores and conidia of *Botryosphaeria* spp. trapped during 1984 and 1985

	1984				1985			
	Diseased tree[a]		Caged prunings[b]		Diseased tree[a]		Caged prunings[a]	
Spore type	no.[c] (×10²)	%[d] ± SD	no. (×10²)	%	no. (×10²)	% ± SD	no. (×10²)	% ± SD
B. dothidea conidia	1,866	43.27 ± 34.61	22	0.53	21,710	96.81 ± 1.47	128	0.32 ± 0.21
B. dothidea ascospores	308	7.49 ± 3.65	97	2.34	3	0.02 ± 0.02	11	0.02 ± 0.04
B. obtusa conidia	1,123	30.90 ± 19.30	2,800	67.41	550	2.15 ± 1.59	41,466	95.93 ± 1.46
B. rhodina conidia	345	7.93 ± 6.38	241	5.81	33	0.14 ± 0.11	431	1.50 ± 1.65
B. obtusa & *B. rhodina* ascospores	384	10.41 ± 7.52	993	23.91	113	0.89 ± 1.00	1,022	2.23 ± 1.47

[a] Data are means for four traps.
[b] Data for one trap only.
[c] Total number of spores trapped.
[d] Percentage of total number of waterborne spores trapped; data from four traps given as means ± standard deviation.

GA, indicate that the discharge of ascospores was frequently triggered by periods of wetness not due to measurable rainfall, but rather to dew or mist conditions. Release of ascospores of *B. obtusa* or *B. rhodina* occurred during periods of wetness and release of ascospores of *B. dothidea* generally occurred soon after periods of wetness. The latter observation differed from those of Sutton (12), who found that ascospores of *B. dothidea* were usually more abundant at the beginning of a rain period. Differences in the two reports may be explained by differences in geographical location, fungal biotype (10), or plant host.

Although a consistent trend in seasonal water dispersal of spores was not shown for 1984 and 1985, waterborne spores of *Botryosphaeria* were detected throughout a major part of each year when there was adequate rainfall to collect water runoff. Generally, the numbers of waterborne spores were low for all three *Botryosphaeria* spp. during December and January and from August to October. Low numbers in September and October can be attributed to low precipitation in 1984, but not in 1985. The absence of spores of *B. dothidea* in rainwater runoff during January and February is consistent with a previous study by Weaver (17). Also, Britton and Hendrix (4) were unable to isolate *B. dothidea* in January.

The fact that the greatest proportion of spores in rainwater runoff from trees were of *B. dothidea* and the greatest proportion in runoff from dead prunings were of *B. obtusa* suggests one of the following: the fungi differ in their capacity to grow and sporulate on bark of different ages or the fungi differ in their capacity to grow and sporulate on living versus dead peach bark.

The proportion of spores of *B. dothidea* from trees in 1984 might have been higher if rainfall had not been unusually low in late summer and early fall. Eighty percent of the total catch for 1985 was trapped at the end of October and beginning of November.

Because of the abundance of spores of *Botryosphaeria* dispersed from dead peach bark, sanitation measures are important in disease management. Dead trees and branches should be removed from the orchard and burned. A possible alternative for reducing inoculum from dead wood on the orchard floor would be to use a flail mower (13). The importance of fresh prunings as a source of inoculum of *B. dothidea* in the orchard during the first season is unknown, since prunings were already 10-mo-old beginning in each year of the study. Sutton (14) showed that apple prunings can be colonized and produce inoculum during the same growing season. Spores of *B. dothidea* could possibly be more abundant on peach bark that is dying or has recently died than on peach bark that has been dead for longer than 1 yr.

Opportunities to reduce disease incidence based on spore availability during the year appear limited. To avoid wound invasion by *B. dothidea*, it may be advantageous to prune in January or February when the number of spores of this fungus are low or absent. Because peach tree short life is another major problem to consider in a management program, pruning in February would be preferable (12).

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