Biology and Epidemiology of *Mycosphaerella pomi*, Cause of Brooks Fruit Spot of Apple

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ABSTRACT

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The disease cycle of *Mycosphaerella pomi*, cause of Brooks fruit spot of apple, was elucidated. The fungus overwinters in apple leaves, and in the spring and early summer ascospores are discharged from pseudothecia during rain and dew periods and infect fruit and leaves. Leaf infections remain quiescent until late summer, when they appear as small purple flecks on the leaves. Extensive colonization of the leaf does not occur until after leaf fall. There was no evidence of secondary spread. Ascospores of *M. pomi* germinated within 6 hr at 16–24 C; germ tube elongation was greatest at 24 C. Penetration of the leaf occurred only through the stomata; appressoria formed over the stomatal openings. Ninety-six hours of continuous wetting at 20 C was necessary for leaf infection to occur. A 12- or 24-hr dry period after germination either had no effect on the incidence of infection or resulted in an increase in infection. This suggests that long continuous wetting periods are not necessarily required for infection to occur and that infection may be enhanced under alternating wetting and drying conditions in the orchard. Leaf age did not affect susceptibility. After leaf infection, thick-walled vesicle- or chlamydospore-like structures developed in the mesophyll beneath the stomata. Mycelium was observed growing from some of these structures into the leaf mesophyll 12–16 wk after infection. Leaf tissues are colonized during the winter and pseudothecia can form on either leaf surface. The use of ergosterol biosynthesis inhibiting fungicides in the post bloom period for Brooks spot control is discussed.

Additional key words: *Malus domestica*, Phoma rot.

Brooks fruit spot, or Phoma rot, caused by *Mycosphaerella pomi* (Pass.) Lindau, is a minor disease of apples (*Malus domestica* Borkh.) throughout most of the eastern United States. The disease frequently occurs in North Carolina on the cultivars Rome Beauty and Stayman. Jonathan, Delicious, Golden Delicious, and other cultivars are also affected (2,18). Because fruit lesions are often small and result in shallow depressions, light infection is often not noticed at harvest, particularly on cultivars with deeply pigmented red skins. However if numerous infections occur, fruit are downgraded and severe infection can result in pitted and cracked fruit (Fig. 1A).

The disease cycle of *M. pomi* has not been completely determined. *M. pomi* overwinters in infected leaves on the orchard floor and ascospores are discharged from pseudothecia during rainy periods in late spring and early summer (1,15). *M. pomi* ascospores mature later than those of *Venturia inaequalis* (Cke.) Wint. Although pseudothecia are readily found in overwintered leaves, no leaf symptoms have been associated with the disease, and it is not known how or when leaves become infected. In experiments in which fruit were periodically bagged and unbagged, infection occurred during the 6–8-wk period following petal fall (13). Symptoms on fruit usually appear in North Carolina the first 2 wk in July. No fruiting structures have been observed on fruit in the orchard, but after storage, *Cylindrosporium pomi* Brooks and *Phoma pomi* Pass. have been observed sporulating in fruit lesions (2,3). The *Cylindrosporium* stage is commonly produced in culture.

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The objectives of this study were to elucidate the role of leaf infection in the disease cycle of *M. pomi* and to investigate the factors affecting ascospore production and dissemination.

MATERIALS AND METHODS

Isolations. In 1983 investigations were centered on associating a leaf symptom with *M. pomi* infection. Initially isolations were made on potato-dextrose agar (PDA) amended with 200 μg/ml of penicillin (PPDA) or acidified (APDA) with 50% lactic acid (about four drops per 100 ml). However, *Alternaria* spp. and other saprophytes quickly overgrew the isolations on PDA, obscuring the growth of *M. pomi*. Subsequently we found that *M. pomi* could be successfully isolated on water agar (WA) either amended with 200 μg/ml of penicillin (PWA) or acidified (AWA) with lactic acid (about four drops per 100 ml). *M. pomi* colonies were usually visible at the margins of infected tissue after 3 wk at room temperature (20–24 C) and light; plates were observed during a 5–6-wk period for colony development.

From August to October 1983, isolations were made from various small and large necrotic areas, chlorotic spots, and purple flecks observed on leaves collected from unsprayed trees at the Mountain Horticultural Crops Research Station (MHCRS), Fletcher, NC, as well as from orchard locations in Henderson, Haywood, and Mitchell counties. Tissue sections, about 2 mm diameter, were cut from the margins of lesions, dipped in 0.525% NaOCl solution for 10 sec, blotted dry, and plated on PWA or AWA. Subsequent isolations in 1984 and 1985 were made in a similar manner.

Inoculation with ascospores. *M. pomi* ascospore inoculum was obtained from mature pseudothecia in overwintered apple leaves collected in early May from the floor of an orchard in which fruit had been severely infected with Brooks spot the previous summer.

On 19 May 1983, the leaves were spread onto 0.6- × 1.0-m wire mesh frames and soaked in water for 5–7 min. The excess water was shaken off and the frames were suspended over 10 Rome Beauty seedlings in an inoculation chamber. Temperature in the chamber ranged from 19 to 24 C and the relative humidity (RH) was maintained at 100%. The inoculum source remained in the chamber for 32 hr and the seedlings for 50 hr. The youngest unfolded leaf on each seedling was tagged when the seedlings were removed from the chamber.

The inoculated seedlings were placed in a cold frame outside a greenhouse at MHCRS and watered and fertilized on a maintenance schedule through the summer. Ten uninoculated Rome Beauty seedlings were also placed with the inoculated seedlings. Before leaf fall, trees were pruned so that only inoculated leaves remained on the seedlings. Uninoculated seedlings were pruned to approximately the same height as the inoculated ones. Leaves were collected from the seedlings on 1 October, at 50% leaf fall. Ten leaves were arbitrarily picked from the ground around each pot and 10 were arbitrarily picked from each tree. All leaves were placed on cheesecloth packets and overwintered in Saran cloth (Chicopee Mfg. Co., Cornella, GA) cages on the orchard floor. The leaves were observed in mid-May 1984, and the percentage of the each leaf's surface that was covered with pseudothecia was estimated.

Effect of temperature on ascospore germination. Leaves containing mature pseudothecia of *M. pomi* were soaked for 5–6 min in water, blotted dry, and placed in a spore discharge tower (7). Water agar plates preconditioned at 4, 8, 12, 16, 20, 24, 28, or 32 C were placed in the tower for 2 min and about 50–200 ascospores were deposited in four 0.5-mm-diameter circles on each plate. Plates were incubated in the dark at the temperatures indicated above. After 2, 4, 6, 8, 12, 18, 20, and 24 hr, spores were fixed by placing a drop of cotton blue in lactophenol in the center of the group of spores. Germination was determined by observing 25 spores within each group. A spore was considered germinated if the germ tube length was one half the length of the spore. Germ tube length of 10 spores per group was measured after 8, 12, and 24 hr at 20, 24, and 28 C. Each temperature treatment was replicated three times and the experiment was repeated once.

Time of leaf infection. Potted seedlings of Rome Beauty were used to determine the time of leaf infection during 1983 and 1984. In 1983, one seedling with one to three shoots each was placed in two orchards in Henderson County and two orchards in Haywood County weekly from 5 May through 7 July and every 2 wk from 7 July through 6 September. Brooks spot had been prevalent on fruit in these orchards in 1982. After removal from the orchards, seedlings were maintained outside the greenhouse at MHCRS. Five seedlings placed outside the greenhouse on 21 May served as controls. On 1 October, six to 10 leaves were picked off each seedling, placed in cheesecloth packets, and overwintered in Saran cloth cages on the orchard floor. Leaves were examined in May 1984 for pseudothecia. The percent surface area infected was estimated visually.

In 1984, sets of three seedlings were placed in a Rome Beauty orchard at MHCRS from 2 May through 25 June. Seedlings remained in the orchard for varying times, depending on number and duration of rainy periods, and were then placed in a greenhouse. In early September seedlings were placed in a bed outside the greenhouse, and in October all leaves exposed during May or June were removed, placed in cheesecloth packets, and overwintered in Saran cloth cages. Leaf infection was assessed in May and June 1985 as described for 1984.

The effect of leaf age on infection. Potted seedlings of Rome Beauty were placed in the orchard during six periods from 2 May through 25 June 1984. Four plants with one to three actively growing shoots each were placed in the orchard from 2–4, 7–10, 21–25, and 28–31 May and 4–15 and 15–25 June. The six youngest leaves were identified by attaching colored tape to their petioles. Leaves in position seven and older were considered in one age class. After exposure in the orchard, trees were kept in a greenhouse until 1 September and then were placed in a cold frame outside the greenhouse. On 4, 11, 18, and 31 October, leaves that had abscised were collected, placed in cheesecloth bags, and overwintered in Saran cloth cages. In May 1985, leaves were examined for pseudothecia of *M. pomi*. Some leaves had blown away and were lost before collection, whereas others were badly decomposed, thus the number of leaves examined for each age and exposure period ranged from 0 to 13 with a mean of 5.4 leaves.

The effect of leaf age on infection was studied in the greenhouse by inoculating six Golden Delicious seedlings with 10–12 expanded leaves. A spore suspension of 4×10^5 conidia per milliliter was atomized onto the leaves to the point of drip, trees were allowed to dry for approximately 15 min, and then were placed in a moist chamber at 100% RH and 20–22 C for 192 hr. After the inoculation period, all leaves were removed from the trees and the position of each leaf was recorded. Leaves were dipped into a 0.525% NaOCl solution for 10 sec, blotted dry, and 10 leaf disks (4.1 mm diameter) were punched from each leaf at random and plated on PWA. Plates were incubated at 20–22 C and leaf pieces were observed after 3 and 6 wk for growth of *M. pomi*. Leaf disks from one uninoculated tree served as a control. The experiment was repeated once.

Effect of duration of wetting on leaf infection. Twelve Golden Delicious apple seedlings with one to two shoots each were inoculated with a spore suspension (4×10^5 conidia per milliliter) of *M. pomi*. Both leaf surfaces were atomized to the point of drip and trees were placed in a moist chamber at 100% RH and 20–22 C for 24, 48, 72, 96, 120, or 192 hr. After each wetting period two seedlings were removed from the chamber and five leaves were removed arbitrarily from each seedling. One leaf disk (11.2 mm diameter) was punched from each leaf, stained in acidified trypan blue (45 ml of acetic acid, 55 ml of water, 0.1 g of trypan blue), and

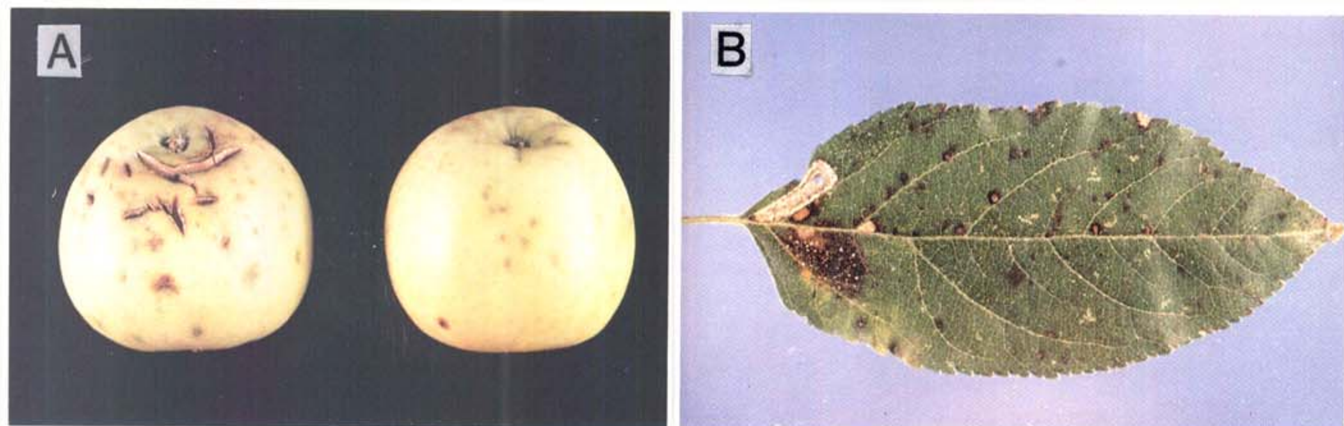

Fig. 1. Golden Delicious fruit and leaf infected with *Mycosphaerella pomi*. **A,** Severely infected fruit (left) showing cracking at points of infection. Moderately infected fruit (right) showing small sunken lesions associated with lenticels. **B,** Infected leaf showing purple flecks characteristic of *M. pomi* infection.

the percentage of germinated conidia that penetrated stomata on the abaxial surface was recorded. An additional 48 leaf disks (4.1 mm diameter) were punched at random from the leaves, washed for 30 min in running water, blotted dry, and plated on PWA. The same leaves were then dipped into a 0.525% NaOCl solution for 10 sec, blotted dry, and 48 leaf disks (4.1 mm diameter) were punched at random from them and plated on PWA. Plates were incubated at 21–23 C and tissue pieces were observed after 3 and 6 wk for growth of *M. pomi*.

Effect of interrupted wetting on leaf infection. Forty Golden Delicious apple seedlings were inoculated with a conidial suspension (4×10^5 spores per milliliter) of *M. pomi* by atomizing both leaf surfaces to the drip point. Four seedlings were aspirated with distilled water as a control. All trees were allowed to dry, then inoculated seedlings were placed into a mist chamber at 20–22 C and 100% RH. Eight trees were removed from the mist chamber after 12, 24, 48, and 84 hr and placed into another chamber with 80% RH. Four trees from each group were returned to the mist chamber after 12 and 24 hr. Trees then remained in the mist chamber with 100% RH until they had accumulated 144 hr total wetting.

Three leaves were collected from each of the four trees per moisture treatment. One leaf disk (12 mm diameter) from each leaf was stained with acidified trypan blue for 15 sec and blotted dry. Approximately 10% of the area of each leaf disk was examined at 400× by making two parallel passes through the disks and the number of mycelial penetrations of stomata were recorded. Thirty leaf disks were punched from each three-leaf sample, surface sterilized in 0.525% NaOCl solution for 10 sec, blotted dry, and plated on PWA. Plates were incubated at 21–23 C and after 3 and 6 wk leaf disks were examined for growth of *M. pomi*.

Ascospore dispersal in the orchard. Airborne dispersal of ascospores of *M. pomi* was studied at MHCRS from 1 May to 16 June 1982, 28 April to 28 June 1983, and 1 May to 16 June 1984; at the Saylor Orchard from 14 April to 14 June 1982 and 13 April to 28 June 1983; at Barber's Orchard from 12 April to 6 July 1983; and the Stepp Orchard from 13 April to 28 June 1983. The MHCRS and Stepp Orchard are in Henderson County, Barber's Orchard is in Haywood County, and the Saylor Orchard is in Mitchell County. Ascospores were trapped at each location with a Burkard volumetric spore trap (Burkard Scientific Sales, Ltd., Rickmansworth, Hertfordshire, England). Traps were adjusted to sample 10 L of air per minute; the trap orifice was approximately 40 cm above the orchard floor. Tapes from the trap were cut into 48-mm sections, mounted on glass slides, stained with cotton blue in lactophenol, and ascospores were counted under 400× by making one traverse through the center of each hourly exposure. Temperature and relative humidity at each location were monitored with a recording hygrothermograph (Belfort Instruments Co., Baltimore, MD) located in a standard instrument shelter, and rainfall was measured with a top-weighing recording rain gauge (Belfort Instruments Co., Baltimore, MD). Leaf wetness was measured at MHCRS and the Saylor Orchard with a DeWitt leaf wetness meter (Valley Stream Farm, Orono, Ontario, Canada).

Histopathology. Leaves from greenhouse inoculations and naturally infected Golden Delicious leaves were collected for microscopic examination. Leaves from the greenhouse were from trees that had been inoculated with conidia of *M. pomi* and incubated in a mist chamber for 192 hr. Numerous purple flecks were visible on naturally infected leaves collected in mid-September 1985. Leaf disks (4.1 mm diameter) were cut from the leaves, fixed in FPP [a mixture of 2-propanol, water, propionic acid, and formaldehyde (45:45:5:5 v/v)], dehydrated, embedded in Paraplast+ (MP = 56 C. Sherwood Medical Industries, St. Louis, MO), sectioned at 12 μm and stained according to a modified Connant's stain (8).

RESULTS

Isolations. *M. pomi* was isolated from small purple flecks or small necrotic spots during 1983–1985. For example, in a series of isolations from Rome Beauty and Golden Delicious leaves collected on 23 September 1983, *M. pomi* was isolated from 20 of 59 purple flecks and six of 23 small necrotic spots. Purple flecks were 1–3 mm diameter and often were located along the leaf veins (Fig. 1B). They were first noticed on leaves in mid- to late-August and increased in number until leaf fall. The fungus was rarely isolated from large necrotic areas.

Inoculations with ascospores. Pseudothecia were evenly dispersed over the surface of inoculated leaves. Ninety percent of the leaves were infected and an average of approximately 12% of the leaf surface was covered with pseudothecia. All of leaves picked from the ground were infected and pseudothecia covered an average of 15% of the leaf surface; leaves picked off the tree were 80% infected and pseudothecia covered 9% of the leaf surface. Fifteen percent of the leaves from the uninoculated control were infected, but less than 1% of the leaf surface was covered with pseudothecia, which occurred predominantly in clumps.

Effect of temperature on ascospore germination. Some ascospores germinated after 2 hr at 16, 20, 24, and 28 C, and after 6 hr most of the spores germinated at these temperatures (Fig. 2). Less than 10% of the spores germinated at 4 C after 24 hr. After 4 and 12 hr there was no significant difference in germ tube length between 20, 24, and 28 C. After 24 hr the germ tube length at 24 C (432 μm) was significantly longer ($P = 0.05$) than at 20 C (362 μm) and at 28 C (256 μm).

Time of leaf infection. In 1983, infection was recorded during each period from 5 May until 15 June, except 11–18 May (Table 1).

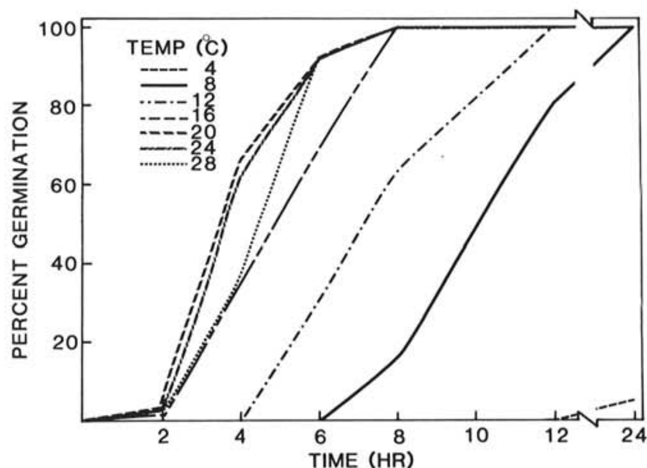

Fig. 2. Effect of temperature on ascospore germination of *Mycosphaerella pomi.*

TABLE 1. Percent leaves infected with *Mycosphaerella pomi* on potted apple trees placed in orchards for varying periods of time during 1983 and 1984

Year	Period in orchard	Leaves infected (%)
1983[a]	May 5-11	43
	May 11-18	0
	May 18-25	13
	May 25-31	3
	May 31-June 8	15
	June 8-15	30
	June 15-21	0
	June 21-28	0
	June 28-July 7	0
	July 7-Sept. 6[b]	0
1984	May 2-4	6
	May 7-13	5
	May 21-25	4
	May 28-31	5
	June 4-15	13
	June 15-25	22

[a] Results combined from three orchard locations.
[b] Two-week intervals combined through this period.

The greatest percent leaf infection was recorded during the period from 5–11 May, although it rained only one day during the period (0.89 cm) with 6 hr leaf wetness. No infection was recorded after 15 June. In 1984, some infection was recorded during all periods; the greatest percent infection was recorded from 15–25 June (Table 1). This was a wet period with rain on 9 days (8.25 cm total) and 127 hr leaf wetness.

Influence of age on leaf susceptibility. Degree of infection was relatively low on the overwintered leaves, averaging 6.9%. There was no significant difference ($P = 0.05$) in the amount of infection on leaves of different ages; the percent infected leaves ranged from 4.5% on the second expanded leaf to 9.8% on the third expanded leaf.

Infection was variable in the first greenhouse test. The percent leaves infected on individual seedlings ranged from 40 to 100%. There was no significant difference in leaf age ($P = 0.05$), although the percent leaf disks infected by age class ranged from 35 to 68%. Infection was more uniform in the second test; all leaves were infected. The percent infected leaf disks ranged from 97 to 100%.

Effect of duration of wetting on infection. Penetration of the stomata by the fungus was not observed until 96 hr of wetting. At that time approximately 5% of the conidia produced germ tubes with distinctly swollen areas, resembling appressoria, over the stomata (Fig. 3A). In some cases, hyphae appeared to enter directly into a stomate without any noticeable swelling. We occasionally observed hyphae originating from the same conidium penetrating two or more stomata after 120 or 196 hr wetting. The number of germinated conidia that penetrated stomata never exceeded

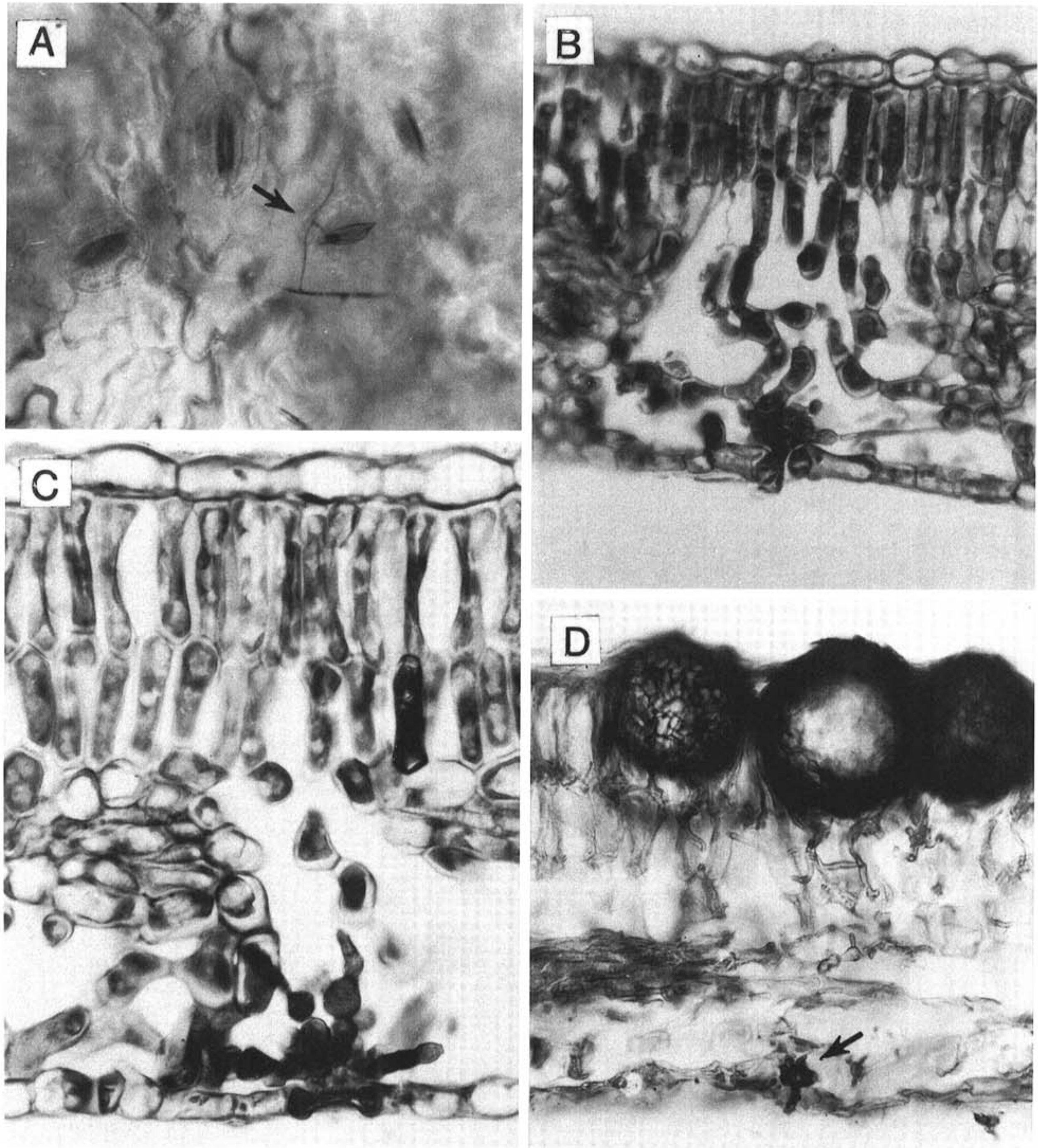

Fig. 3. Light microscopy of leaves infected with *Mycosphaerella pomi*. **A,** Germinated conidium of *M. pomi* with appressorium over stomate (arrow). **B,** Group of vesicle- or chlamydospore-like structures beneath stomate. Note remnant of mycelium in stomatal opening and strand of mycelium growing from one of the chlamydospore-like structures. (×250). **C,** Large group of chlamydospore-like structures beneath a stomate. **D,** Pseudothecia of *M. pomi* in leaf tissue. Note remnant of vesicle-like structure beneath stomate (arrow) and mycelium throughout leaf tissue.

5–10%. In many cases mycelium grew over stomata without any indication of penetration. We did not observe any direct penetration of the cuticle.

M. pomi grew from 24% of the leaf disks after 6 wk, following 24 hr wetting and washing and 70–90% after 48–120 hr wetting (Table 2). The fungus did not grow from leaf disks taken from leaves that remained in the moist chamber for 24 or 48 hr and were surface sterilized. Approximately 10% of the leaf disks taken from leaves that were wet for 72, 96, or 120 hr were infected, and 94.8% of the disks from the 192-hr treatment were infected.

Effect of interrupted wetting on leaf infection. Percent leaf disk infection did not differ significantly from the continuous wet treatment when wetness was interrupted after 12 or 24 hr wetting (Fig. 4A). A 24-hr interruption after 12 hr wetting increased the level of infection ($P = 0.05$) but had no effect after 24 hr wetting. No deviation from the continuous wet treatment was seen when wetting was interrupted after 48 or 84 hr for a 12-hr period, but when the dry period was extended to 24 hr, a greater percentage of the leaf disks were infected than in the continuous wet treatment ($P = 0.05$).

The interruption of wetting for 12 or 24 hr of wetness had no significant effect ($P = 0.05$) on the number of stomata penetrated by mycelium of *M. pomi* when compared with the uninterrupted, constant wet treatment (Fig. 4B). Interruption for 24 hr after 24 hr of wetness resulted in reduced stomatal penetration with respect to both the 12-hr interruption and the continuous wet treatments. There was no difference ($P = 0.05$) between the 12-hr interruption and the continuous wet treatment. Interruption of wetting after 48 hr had no effect on the number of stomata penetrated when the period of reduced relative humidity was 24 hr but, when this period was 12 hr, stomatal penetration was enhanced (Fig. 4B). When wetting was interrupted after 84 hr, there was no difference in the 12- or 24-hr interruption and the continuous wet treatment ($P = 0.05$).

Ascospore dispersal. During the 4 yr that ascospore dispersal was monitored, ascospores were generally first trapped in late April, peak discharge occurred in mid-May, and few spores were trapped after mid-June. Discharge patterns observed in Mitchell County in 1982 and Stepp in 1983 are representative of the patterns of ascospore dispersal observed (Fig. 5). The period of maximum spore discharge occurred in mid-May approximately 2–3 wk after petal fall. Ascospores were trapped in the largest numbers soon after the initiation of rain and usually continued to be trapped for up to 24 hr after the rainfall ended (Fig. 6). We also observed spore discharge in the morning (0400–1000 hours) in the absence of rainfall when the relative humidity was near 100% and dew formation had occurred. These discharges were proportionately lighter than those that occurred during rainfall.

Histopathology. *M. pomi* developed slowly following penetration of the stomata. In September, approximately 12–16 wk after infection, we observed thick-walled vesicle- or chlamydospore-like structures just beneath the stomata (Fig. 3B and C). Hyphae grew from some of these structures, but leaf colonization was not extensive. A purple discoloration was observed on the leaves at the time they were fixed for histological examination despite the limited colonization. After leaf fall, the mycelium of *M. pomi* grew intercellularly into the mesophyll and palisade layer, extensively colonizing the leaf (Fig. 3D). Pseudothecia were produced on either leaf surface but were more common on the adaxial surface.

TABLE 2. Effect of duration of wetting on leaf infection by *Mycosphaerella pomi*

Hr wet	Leaf disks infected (%) Washed[a]	Leaf disks infected (%) Surface sterilized[b]	Mycelium observed penetrating stomates
24	24.0	0	–[c]
48	79.2	0	–
72	67.7	10.4	–
96	91.7	6.3	+
120	94.8	11.5	+
192	···	94.8	+

[a] Leaf disks washed for 30 min in running water and blotted dry before plating on PWA.

[b] Leaf disks dipped into a 0.525% NaOCL solution for 10 sec and blotted dry before plating on PWA.

[c] – = No stomatal penetration observed; + = stomatal penetration observed.

DISCUSSION

Based on the results of this study, we propose the following as the disease cycle for *M. pomi*, cause of Brooks fruit spot of apple. *M. pomi* overwinters in infected apple leaves on the orchard floor, and pseudothecia mature in late April in North Carolina. Ascospores are discharged during periods of rain and in heavy dew from late April through mid- to late June resulting in fruit and leaf infections. Fruit infections first become visible in early July as slightly sunken greenish lesions associated with the lenticels. Leaf infections remain quiescent until late summer, when they appear as small purple flecks in the leaves. Infected leaves fall to the ground in October and November and the cycle repeats itself. We have no evidence of any secondary infection occurring. During the course of the study we never observed *M. pomi* fruiting on infected apples or leaves in the orchard and in 1983 there was no infection on potted trees that remained in the orchard from 21 June until 6 September.

Tsuyama et al (14) inoculated scorched apple leaf tissue with Cylindrosporium spores and in 3–4 wk obtained mature pseudothecia of *M. pomi* with ascospores. Our studies indicate that under orchard conditions the developmental process is much slower. Furthermore, we have no indication that infection through wounded or necrotic tissue is important in the orchard. Under conditions in North Carolina, necrotic tissues are quickly invaded and colonized by saprophytes such as *Alternaria* spp.

Although ascospores germinate quickly, our data suggest that a relatively long wet period is necessary for infection to occur. We

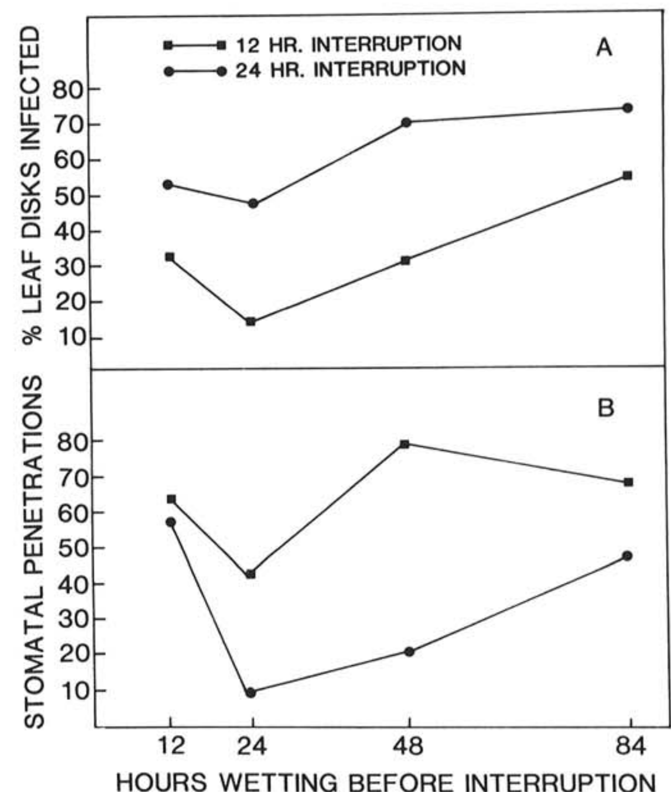

Fig. 4. The effect of interrupted wetting on infection by *Mycosphaerella pomi*. **A,** Percent leaf disk infection by *M. pomi*. **B,** Percent stomatal penetration. Thirty percent of the leaf disks were infected in the continuous wet treatment and there were 46.5 stomatal penetrations.

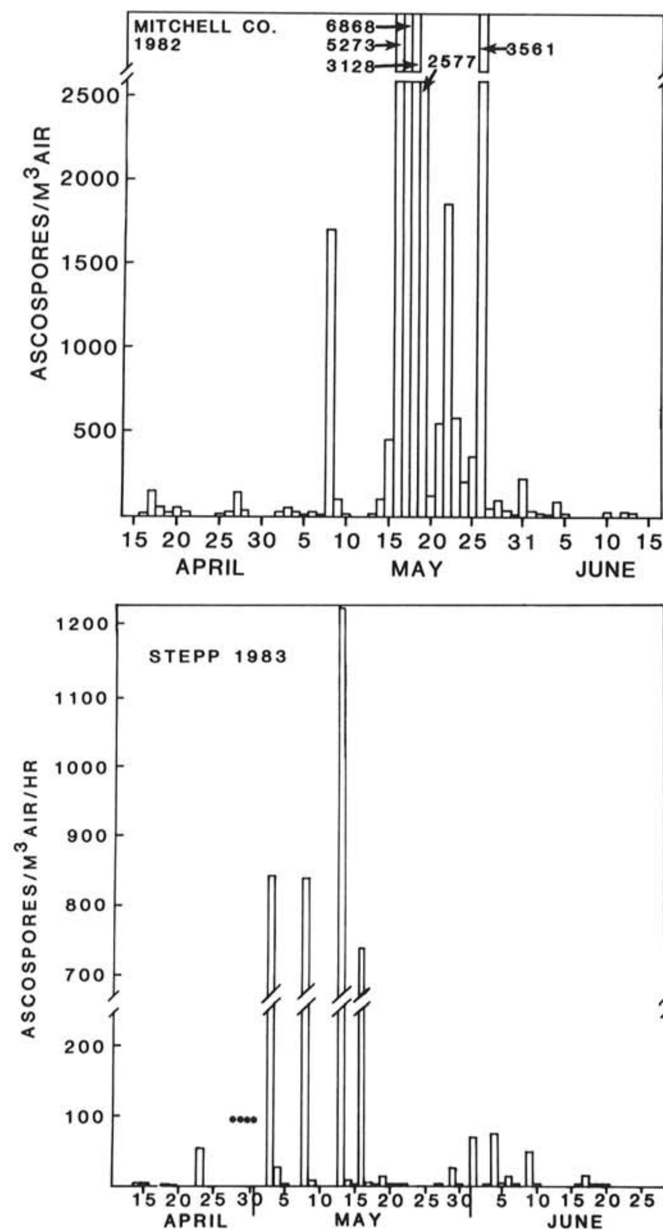

Fig. 5. Ascospore discharge patterns of *Mycosphaerella pomi* from Saylor Orchards in 1982 (Mitchell County) and J. H. Stepp and Sons (Stepp) in 1983.

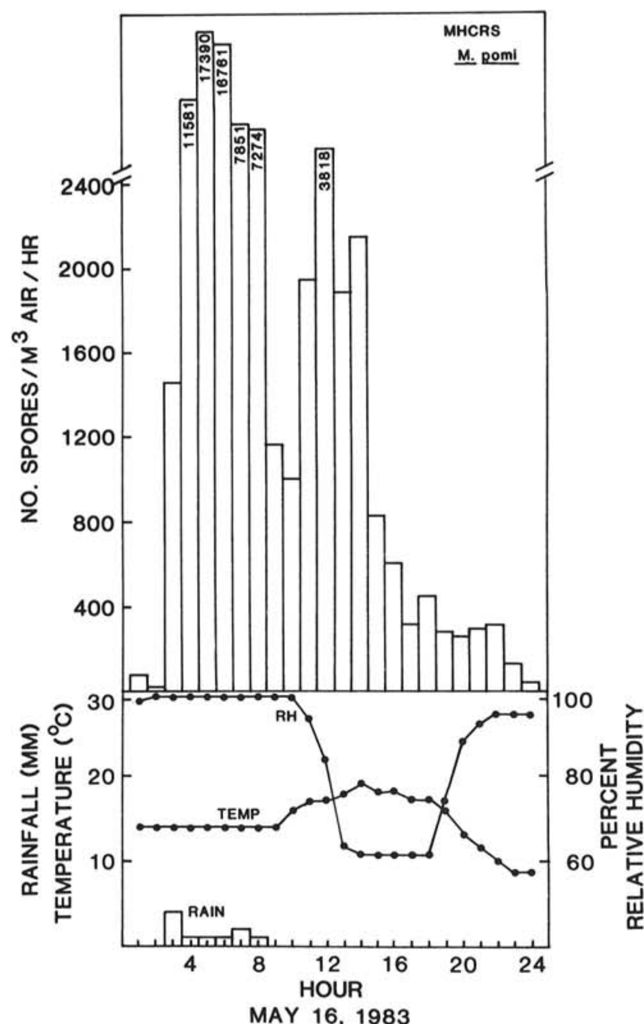

Fig. 6. Ascospore discharge pattern for *Mycosphaerella pomi*, rainfall, temperature, and percent relative humidity on 16 May 1983 at the Mountain Horticultural Crops Research Station (MHCRS) 1983.

did not observe any appressoria formation or penetration until 96 hr of continuous wetting had occurred. However, we found that germinated spores of *M. pomi* were able to withstand drying periods of at least 24 hr without any reduction in infection. This may explain why infection occurs under orchard conditions, although infection requirements are seldom met during one rain period. In some treatments, leaf infection was increased by a 12- or 24-hr interruption of the wetting period. This suggests that, under orchard conditions of wetting and drying, infection may occur in a shorter period of time than our studies indicate. We were successful in obtaining infection from ascospore inoculations when trees were placed behind the greenhouse after only 50 hr incubation in a mist chamber. Whiteside (17) reported that the number of stomatal penetrations by *M. citri* Whiteside was greatly enhanced by daily drying of otherwise continuously wet lemon leaves. Moore (10) found that discontinuous wetting stimulated infection of apple leaves by *V. inaequalis* when dry periods were 8 hr and that leaf infection was only slightly inhibited when dry periods were 24 hr. However, longer dry periods greatly inhibited leaf infection.

The increase in leaf infection by *M. pomi* observed in the 24-hr interrupted wetting experiments was apparently not due to an increase in the number of stomata penetrated. The 24-hr interruption had no effect or resulted in a reduction of stomatal penetrations. This apparent paradox may be explained by factors that affect establishment of the fungus within the leaf. A 24-hr dry period may arrest or retard mycelial growth, thus reducing stomatal penetrations, while triggering the colonization of previously penetrated stomata. A dry period of 12 hr may be long enough to induce formation of an appressorium but not long enough to trigger a shift in fungal activity from mycelial growth to leaf colonization.

The development of leaf symptoms of *M. pomi* is similar to that of greasy spot of citrus caused by *M. citri* (16). After infection, mycelium of *M. citri* slowly grows through the mesophyll and palisade layer and symptoms are not visible for 8–12 wk after infection. We observed purple flecks on apple leaves only after 12–16 wk. During this time, growth of *M. pomi* was restricted primarily to the mesophyll tissues, during which thickened chlamydospore- or vesicle-like structures were formed. We observed only occasional hyphae growing from these structures in leaf sections made in September. Extensive colonization of the leaf apparently does not occur until after leaf fall. In the spring, mycelia were observed growing throughout the fallen leaf beneath clumps of *M. pomi* pseudothecia.

We did not find any variation in leaf susceptibility with leaf age in orchard or greenhouse tests. There is only a limited amount of data available on fruit susceptibilty. Brooks (2) conducted a series of inoculations during the spring and summer in an orchard in New Hampshire and concluded that the possibility of fruit infection decreases after early July. Other studies (9) have found that fruit

infection is greatest early in the growing season; however, results of these studies are confounded with inoculum availability. Because most lenticels on the fruit become cutinized or suberized during the growing season (6), it seems likely that infection through lenticels would be limited. Studies under controlled conditions are required to determine if fruit susceptibility varies with age.

During the 3 yr of this study, ascospores were trapped during rains and heavy dews from mid-April through early July. Peak discharges occurred in mid-May, 2–4 wk past full bloom for Delicious. Therefore, the period from petal fall through the second cover spray is critical for satisfactory Brooks spot control. Metiram, mancozeb, benomyl, and Dikar applied during this period on a 10–14-day schedule provide satisfactory control (18). However, the ergosterol biosynthesis-inhibiting fungicides (EBI) are ineffective (4,5,11,12). These materials are very effective against apple scab (caused by *V. inaequalis*) and powdery mildew (caused by *Podosphaera leucotricha* (Ell. & Ev.) Salm.) and may be used in the postbloom period. Thus in areas where Brooks spot is a potential problem, combinations of an EBI fungicide and a suitable protectant will be necessary during the 4–6-wk period after petal fall.

LITERATURE CITED

1. Anderson, H. W. 1956. Diseases of Fruit Crops. McGraw-Hill, New York. 501 pp.
2. Brooks, C. 1908. The fruit spot of apples. Bull. Torrey Bot. Club 35:423-456.
3. Brooks, C., and Black, C. A. 1912. Apple fruit spot and quince blotch. Phytopathology 2:63-74.
4. Brown, E. M., and Sutton, T. B. 1984. Apple disease control, 1983. Fungic. Nematic. Tests 39:1.
5. Brown, E. M., and Sutton, T. B. 1984. Timing of Vangard and captan applications for Brooks spot control, 1983. Fungic. Nematic. Tests 39:2.
6. Clements, H. 1935. Morphology and physiology of the pome lenticels of *Pyrus malus*. Bot. Gaz. 97:101-117.
7. Gilpatrick, J. D., Smith, C. A., and Blowers, D. R. 1972. A method for collecting ascospores of *Venturia inaequalis* for spore germination studies. Plant Dis. Rep. 56:39-42.
8. Johansen, D. L. 1940. Plant Microtechnique. McGraw-Hill, New York. 523 pp.
9. Martin, W. H. 1929. The Phoma fruit spot of apple. Proc. N.J. State Hort. Soc. 55:57-66.
10. Moore, M. H. 1964. Glasshouse experiments in apple scab. Ann. Appl. Biol. 53:423-435.
11. Sutton, T. B. 1983. Apple disease control, 1982. Fungic. Nematic. Tests 38:154.
12. Sutton, T. B., and Brown, E. M. 1985. Apple disease contol, 1984. Fungic. Nematic. Tests 40:24.
13. Taylor, C. F., and Bennett, T. G. 1950. Infection period of apple diseases as determined by period of coverage with glassine bags. Proc. Cumberland-Shenandoah Fruit Workers Conf. 26th Annual Meeting.
14. Tsuyama, H., Horigome, K., and Sasaki, S. 1973. Study on the life history of *Mycosphaerella pomi* (Pass.) Lindau. Rep. Tottori Mycol. Inst. 10:465-472.
15. Walton, R. C., and Orton, C. R. 1926. The perfect stage of *Cylindrosporium pomi*. Science 63:236.
16. Whiteside, J. O. 1972. Histopathology of citrus greasy spot and identification of the causal fungus. Phytopathology 62:260-263.
17. Whiteside, J. O. 1974. Environmental factors affecting infection of citrus leaves by *Mycosphaerella citri*. Phytopathology 64:115-120.
18. Yoder, K. S. 1982. Fungicide control of Brooks spot of apple. Plant Dis. 66:564-566.