Epidemiology of the Black Knot Disease of Plums

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ABSTRACT

Dibotryon morbosum ascospores were ejected for distances as great as 45 mm shortly after ascocarps were moistened in the laboratory. In field experiments, ascospore ejection occurred only on days with measurable rainfall. The initial ejection period of the season seemed to be related to ascospore maturity. During air-sampling periods (April, May, and June) with a Hirst spore trap in 1965 and 1967, ascospores were trapped on 14 days during each year. In both years, initial catches occurred in late April, with final catches in late June. The time between the onset of rainfall and the deposition of ascospores on Hirst trap slides ranged from 0.3 to 3 hr. Ascospores germinated on agar media, but rarely did germ tubes continue to elongate and produce the imperfect stage. Of the temp studied, 24 C was most favorable for germination. In greenhouse pathogenicity tests, severe infection of *Prunus domestica* L. 'Stanley' trees occurred with postinoculation moisture periods as brief as 6 hr at 21 C. Apparently, ascospores constitute the only significant source of inoculum. In several years of field tests with Stanley trees, it was shown that most natural infection occurred after petal-fall during May and June in southern Pennsylvania. Phytopathology 60:1441-1444.

Additional key words: Dibotryon morbosum, spore dispersal.

The black knot disease of plums and cherries is caused by the ascomycetous fungus *Dibotryon morbosum* (Schw.) Theis. & Syd. (13). Farlow (3) placed the imperfect stage of the fungus in the genus *Cladosporium,* and Koch (9) assigned it to *Hormodendrum.* These genera were combined under the name *Cladosporium* by de Vries (14). Black knot has been known in the United States for more than 100 years; it is probably a disease of American origin (1, 2, 3). It is a serious problem on some varieties of plums in Pennsylvania.

The most significant contributions to the present knowledge of black knot were made by Koch (7, 8, 9, 10). The most intensively studied aspects of this disease have been symptomatology and control. Much remains unknown about black knot, especially its epidemiology and the developmental morphology of the pathogen.

The present work was planned to obtain information on several points of possible significance in recent epidemics of black knot on *Prunus domestica* L. 'Stanley' in southern Pennsylvania. Two preliminary reports have been published (11, 12).

MATERIALS AND METHODS.—*Ascospore ejection and dissemination.*—Ascospore ejection was studied in several ways: (i) Ejection was observed with a stereomicroscope after wetting ascocarps in the laboratory; (ii) moistened ascocarps were placed under glass slides in the laboratory to determine time required for initial ejection and the approximate ejection distance; (iii) Ingold's apparatus (6) was utilized to obtain precise measurements of ejection distance; and (iv) in the field, horizontal ejection was studied by positioning vaseline-coated slides in close proximity to the ascocarps. This method was used with nine knots on living *Prunus serotina* Ehrh. and seven knots excised from *P. domestica* L. 'Stanley' trees. Spore deposition was determined by counting the ascospores in a 22- × 50-mm area on each of the slides.

An automatic volumetric Hirst spore trap (5) was used for ascospore dissemination studies during 1965 and 1967. The trap orifice was 20 inches above the ground, with an air intake of 10 liters/min. Slides were changed daily at 8 AM.

In 1965, the experimental site was arranged as follows: Severely knotted *P. serotina* trees were planted in a circle 18 ft in diam. Two hundred additional knots were excised from Stanley trees and suspended from a wire 2 ft above the soil in a circle 24 ft in diam. Knots from Stanley trees were also distributed on the ground between the concentric circles. The Hirst trap was placed in the center of the circle and operated continuously from 5 April to 30 June 1965.

In 1967, knots from Stanley trees were attached to a wire forming a circle 18 ft in diam; no *P. serotina* trees were included. A Hirst trap was operated continuously from 6 April until 2 July 1967.

During 1965, temp and rainfall data were collected at the experimental site. The rainfall data for 1967 were obtained at a weather station located 3 miles from the trapping site.

Effect of temp on ascospore germination.—Since *D. morbosum* does not produce ascospores in vitro, it was necessary to obtain ascospores from naturally infected trees. To stimulate ascospore ejection from ascocarps, *P. serotina* knots were immersed for 2-5 min in glass-distilled H_2O, drained of excess H_2O, and placed above petri dishes containing 1.5% H_2O agar. Ascospore suspensions were prepared by immersing agar sections with heavy spore deposits in glass-distilled H_2O. The resultant ascospore suspension was adjusted to 50,000/ml.

The germination medium was Difco nutrient agar (23 g/liter) + penicillin G, potassium form (400,000 units/liter). Nutrient agar was autoclaved and penicillin added after the medium cooled to 60 C. Droplets of ascospore suspension were placed on the medium and the plates were exposed to various temp.

Germination counts were made after 6, 12, 24, and 48 hr. Each treatment was replicated 4 times, and every spore in each randomly selected field was counted until 100 or more spores were observed for each replicate.

Effect of postinoculation moisture period on infection.—Two-year-old Stanley trees were planted in clay pots containing a 2:1 soil:sand mixture. Trees were inoculated by atomizing each tree with 6 ml of a water suspension of ascospores (50,000/ml) obtained from naturally infected Stanley trees. After inoculation, trees were placed in a 21-C moisture chamber for the designated time period, then returned to the greenhouse bench.

Effect of various propagules on infection.—Four different propagules were tested; viz., conidia from a petri dish culture, ascospores from *P. domestica* 'Stanley', ascospores from *P. serotina,* and a mixture of ascospores from *P. domestica* 'Stanley' and *P. serotina.*

Time of natural infection in the field.—The time of infection in the field was estimated within a few days with weather data, spore dissemination data, and the amount of infection on Stanley trees which were not treated with a protectant fungicide until successively later dates in the growing season. The experimental design for each test consisted of randomized blocks with 6-10 single-tree replicates/treatment. To provide inoculum, one knot bearing active ascocarps was placed in the top of each tree during April. Fungicide treatments consisted of one to seven weekly applications of 75% zineb [zinc ethylenebis (dithiocarbamate)] at 2 lb./100 gal. Because of the lengthy incubation period exhibited by *D. morbosum,* disease data were not collected until 4-12 months after the last fungicidal spray.

RESULTS.—*Ascospore ejection and dissemination.*—Forcible ejection occurred soon after ascocarps were moistened. Horizontal ascospore ejection for distances as great as 19 mm was demonstrated with vaseline-coated slides in field tests. In four laboratory tests with Ingold's apparatus, 0.5% of the ascospores were deposited 45 mm from the ascocarp, and 25% were deposited 21 mm or more from the source (Fig. 1).

Ejection of ascospores from *P. serotina* ascocarps occurred in controlled-temp chambers at temp ranging from 3-36 C. Ascospore ejection was 135 times as great at 21 and 29 C as at 5 C. With ascocarps from *P. domestica* 'Stanley', ejection was negligible at 5 C, heavy at 21 C, and light at 36 C. The number of ascospores ejected varied markedly from knot to knot. In the field, some spores were trapped at 6, 8, and 9 C. But during the four periods of max deposition in 1965, temp 1 hr prior to initial spore deposits varied from 14 to 27 C; in 1967 they varied from 16 to 27 C.

The initial ascospore ejection period in the spring seemed to be related to ascospore maturity. Temperature and rainfall were adequate early in the season;

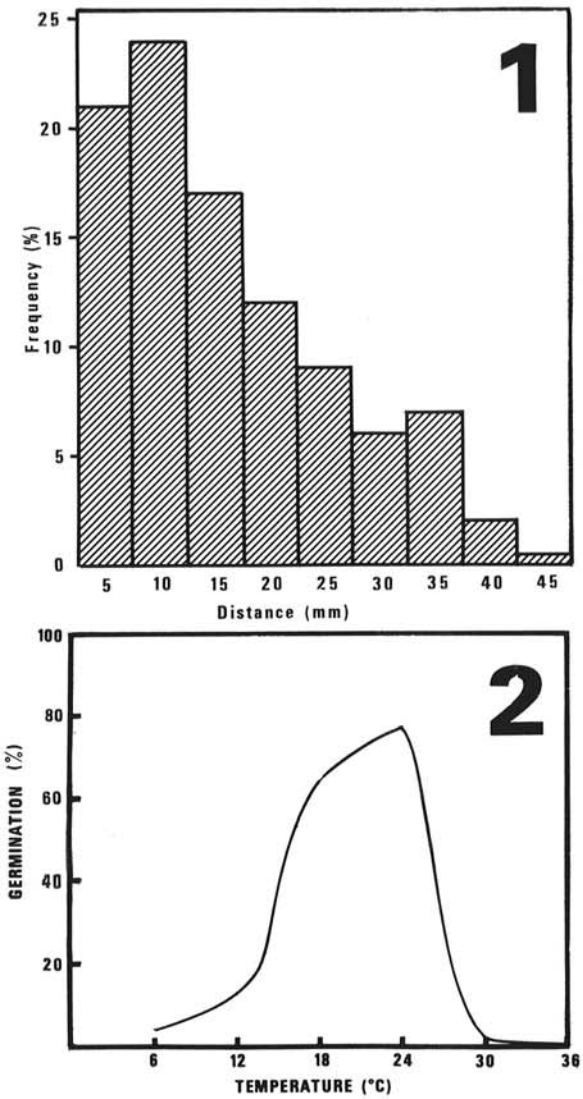

Fig. 1-2. **1)** Horizontal distance of ascospore ejection using Ingold's apparatus. **2)** Effect of temp on ascospore germination.

but ascospores were not trapped until 26 April 1965 and 22 April 1967.

Ascospores were trapped only on days with measurable rainfall, except for one instance when ejection probably occurred during a rain ending near the time when the trap slide was changed on 10 May 1965. There was a lag period varying from 0.3 to 3 hr between the onset of rainfall and the deposition of spores in the Hirst trap. Spores were trapped within 0.5 hr after rain began on 22 May 1965; peak deposition occurred within 2 hr (Table 1).

The results of these studies clearly demonstrated that ascospores were wind-disseminated. Ascospores were trapped in significant numbers (max 10,652/24 hr) in a Hirst trap located 9 ft from a large number of knots. Moreover, spores were trapped for 1-5 hr after the cessation of rain.

TABLE 1. Hourly records of *Dibotryon morbosum* ascospore deposits on a Hirst slide after the onset of rain at 8 PM on 22 May. Ambient temp at 8 PM was 24 C. Total rainfall was 13 mm with a 14 to 27-C temp range from 8 AM 22 May-8 AM 23 May

Hr after onset of rain	No. ascospores
1	825
2	2906
3	555
4	158
5	90
6	23
7	16
8	21
9	15
10	12
11	19
12	7

During nearly 3 months of continuous air-sampling in April, May, and June 1965 and 1967, ascospores were deposited on Hirst trap slides on 14 days during each year. The data for 1965 are shown in Table 2. Initial deposition periods occurred in late April, with the final deposit in late June. The max deposit during 1965 occurred on 17 May, with heavy deposits on 8 and 23 May. The max deposit for 1 day in 1967 was on 3 May, but significant deposits occurred during every rain period from 11 May to 15 June.

TABLE 2. *Dibotryon morbosum* ascospore collections during 1965 with a Hirst spore trap

Date	No. ascospores[a] trapped/ 24-hr period	Rainfall[a]	Temp 1 hr before initial spore deposit	Temp range[a] 8:00 AM-8:00 AM
		mm	*C*	
April				
7	0	4.25		4-10 C
8	0	0.50		4-7
9	0	0.75		7-13
12	0	0.25		10-13
16	0	9.75		7-13
19	0	9.25		2-13
20	0	0.25		3-8
22	0	0.25		10-21
24	0	0.25		3-16
25	0	1.25		4-12
26	5	6.50	6	4-7
27	0	0.50		6-20
May				
6	0	0.25		8-17
7	435	10.75	16	11-23
8	1,638	9.25	14	8-14
9	134	0.25	9	9-14
10	72	0.00	13	13-26
17	10,652	19.00	27	14-29
20	0	0.50		16-28
23	4,647	13.50	25	14-27
25	0	0.50		13-18
26	80	2.25	25	15-28
27	0	0.25		17-31
28	71	14.25	29	17-29
29	195	4.00	19	13-24
June				
3	20	6.75	26	13-27
9	89	6.50	23	19-30
16	25	2.75	16	11-21
17	0	0.25		11-14
18	0	3.25		9-13
19	0	1.25		9-21
24	29	11.25	31	19-33
30	0	3.00		19-33

[a] Denotes data for previous 24 hr.

Effect of temp on ascospore germination.—Although ascospores germinated, continued germ tube elongation and production of the *Cladosporium* stage rarely occurred. No ascospores germinated after 6 hr at 6, 12, 30, and 36 C, while germination was 0.2 and 4% at 18 and 24 C, respectively. Germination was 0.0, 0.7, 17.5, 33.9, 0.5, and 0.0% after 12 hr at 6, 12, 18, 24, 30, and 36 C, respectively. After 24 hr, germination was 0.2, 3.1, 36.6, 53.9, 0.9, and 0.0 at 6, 12, 18, 24, 30, and 36 C, respectively. The most favorable temp for ascospore germination was 24 C. The 48-hr results are shown in Fig. 2.

Effect of postinoculation moisture period on infection.—Severe infection occurred on Stanley trees in the greenhouse with postinoculation moisture periods ranging from 6 to 30 hr at 21 C. Of 100 trees inoculated with suspensions of ascospores from *P. domestica* 'Stanley', 98 became diseased. The min moisture period required for infection remains unknown. Noninoculated trees in these experiments remained free of disease except in one instance, where four knots developed on the controls as compared with 168 to 227 on the inoculated trees.

Effect of various propagules on infection.—Of 91 Stanley trees inoculated with conidial suspensions, one became diseased. No knots formed on 36 Stanley trees inoculated with ascospores from *P. serotina*. A mixture of ascospores from *P. serotina* and *P. domestica* 'Stanley' caused infection on 44 of 53 Stanley trees.

Time of natural infection in the field.—In several years of field tests with Stanley trees, it was clear that most natural infection occurred after petal-fall during May and June in southern Pennsylvania.

In a 1960 test, Stanley trees were at the full-bloom stage of development on 24 April. Trees which received the first fungicidal spray on 10 May or later developed 2.4 to 3.9 knots/terminal. There was no clear evidence of additional infection after 24 May.

In 1962, Stanley trees were at the petal-fall stage of growth on 1 May. Trees which received the first spray on 5 May were free of disease. About one-half of the infections apparently occurred after the last spray on 29 May. In another 1962 test with a large amount of inoculum present, trees sprayed for the first time on 5 May developed 3 knots/tree as compared with 48 knots/tree on those sprayed for the first time on 18 May.

In 1963, no infection occurred on trees which received the first spray at petal-fall. First infection occurred within 10 days after petal-fall, with severe infection between 9 and 20 May. This was 10 days to 3 weeks after petal-fall. The number of infections did not increase after 20 May.

During 1965, Stanley trees were at the full-bloom stage of development on 6 May. A few knots developed on trees which received the first fungicidal spray on 12 May. Approximately 44 knots/tree developed on those which received the first spray on 21 May. It

should be noted that the peak ascospore deposit occurred on 17 May (Table 2). The average number of knots (95/tree) on the unsprayed trees suggested that a substantial amount of infection occurred in June.

DISCUSSION.—Prior to the present study, the only successful investigation of *D. morbosum* ascospore dissemination was conducted by Koch (7). Koch's results may be interpreted as evidence of forcible discharge. Koch trapped an unstated number of ascospores on four occasions with a trap located 30 ft from the nearest plum tree. We trapped a few ascospores with Hirst and Rotorod traps in a Stanley orchard during 1963 and 1964. But large numbers of ascospores were not trapped until 1965, when several hundred knots were placed 9 to 12 ft from the Hirst trap. This is the first study where large numbers of *D. morbosum* ascospores have been trapped at some distance from the ascocarps. Ascospores from diseased Stanley trees were the primary source of inoculum in these studies. Our data and those of Gourley (4) suggest that conidia are not an important source of inoculum. The unsuccessful attempt to infect Stanley trees with ascospores from *P. serotina* may be evidence of physiological specialization within *D. morbosum*.

Koch (7) stated that over a period of 4 years the initial ascospore discharge by *D. morbosum* on *P. domestica* in the Niagara Peninsula of Canada varied from 23 March to 6 April, and the final discharge occurred on 6 or 7 June. Many of the control programs since that time have been founded on the supposition that infection can occur at any time after initiation of growth.

The results of this study indicate that most of the infection occurs in May and June after the petal-fall stage of development. While some caution is necessary, this information raises doubt about the value of fungicidal sprays prior to full-bloom for black knot disease control.

It seems evident that little or no black knot infection may be expected until a relatively warm (above 11 C) rainy period occurs when ascospores are mature. In southern Pennsylvania, this has been from about the time of petal-fall on Stanley trees until sometime in June, a period of 4-6 weeks. The dates have varied, but not the relationship to the stage of tree development.

Infections observed in the field occurred during moisture periods significantly longer than the 6 hr reported for the greenhouse study. Additional research will be required to fully elucidate the moisture requirement for infection.

LITERATURE CITED

1. ANDERSON, H. W. 1956. Diseases of fruit crops. McGraw-Hill Book Co., N. Y. 501 p.
2. BAILEY, L. H. 1892. The black knot of plum and cherry. N. Y. (Cornell) Agr. Exp. Sta. Bull. 49:347-350.
3. FARLOW, W. G. 1876. Black knot. Bull. Bussey Inst. 24:440-453.
4. GOURLEY, C. O. 1962. A comparison of growth, life cycle, and control of *Dibotryon morbosum* (Sch.) Th. & Syd. on peach and plum in Nova Scotia. Can. J. Plant Sci. 42:122-129.
5. HIRST, J. M. 1952. An automatic volumetric spore trap. Ann. Appl. Biol. 39:257-265.
6. INGOLD, C. T. 1965. Spore liberation. Clarendon Press, Oxford. 210 p.
7. KOCH, L. W. 1933. Investigations on the black knot of plums and cherries. I. Development and discharge of spores and experiments in control. Sci. Agr. 13: 576-590.
8. KOCH, L. W. 1934. Investigations on the black knot of plums and cherries. II. The occurrence and significance of certain fungi found in association with *Dibotryon morbosum* T. & S. Sci. Agr. 14:80-95.
9. KOCH, L. W. 1935. Investigations on the black knot of plums and cherries. III. Symptomatology, life history, and cultural studies of *Dibotryon morbosum* (Sch.) T. & S. Sci. Agr. 15:411-423.
10. KOCH, L. W. 1935. Investigations on the black knot of plums and cherries. IV. Studies in pathogenicity and pathological histology. Sci. Agr. 15:729-744.
11. SMITH, D. H., & F. H. LEWIS. 1965. Effects of temperature on *Dibotryon morbosum* ascospore germination. Phytopathology 55:506 (Abstr.).
12. SMITH, D. H., & F. H. LEWIS. 1966. Some factors involved in outbreaks of black knot on plums. Phytopathology 56:586 (Abstr.).
13. THEISSEN, F., & H. SYDOW. 1918. Vorentwurfe zu den Pseudosphaeriales. Ann. Mycol. 16:1-34.
14. VRIES, G. A. DE. 1952. Contribution to the knowledge of the genus *Cladosporium* Link ex Fr. Uitgeverij and Druckerij Hollandia, Baarn. 121 p.