Factors Affecting Release and Dissemination of Physalospora obtusa Spores in a New Hampshire Apple Orchard

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ABSTRACT

Humidity and temp range within a 24-hr period affect the release of *Physalospora obtusa* spores. Most spore releases occurred between 6 and 16 C. A significant number of conidia were released from the pycnidia 1 week earlier in the spring than ascospores were released from the pseudo-perithecia. Pycnidia and pseudoperithecia also retained approximately 25% of their spores in the fall. Spore release was 13 times greater when rains occurred at night than during the daytime. Dissemination of *P. obtusa* spores was mainly by splashing rain. Windblown spores occurred only rarely in spore traps during 1967 and 1968. Spores can be disseminated by the ladybird beetle (*Hippodamia convergens*). Phytopathology 60:1052-1054.

Physalospora obtusa (Schw.) Cooke, incitant of black rot of apple, produces three syndromes: canker, leaf spot, and fruit rot. The black rot disease, once considered unimportant, has caused losses in several commercial orchards in New Hampshire in recent years.

The wide range of wild hosts for *P. obtusa*, covering 55 plant families (3, 5, 14, 15), makes it increasingly important to know what factors affect spore dispersal. Three methods of dissemination are discussed in the literature: splashing rain, wind-blown mist, and insects (1, 7, 8). Dissemination by splashing rain and insects is generally accepted, whereas wind-blown mist is questioned (1, 7, 8, 17).

Walton (16) stated that frogeye leaf spot infection is correlated with 3-day rainy periods when the temp is sufficiently high for spore germination. Recently matured conidia will germinate within 5 or 6 hr, while overwintering ones require longer periods. The most favorable temp for spore germination are about 23-27 C (6). Foster (6) showed that the optimum temp for frogeye leaf spot infection was 20 C for a 24 hr period.

The object of this research was to clearly define some of the main climatic factors that affect the release of *P. obtusa* conidia and ascospores in the field, and to evaluate the methods of their dispersal.

MATERIALS AND METHODS.—Spore collection stations were maintained in a neglected apple orchard from 15 April to 20 October 1967, and from 1 April to 1 August 1968. Microscope slides coated with silicone (12) were used as spore traps at all stations. Slides were collected daily and the spores deposited over a 0.5 cm^2 area during the 24-hr period were counted. A battery-operated Kramer-Collins spore sampler (10) designed for automatic quantitative collection of fungus spores every 15 min over 24-hr periods was used at one station in 1967. The remaining stations employed exposed microscope slides set on plastic-covered boxes 50 cm above the ground. Station locations from 15 April to 20 October 1967 were as follows: Station A; Kramer-Collins spore sampler operated from 15 April to August 3, 1967, and was located directly under the canopy of a large apple tree heavily infected with *P. obtusa*. Station B; located 25 cm from station A to check on the efficiency of the Kramer-Collins spore trap. Due to lack of data from 15 April to 12 June 1967, caused by the inability of rain to penetrate the heavy foliage, infected limbs bearing pseudoperithecia were placed 15 cm above stations A and B from 12 June to 20 October 1967 to provide an inoculum source. Station C; located in an open area 10 m from the nearest apple tree and 20 m from the brush pile at the edge of the orchard. Station D; located 15 cm under infected pruned limbs in the brush pile. Station E; located 2 m east of the brush pile. Station locations from 1 April to 1 August 1968 were as follows: Station F; located in the same place as station C. The slide was placed under a wire cage and 25 immature apple mummies infected with *P. obtusa* were placed on the wire mesh 6 cm above the slide. Station G; located in the same place as station D.

Weather data were collected with a temp recorder, a recording hygrothermograph, and a rainfall recorder at the spore station area. The spore deposition and weather data were treated as a linear regression and the correlation coefficient was derived (13).

To check the presence and dissemination of spores on the bodies of insects in the orchard, the ladybird beetle (*Hippodamia convergens* Guerin) was chosen because of its presence in orchards from early to late spring during the time of primary and secondary spore release of *P. obtusa*. Beetles were collected individually from early March 1968 to late May 1968 in sterilized test tubes plugged with cotton. The insects were placed on potato-dextrose agar in petri plates and incubated for 2 weeks at 21 C. The plates were examined for presence of *P. obtusa*.

RESULTS.—Frogeye leaf spots appeared on apple leaves approximately 19 days after the first large release of conidia in the spring of 1967 and again in 1968. This occurred at approximately mid-bloom in both years, which varied from 25 May in 1967 (a late spring) to 11 May in 1968 (an early spring).

Data from release and dissemination experiments are given in Tables 1 and 2. Stations C and E were not

TABLE 1. Hours of 100% relative humidity (RH) and spore deposition of *Physalospora obtusa* for days when spore deposition occurred in the apple orchard

Hours of 100% RH/day	Mean spore deposition 1 May-31 July 1968[a]		
	Station F[b]	Station G	Combined stations
0-6	18		9
7-12	82	17	49
13-18	373	158	265
19-24	461	211	336

[a] The correlation between hr of 100% RH and the number of spores deposited is significant at the .05 level.
[b] Both spore trap stations were located under inoculum sources.

represented in tabular form due to the very low spore deposition at these locations.

The number of hr of 100% relative humidity (RH) within a 24-hr period and also the daily temp range correlated significantly (.05 level) with the number of spores collected. The highest spore counts for all stations were obtained with 20 hr of 100% RH/day, and a daily temp range of 2 C (Tables 1, 2). While not statistically different from each other (.05 level), the highest spore counts for stations D, F, and G (1,000-4,000 spores deposited) occurred in conjunction with the following: 1 inch of rain, 14 hr of estimated wet period, a mean daily temp of 11.2 C, maximum daily temp of 12.6 C, and a minimum daily temp of 9.8 C. Rain was needed for release of either conidia or ascospores, but the amount of rain was not significantly correlated with the number of spores released. The mean temp for the days of maximum spore release were 9 C in May, 15 C in June, and 20 C in July. The number of spores released was highest in May, followed by June and July. The largest spore release in May occurred during blossom time, which has long been identified as the time of greatest primary infection (1, 7, 8, 16). In 1968, which had an early spring, initial spore release occurred on 31 March. The earliest spore release in 1967 was 23 April. The first major spore release from infected immature apple mummies (pycnidia present) occurred 1 week earlier in the spring than the first major spore release from infected prunings (pseudoperithecia present).

At station C, located in the open 10 m from the nearest apple tree, a very small spore deposition of 5 spores occurred during 2 successive days of very high winds in June 1967. At station E, located 2 m from the brush pile in the open, 37, 64, and 2 spores were deposited on 3 successive days, respectively, of very high winds during the same rainy period. Data obtained from these two stations show that only a very limited amount of spore dissemination occurs by wind-blown mist, and that this is limited to rainy periods of high wind velocity.

Spore release was 13 times greater when rains occurred at night than during the daytime (mean spore deposition of 891 at night vs. 67 spores during the day). During autumn it was observed that even with heavy rains and seeemingly favorable temp and humidity, there were very few spores released.

Of the ladybird beetles (*H. convergens*) collected in 1968, 18% were infested with *P. obtusa*.

DISCUSSION.—It is interesting that the highest single spore count obtained in this study occurred at a mean daily temp of 7.2 C with a daily temp range of only 1.1 C. Walton's (16) data in 1920 indicate that the first large infection probably occurred at a mean daily temp of 7.2 C with a daily temp range of 1.6 C, although he did not draw this conclusion. Foster (6) also found that considerable leaf infection developed at 8 C for 48 hr.

Anderson (1) and Hesler (8) indicated that in laboratory studies the most favorable temp for spore germination was about 23-27 C, while at a temp of 15 C, spores germinated so slowly that infection usually failed. Apparently the greatest number of spores are released at a relatively low mean daily temp varying from 6-16 C, with a very low (1.1-4.9 C) daily temp range (Table 2). This means that major spore releases usually occur during low daily mean temp that hold almost stable for long periods of time, due in part to the presence of 100% RH (Tables 1, 2). Thus, the humidity, besides providing moist conditions for spore release, also helps provide the long periods of stable temp required for heavy spore releases.

TABLE 2. Daily temp range and *Physalospora obtusa* spore deposition on days when spore discharge occurred in the apple orchard

Difference between daily minimum and maximum temp (C)	Mean spore deposition[a]					
	Station A 6-15-67 to 7-31-67	Station B 6-15-67 to 7-31-67	Station D 5-1-67 to 7-31-67	Station F 5-1-68 to 7-31-68	Station G 5-1-68 to 7-31-68	Mean for combined stations
1.0-2.9	870	373	1464	681	271	732
3.0-4.9	130	234	183	669	384	320
5.0-6.9	31	19	88	550	117	161
7.0-8.9		31	260	5	1	59
9.0-10.9	225	58	125	62	8	96
11.0-12.9		5	174	76	9	53
13.0-14.9			87	38	18	29
15.0-16.9				137	160	59
17.0-18.9				158	27	37
19.0-20.9			315	167	40	104

[a] The correlation between daily temp range and the number of spores deposited is significant at the .05 level. All spore trap stations were located under inoculum sources.

Data from stations C and E show that a limited amount of either conidium or ascospore dissemination by wind-blown mist occurs during rainy periods of high wind velocity. This very infrequent deposition of spores by wind-blown mist, even close to a major source of inoculum such as a brush pile, could explain why Wolf (17) failed to trap *P. obtusa* in 1910.

This study showed that conidium and ascospore release was 13 times greater when rains occurred at night than during the daytime. Since the number of hr of 100% RH was significantly correlated (.05 level) with the number of spores released (Table 1), nights would logically be the period of largest spore release. Ingold (9) suggested that four major factors influencing the release of spores (temp, humidity, light, and wind) tend to exhibit a daily cycle of behavior. During the day, light, temp, and wind velocity are usually maximal, while humidity is usually high at night and associated with lower temp and decreased atmospheric turbulence. Walton's (16) meteorological data in 1920 also appeared to show a similar increase in infection following rains which occurred at night.

Examination of fruiting bodies (pseudoperithecia and pycnidia) in the laboratory revealed that approximately 25% of the spores were still present in the autumn. The retention of spores in the asci of *Venturia inaequalis* was also reported by Miller & Waggoner (11), who stated that the appearance of ascospores in the air ceased in late spring, while an estimated 25% of the spores still remained in the asci. Our spore collections were continued until 20 October 1967; by this time all apples had been picked and stored. This failure to release spores in the autumn at a time when the apple is exposed to injury and infection, during the process of picking and storage, is thought to be of critical importance. If spore release did occur at this time, black rot of stored apples would probably be a much greater problem than at present. The exact reason for the retention of spores in the fruiting bodies in the fall under seemingly favorable conditions for spore release is not understood. It may result from selection for a mechanism which assures a reserve for the initial spore release in the spring of the following year.

The first major spore release from infected immature apple mummies (pycnidia present) occurred 1 week earlier in the spring than the first major spore release from infected prunings (pseudoperithecia present). Thus, the immature apple mummies in the trees, being in strategic positions close to the leaves and fruit, provide inoculum for primary infection of leaves and fruit.

Ladybird beetles are prevalent during pollination of the apple flowers, and the only way to prevent the local spore dissemination by these insects is to minimize the amount of inoculum present in and around an apple orchard, as suggested by other writers (1, 2, 4, 7, 8).

Thus, high humidity and a narrow temp range within a 24-hr period were found to be the main factors favoring spore release, and spores were disseminated mainly by splashing rain, at least one species of insect, and only rarely by wind-blown mist.

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