Eutypella Canker of Maple: Ascospore Discharge and Dissemination

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ABSTRACT

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Free moisture on mature perithecia of *Eutypella parasitica* induced discharge of viable ascospores in groups of eight (spore octads). At least 0.03 cm of rain penetrating the tree crown canopy was required to initiate discharge; high relative humidity alone was not sufficient, but did prolong the spore ejection from moist perithecia. Previous moisture content of stromata determined the magnitude of spore discharge. In the laboratory, spores were discharged from moistened perithecia at 4–36 C. Discharge from perithecia under controlled laboratory conditions resembled that observed in the field. Stromata collected during any season ejected spores when bark was moistened. Storage of stromata at −24 C for 72 wk did not affect spore discharge or germination adversely. Ascospore octads were trapped with a Hirst spore trap and with Rotorod samplers 25 m downwind from maple cankers in a mixed hardwood stand in northern Wisconsin. The number of spores trapped decreased rapidly with increasing distance from the source. The general dispersal pattern depended upon wind direction, but was modified by updrafts and other types of turbulence within the stand.

Additional key words: epidemiology, Pyrenomycetes, Xylariaceae.

Eutypella parasitica Dav. and Lor. incites a serious stem canker on *Acer* spp. in the northern hardwood forest type. This canker often girdles and kills small, weak trees and persists for many years on larger, otherwise vigorous trees. Cull results from wind breakage and from canker development in the most valuable basal portion of the stem. Damage is greatest in stands with a high proportion of sugar maple, *A. saccharum* Marsh. (2,3,11).

The mode of infection by *E. parasitica* is unclear. Ascospores are considered to be the major inoculum, but little work has been done on the epidemiology of the disease since the original description of the canker by Davidson and Lorenz (2) in 1938. Clarification of the requirements for ascospore discharge and dissemination is needed to determine when infection occurs in this important disease of maples.

Lachance (12) reported that rainfall triggered spore discharge, but he found no consistent relationship between the amount of rainfall and the intensity of discharge. French (3) reported that he trapped spores only during periods of high relative humidity (RH) and at temperatures of 4.4 C or above. At least 0.10 cm of rainfall had to penetrate the tree canopy to induce discharge. Temperature was not a critical factor in determining whether or not spores were discharged, but did influence the rate of discharge.

The ejection of spores, as reported for other ascomycetes, depends upon several interrelated factors including the amount and duration of rainfall, temperature, and RH, as well as moisture content of the fungus stroma (4,14).

The dissemination of ascospores is well-known (1,4,5,14), however, no studies have been conducted for Eutypella canker within forest stands. In the present study, the discharge and dissemination of ascospores of *E. parasitica* under natural and controlled environment conditions were monitored in order to clarify aspects of the epidemiology and distribution of maple canker.

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MATERIALS AND METHODS

Field studies were conducted in second-growth northern hardwood stands on the American Legion and Northern Highland State Forests in Wisconsin. Cankered sugar maples and red maples (*A. rubrum* L.) were selected for study and for sources of inoculum.

Ascospore discharge. *Field studies.* Ascospore discharge from 10 trunk cankers was studied from 25 May to 10 September 1967. Tree diameters ranged from 10.2 to 25.7 cm at breast height. A total of 17 spore traps consisting of clean, dry microscope slides were positioned on supporting nails in front of perithecia. Hygrothermographs, rain gauges, and one battery-powered weather-recording station were positioned near cankered trees to record temperature, RH, rainfall, and wind direction. Rain gauges were checked daily between 0800 and 0900 hours CST. At that time, spore traps were collected and replaced with clean slides. Spore traps were placed over the same area of the stroma after each collection. Spore discharge was rated on a scale (Spore Discharge Index—SDI) of 0 to 3 (0 = no spores; 1 = few scattered spores in groups of eight (a spore octad); 2 = moderately heavy deposit, one layer deep; 3 = heavy deposit, two or more layers deep) with a compound microscope. This method of evaluating spore discharge was used instead of exact spore counts because of the irregular distribution of spores on the trapping slides.

Germinability of ascospores was determined by washing spores from the trapping slides onto the surface of 2% water agar. Plates were incubated at room temperature (22–24 C) for 48 hr and percent germination was determined.

A Unico-Casella Hirst spore trap (8) (Union-Industrial Equip. Co., Fall River, MA 02722), regulated with a 187.4-W (1/4 hp) vacuum pump to sample 10 L air/min, provided additional detailed information on spore discharge and dissemination from cankers. Sampling was done at canker height (2 m) and downwind 1 m from a sugar maple canker during periods of rainfall. The numbers of spores trapped on vaseline-coated slides were determined at ×100 magnification by counting all *E. parasitica* spores on the slide per unit time.

Controlled environment studies. To compare spore discharge in

the field with that under controlled laboratory conditions, a Hirst spore trap was operated with the orifice positioned at canker height 1 m away from sections of cankered maple stems in a large mist chamber. Cankers were sprayed with water at 6 hr intervals over a period of 24 hr. A hygrothermograph was used to record temperature and RH throughout the test period. This study was conducted twice.

To determine the distance spores were ejected in still air, excised bark sections bearing perithecia were attached with melted paraffin to clean microscope slides, with the perithecial necks oriented parallel to the slide surface. The slides were placed in petri plates containing moistened filter paper and incubated in the dark at 4 to 36 C. Observations were made on the relative numbers of spores discharged and the distance spores were ejected horizontally over a 3-day period on two separate occasions.

Temperature effects upon ascospore ejection were investigated by incubating dry bark sections bearing perithecia for 2 days at 4, 8, 16, 20, 24, and 28 C. Bark sections then were immersed in water for 1 hr, placed upright in petri plate moist chambers, and incubated at their same temperatures. Spore discharge was assessed at 1 hr intervals and rated on the SDI scale.

The effect of moisture content of stromata on spore discharge was investigated by moistening bark sections bearing active perithecia according to a technique similar to that described by Moller and Carter (14). Productive stromata were selected, air-dried for 2 to 3 days, then stored at room temperature until needed. Bark pieces were divided into three groups for treatment: one group was placed stromata-side up on moist sand in a covered chamber to allow uptake of moisture by the base. Another group was treated similarly but the bases were coated with paraffin to prevent uptake of moisture. A control group was held at room temperature. After treatment for 12 hr, all pieces were immersed in water for periods of 0, 15, 30, 60, and 120 min. Discharge of spores onto petri plate lids was rated on the SDI scale at 1 hr intervals following immersion in water.

Areas of productive stromata on natural cankers studied in 1967 also discharged viable spores in 1971. To determine if spores were produced from the same perithecia over this time, or whether new perithecia had developed within older stromatic tissues, bark sections bearing *E. parasitica* were excised from cankers periodically between June 1969 and October 1970. Bark was cut into 3–5 mm^2 pieces, fixed in formaldehyde-alcohol-acetic acid under vacuum overnight, dehydrated by the tertiary butyl alcohol (TBA) method (16) and embedded in Fischer Tissuemat paraffin (mp 56.5 C). Serial sections were affixed to slides with Cobe's adhesive and stained with Heidenhain's hematoxylin following the schedule outlined by Rogers and Berbee (15).

Spore production by stromata after storage at several temperatures over prolonged periods was compared by drying bark sections (10–15 mm^2) with perithecia for several days in a desiccator over anhydrous calcium chloride. Stromata then were placed in small plastic-capped glass vials with the drying agent at 24, 4, and −24 C. Samples were removed periodically and induced to discharged spores. Ascospores were plated on WA and percentage germination was determined after 24 hr of incubation at 24 C.

Ascospores also were stored in distilled water at 4 C and percentage of germination was determined after 24 hr incubation at 24 C on WA.

Ascospore dissemination. The numbers of spores, duration of discharge, and pattern of ascospore dispersal from maple cankers were studied in a mixed hardwood stand. The ejection of ascospores was initiated during dry periods, when natural spore discharge was not occurring, by spraying stem sections bearing productive stromata with water from a portable backpack pump. Canker height was approximately 1 m. Artificial wetting of cankers at night approximated conditions that would exist during actual rainfall since temperature and RH in both situations are very similar (4). The range in temperature and RH during these tests was 6–22 C and 72–100%, respectively.

Spores were trapped by positioning Rotorod spore sampling devices (5) downwind at 1.2, 4.8, 9.8, and 19.5 m from cankers and at heights of 1.2 and 2.4 m. Samplers were located along compass lines radiating SE to NW, S to N, and SW to NE from the cankers. The samplers were rotated by DC motors (Model D-367, Brevel Products Corp., Carlstadt, NJ 07072) powered by a 12 V battery. The rotation rate was controlled by installing a variable resistor (2W - 100Ω) which was manually adjusted to maintain 2,500 rpm. All samplers were adjusted with a strobe light prior to use in the field. A vibrating reed tachometer, consisting of a length of piano wire (1.2 mm diameter), was mounted on the side of the sampler housing and adjusted to vibrate at maximum amplitude at 2,500 rpm. With this apparatus the authors could check and readjust the motor speed of each sampler in the field by making a corresponding adjustment in the variable resistor.

The collection bars consisted of a pair of upright brass arms (0.2 cm wide and 6.0 cm long) rotating at a distance of 4 cm from the axis of rotation. The volume of air sampled was 120 L/min. A uniform coating of an adhesive mixture of one part rubber cement and two parts xylol was applied to the arms. Sampling bars were changed every 1 or 2 hr. Adhering particles were stripped from the leading edges by applying transparent cellulose tape to the bar surface. The tape was placed on a microscope slide and the number of spore octads trapped was determined by counting all octads in 10 microscope fields at ×100 magnification. These spores could readily be identified by their smokey-brown color and allantoid shape and because they were usually deposited in groups of eight.

RESULTS

Ascospore discharge. Free moisture (rainfall) on mature perithecia induced discharge of ascospores, usually in groups of eight, which were trapped on dry microscope slides positioned a few millimeters in front of perithecia. Apparently the entire contents of a single ascus are discharged and remain together as a unit in flight.

Spore trap data for 10 cankers for the period from 25 May to 10 September 1967 are presented in Fig. 1.

The total SDI on any selected date was dependent upon amount of rain and previous rainfall (Table 1). At least 0.03 cm rain had to penetrate the tree canopy to initiate discharge. Discharges during periods of low rainfall were light. After long, dry periods of little or no rainfall, a considerable amount of bark-wetting (by rain) was necessary to initiate discharge. If rain lasted 2–3 days or RH remained high, spores often were trapped up to 2 consecutive days following the rain (Fig. 1).

High humidity alone was not sufficient to induce discharge. For example, no spores were trapped on 29 August 1967 when 20 hr of RH above 90% were recorded (Fig. 1). High RH, however, did influence the rate of drying of bark on cankers and prolonged discharge after periods of measurable rainfall.

TABLE 1. Rainfall and *Eutypella parasitica* ascospore collections on selected dates during the summer of 1967

Date	Time since last rain (days)	Amount of rain (cm)		Duration of relative humidity over 90% (hr)	Total SDI[a]
		Under canopy (cm)	In open (cm)		
30 June	0	0.03	0.08	8	0
29 June	1	0.05	0.10	24	4
12 July	4	0.03	0.18	12	0
30 July	4	0.03	0.08	15	2
14 June	0	0.48	0.69	16	44
24 June	1	0.46	0.64	22	37
19 June	3	0.43	0.74	20	31
7 July	4	0.46	0.64	8	36
26 August	0	2.84	2.69	24	19
8 August	1	3.58	3.48	19	49
25 July	2	2.79	2.77	12	46
21 July	4	2.16	2.41	21	47

[a]Total Spore Discharge Index for 17 spore traps on 10 cankers over a 24 hr trapping period.

Ascospore octads also were trapped with a Hirst spore trap. Data for the period from 31 August to 1 September 1968 are presented in Fig. 2. There had been no rainfall in the previous 6-day period. Spores appeared on the slide at the end of the first hr (after 0.41 cm rainfall). The main peak in discharge occurred about 9 hr after the onset of rainfall. The numbers of spores trapped decreased steadily during the following 7 hr period. No spores were trapped in the second 24 hr period of sampling.

A similar test was run from 31 August to 1 September 1969 on the same canker at the same sampling position. It had rained 0.66 cm the previous day. The pattern of discharge was somewhat different from that observed in the previous experiment (Fig. 3.). Spores appeared on the trapping slide at the beginning of the second hour (after 0.33 cm of rain). The main peak in discharge occurred only 3.0–3.5 hr after the onset of rain.

The pattern of ascospore discharge from cankered sections of maple in laboratory tests was similar to that observed from cankers in the field. Cankers that had been sprayed with water at 6 hr intervals in a closed chamber discharged spores within the first hr (Fig. 4). The RH in the chamber increased immediately after each spraying and then decreased rapidly. Discharge remained at a low level during the first 6 hr period. After spraying the canker the second time, discharge increased to a peak within the next 6 hr period.

When the chamber mist system was operated continuously (free moisture in the air), no spores were trapped.

Ascospores were discharged from moistened perithecia in petri plates at 4–36 C. The horizontal distance that spores were ejected from 3–9 mm. The average distance discharged at room temperature (20–22 C) was 7 mm. Ascospores were discharged very slowly from moist perithecia at 4 C; a few spores were detected after 3 hr. Spores were discharged at temperatures above 4 C within the first hr. The greatest numbers of spores were ejected at 24 and 28 C.

Ascospores were discharged within the first hr from bark sections bearing stroma that had been placed in moist sand 12 hr prior to immersion in water. Discharge continued over a 24 hr period. Discharge from bark sections basally-coated with paraffin and from air-dry samples occurred only when stromata were immersed for at least 30 min. Paraffin-treated bark did not discharge spores until 3 hr later. Discharge continued over the next 5 hr, then ceased. Similarly, samples that received no treatment (air-dry bark) prior to immersion discharged spores in 3–4 hr.

Viable ascospores were discharged intermittently over a period of 8 wk from perithecia which were placed in petri plate moisture chambers and remoistened each day after assessing discharge. Germination varied from 58–90+%. The numbers of spores discharged during this period decreased with time and ceased at the end of 8 wk.

Histological examination of bark sections revealed that asci within a single perithecium or perithecia within the same or adjacent stromal tissues, were at all stages of maturity. It was not determined if spores were produced within the same perithecium

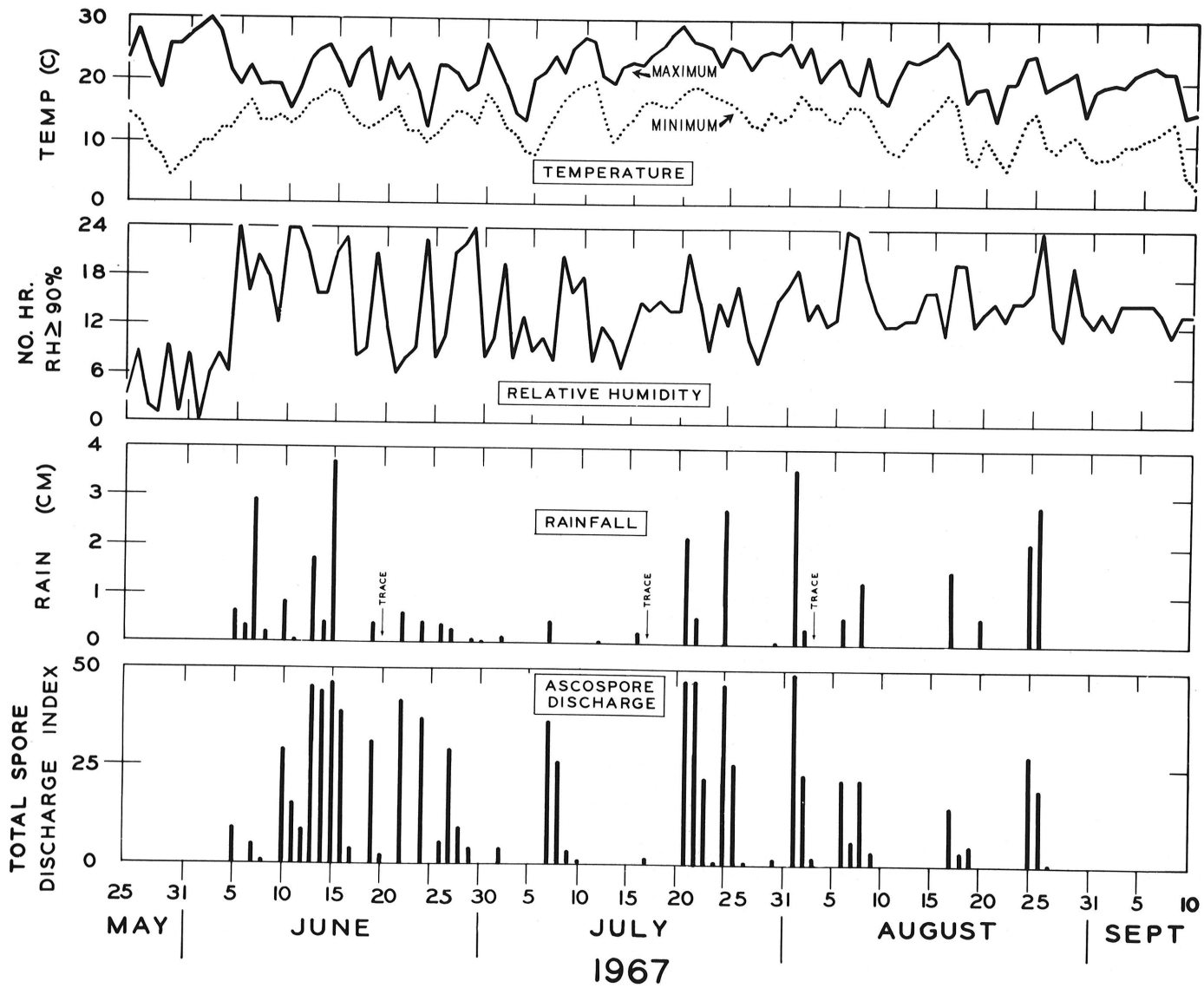

Fig. 1. Daily ascospore discharge from 10 Eutypella cankers in relation to meterological data for summer, 1967.

for more than one season, because perithecia were densely clustered in these tissues and discharge from a single perithecium was not observed.

Ascospore discharge occurred from stromata moistened after dry storage for long periods at different temperatures. The germinability of discharged spores did not change appreciably with storage time at different temperatures, except at 24 C (Table 2). In all cases, spores were discharged within 1–2 days from moistened stromata. Germinability of spores discharged from stromata stored at 24 C was affected after 24 wk of incubation. The drying agent in the vials was moist, probably as a result of a temperature or moisture change in the incubator between 24 and 32 wk. No spores were ejected from these stromata after 40 wk.

Germinability of ascospores stored in distilled water up to 14 wk at 4 C also was not adversely affected (85–90%).

Ascospore dissemination. In one test performed from 29 to 30 August 1969, two cankers were sprayed once for 15 min with water. The data on spore collection for a single Rotorod sampler located 1.2 m downwind at a height of 1.2 m are presented in Table 3. Spores were trapped in the first hr of sampling. Spore discharge was greatest about 2 hr after spraying and then declined. No spores were trapped after 9.5 hr. The range in temperature and RH during the test was 21.7 to 29.4 C and 48 to 100%, respectively.

In other tests conducted in the same area, data were similar. The elapsed time to the main peak in discharge, however, varied from 1 to 6 hr after the initial wetting of the cankers.

TABLE 2. Germinability[a] of *Eutypella parasitica* ascospores discharged from moistened perithecial stromata stored over anhydrous calcium chloride at different temperatures

Time of storage (wk)	Germination after storage at: 24 C (%)	4 C (%)	−24 C (%)
0	100	98.5	95.6
4	97.8	96.1	98.5
8	99.7	99.0	91.9
16	100	99.4	95.3
24	99.4	96.9	94.3
32	75.0	95.6	90.4
40	72.9	98.4	99.0
52	...[b]	99.3	99.0
64	...[b]	100	97.5
72	...[b]	99.6	98.1
88	...[b]	99.2	...[c]
105	...[b]	99.4	...[c]

[a] Determined by percentage of germination after 24 hr at 24 C on 2.5% water agar medium.
[b] No spore discharge occurred. It was noted that the drying agent was moist.
[c] No determination of spore discharge or germinability was made.

TABLE 3. Numbers of *Eutypella parasitica* ascospore octads collected by a Rotorod spore sampler positioned at canker height 1.2 m downwind at a height of 1.2 m[a]

Sampling time	Ascospore octads
2245–2345 hours	2,464
1200–0100 hours	8,064
0115–0215 hours	3,892
0230–0330 hours	364
0345–0445 hours	112
0500–0600 hours	84
0615–0715 hours	0

[a] Cankers were sprayed once with water for 15 min pior to operation of the sampler at 2245 hours. Data are for the period 29 to 30 August 1969.

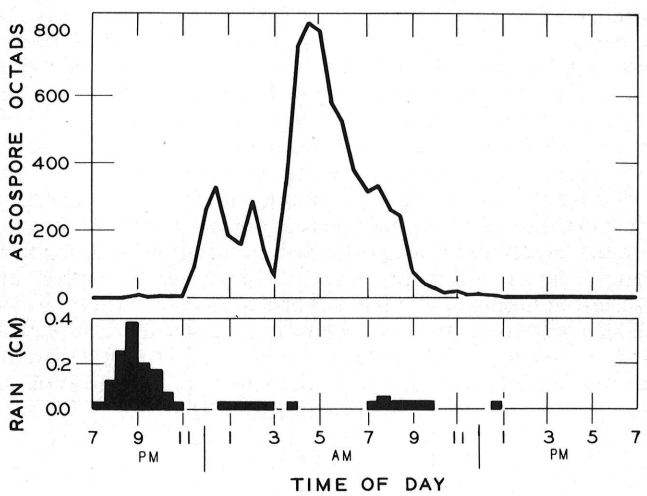

Fig. 2. Numbers of *Eutypella parasitica* ascospore octads collected with a Hirst spore trap positioned at canker height 1 m downwind from a sugar maple canker during rainfall 31 Aug to 1 Sept 1968. There had been no rainfall in the previous 6-day period.

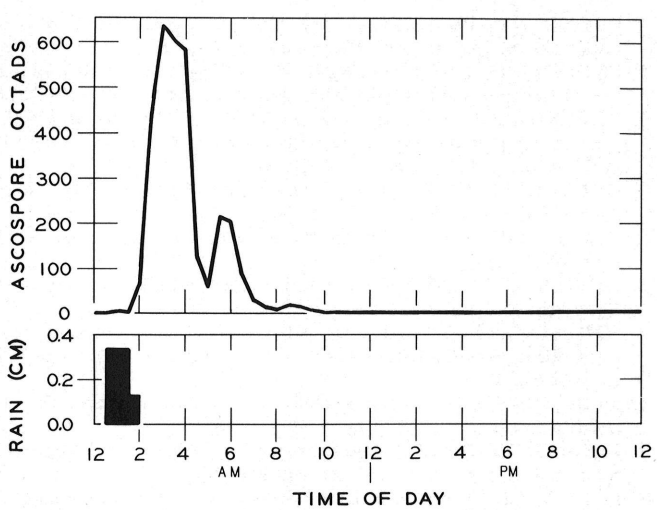

Fig. 3. Numbers of *Eutypella parasitica* ascospore octads collected with a Hirst spore trap positioned at canker height 1 m downwind from a sugar maple canker during rainfall on 31 Aug to 1 Sept 1969. There had been 0.66 cm of rainfall in the previous 24 hr period.

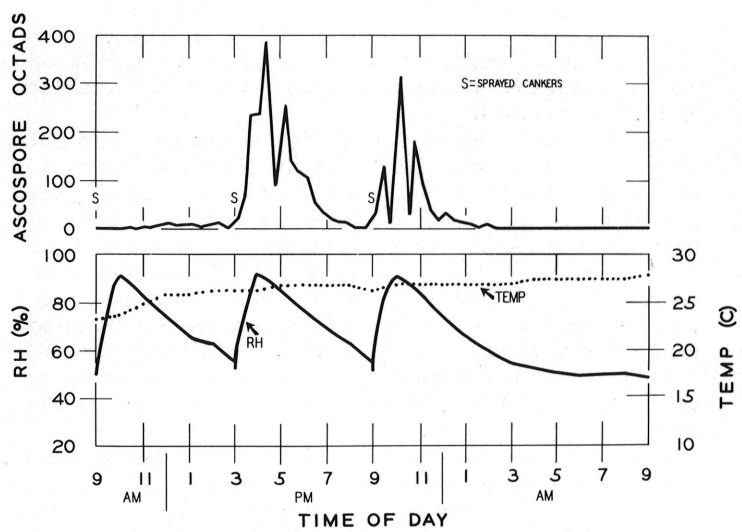

Fig. 4. Numbers of *Eutypella parasitica* ascospore octads collected with a Hirst spore trap positioned at canker height 1 m from a sugar maple canker in a closed chamber in the laboratory. The canker was sprayed with water at 6 hr intervals beginning at 0900 hours.

The numbers of spores trapped at a height of 1.2 m downwind from cankers decreased rapidly with increasing distance. Spores trapped at distances of 4.8, 9.8, and 19.5 m downwind from cankers were 17, 8, and 3% of the total number trapped at 1.2 m. In other studies total spores trapped at 19.5 m ranged from 0.4 to 3.6% of the total trapped at 1.2 m.

Spores also were trapped at the 1.2-m level 25 m downwind from the canker. The numbers of spores collected at this distance during a 2 hr period were very small, thus no sampling was attempted at greater distances.

The numbers of spores trapped at heights of 1.2 and 2.4 m varied with increasing distance from the source. Greater numbers, however, were trapped consistently at 1.2 m (2 to 70 times as many spores) than at 2.4 m. Differences in numbers of spores collected at the two levels decreased with increasing distance.

In one experiment, samplers were placed on a tree trunk at intervals of 1.8 m and 13.4 m downwind from cankers. The numbers of spores trapped during a 1 hr sampling period at heights of 1.8, 3.7, 5.5, 7.3, and 9.2 m were 308, 224, 84, 168, and 28, respectively.

Variation in wind direction in the study area at a height of 4.6 m occurred more frequently during the day than at night. During most spore collections, wind was primarily from the southwest and west; consequently greater numbers of spores were trapped downwind along compass lines radiating in directions northeast and east of the cankers.

DISCUSSION

The great majority of Pyrenomycetes are drought-enduring xerophytes which become inactive during dry periods, but rapidly produce and discharge spores upon wetting (9). *Eutypella parasitica* fits this description well. There was no evidence for diurnal periodicity of ascospore release as reported for other Pyrenomycetes (17). Sufficient rainfall at any time of day or night initiated spore discharge. Spores were detected within the first hour of rainfall, but magnitude of discharge was dependent upon the previous moisture content of the stromata. In the laboratory spore discharge was greater after cankers were moistened the second time. Longer and more abundant discharge occurred outdoors after heavy or continuing rains and in the laboratory after stromata were allowed to imbibe water. Similar results were reported by Moller and Carter (14) for *Eutypa armeniacae*.

In field studies with Eutypella canker, at least 0.03 cm of rain had to penetrate the tree canopy to initiate discharge. Few spores were discharged during light rains, whereas discharge during periods of heavy or continuous rainfall resulted in layers of spores on the slides. Ascospore release also was modified by temperature; fewer spores were ejected at 4 C than at 24 or 28 C. Consequently, spore discharge and dissemination could take place any time in the presence of suitable moisture as reported by Wood and French for Hypoxylon canker (18).

Secondary peaks among major peaks in numbers of discharged spores commonly were observed in both field and laboratory experiments. The buildup of spores in layers on trapping slides positioned a few millimeters in front of perithecia also appeared to be the result of a series of peaks in discharge. Froyd (4) observed a similar pattern in ejection of ascospores from Hypoxylon cankers and concluded that ascospores were formed and matured only when perithecia were moist. The rate of spore formation decreased with time. The buildup of pressure within the perithecium due to crowding of asci and release at intervals as spores are ejected may be a better explanation for the secondary peaks noted in our experiments.

Stromata collected during any season ejected spores when bark was moistened and placed in moisture chambers.

Collection and storage of stromata for long periods in a dry condition did not adversely affect germinability of spores. This is in good agreement with a previous report by the authors (10) that cankered sugar maple trees that had been felled, deep-girdled, or chemically-girdled with sodium arsenite continued to discharge viable ascospores over a 2 yr period.

Spores were trapped from the same areas on cankers for three consecutive years. Shiny-black, newly-formed perithecia were observed among the older, dull-black structures of previous seasons. In addition, perithecia in all stages of maturity were noted within the same or adjacent stroma. Similarly, Moller and Carter (14) reported that new crops of perithecia developed between exhausted perithecia of previous years. This explains the productivity of stromata on specified areas of cankers for more than one season.

Ascospores were trapped primarily in octads, indicating that all spores from each ascus are released simultaneously. This confirms a previous report by Lachance (12).

Spores were ejected up to 9 mm horizontally or vertically. This active discharge mechanism can provide an efficient means of launching ascospores across the laminar boundary of air overlaying the substrate and ensures that spores are dispersed from their source (7). The mechanism of discharge from the ascus may be similar to that described for *Hypoxylon fragiforme* by Greenhalgh and Evans (6). They described an annulus-like structure that acted as an "elastic sphincter" in spore ejection. Lachance and Kuntz (13) reported an ascal crown in *E. parasitica* which may be a similar structure.

Spore dispersal patterns may explain why most cankers occur within the first 3.7 m above ground (11). In this study, spores also were trapped 13.4 m downwind at a height of 9.2 m. This indicates that updrafts may disrupt normal patterns of spore dissemination. Most of the spores carried aloft by updrafts may be screened out by tree crowns. Cankers located in or just below the base of the crown may release spores which are disseminated for longer distances.

Wind direction patterns changed constantly during the day, indicative of updrafts and other turbulence. In contrast, night wind direction was fairly constant, except during storms. Convection currents necessary for long distance dissemination occur predominantly during warm, dry days (7). *Eutypella* ascospores are discharged during rainy periods when convection currents would be less likely to occur, thus it would appear that spore dissemination is limited to short distances. Felling cankered trees would further limit long distance spore dissemination if cankered sections could not be removed from the stand (10,11).

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