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Title: ASCOSPORE DISCHARGE AND GERMINATION IN XANTHORIA POLYCARPA

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Xanthoria polycarpa is a foliose lichen, commonly found as an epiphyte in the Willamette Valley of western Oregon. This study was undertaken to determine some of the effects of environmental factors on ascospore discharge and germination in X. polycarpa.

Ascospores were collected on glass slides suspended above lichen thalli. Abundant discharge occurred in the fall, winter, and spring. Discharge in the summer was rare, and occurred only when thalli were wet. Discharge was correlated with rainfall.

No striking time pattern of discharge was exhibited by apothecia incubated on moist filter paper. Horizontally discharged ascospores travelled from 1 to 24 mm away from the apothecia. Spores commonly occurred in groups of eight, indicating that all of the spores in one ascus can function as one projectile. Ascospore discharge occurred when apothecia were incubated in the light or in the dark. Discharge occurred at 5, 10, 15, and 20 C. No discharge occurred at 25 or 30 C.

Saturated salt solutions were used to produce atmospheres of various relative humidities. Discharge occurred at 100% RH and when apothecia were placed on moist filter paper. No discharge occurred at relative humidities less than 100%.

Ascospores inoculated on water agar germinated within 3 to 4 days when incubated at 15 C. The highest percentage of ascospores germinated at 10, 15, and 20 C. Germination values at 5 and 25 C were lower. No germination occurred at 30 C. Little or no germination occurred when spores were subjected to alternating 15 and 30 C. Ascospore germination occurred equally well in the light as in the dark.

A citrate-phosphate buffer was used to adjust the pH of water agar. The highest percentage of spores germinated at pH 5 and pH 6. Germination also occurred at pH 4, pH 7, and pH 8. Germination at pH 3 was poor.

Ascospores dried on coverslips and incubated at various relative humidities did not germinate. Germination did occur when spores were incubated in water or on an agar medium. The percentage of spore germination was higher on water agar than in water.

No difference in germination was found between spores inoculated on washed and unwashed Noble agar. The percentages of spores germinating on water agar and lichen extract were higher than the percentages of spores germinating on bark extract agar and malt-yeast extract agar.

Ascospore Discharge and Germination in *Xanthoria polycarpa*

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Typed by Mary Jo Stratton for Andrea Ostrofsky

THIS THESIS IS DEDICATED TO
MY PARENTS

Henry J. Constantine
Sadie Constantine

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ASCOSPORE DISCHARGE AND GERMINATION IN *XANTHORIA POLYCARPA*

I. INTRODUCTION

Xanthoria polycarpa (Ehrh.) Oliv. is a bright yellow lichen commonly found on trees and shrubs in the Willamette Valley. The thallus is foliose and grows up to 3 cm in diameter. It is characterized by the lack of soredia and by the presence of numerous apothecia (Thompson, 1949; Hale, 1961). The ascospores are hyaline, ellipsoid, and polarilocular. Parietin, an anthraquinone pigment, is responsible for the purplish-red color produced when the thallus is spotted with KOH.

Although *X. polycarpa* is a common epiphyte, little is known about its basic biology. The production, discharge, and germination of ascospores is undoubtedly an important phase in the life cycle of lichens, especially those lacking asexual diaspores such as *X. polycarpa*. Since lichens are difficult to grow in culture, any spore discharge or germination work must be done with material collected from nature. This is one of the reasons that the progress of discharge and germination studies in the lichens has lagged far behind that of such studies on other fungi.

Since *X. polycarpa* is a common lichen, and numerous apothecia are present in all seasons of the year, it is a good choice for lichen

ascospore studies. This study was undertaken to determine some of the environmental conditions that could influence discharge and germination in X. polycarpa in the Willamette Valley in western Oregon.

Both field and laboratory studies were conducted. The effect of such factors as temperature, light, pH, and relatively humidity were studied. The time and distance of discharge, the time of germination, and the effect of substrate on germination were also examined. These studies on the factors affecting ascospore discharge and germination should facilitate further studies on the actual fate of the ascospores in nature and the role they play in the life cycle of Xanthoria polycarpa.

II. LITERATURE REVIEW

Many workers have described spore discharge and germination in nonlichenized fungi. The literature is extensive, and several books on these two important processes are available (Madelin, 1966; Sussman and Halvorson, 1966; Ingold, 1971; Weber and Hesse, 1975). In contrast, the amount of information available on spore discharge and germination in lichenized fungi is quite sparse.

Many of the early observations on lichen ascospores were made in the course of investigations on the nature of the lichen symbiosis. Tulasne (1852) published the first report on the germination of lichen ascospores. Spores were collected on glass slides, transferred to moist sand and stone fragments, and observed for germination. Lichen ascospores were observed germinating on glass slides by de Bary (1866), on cultures of Nostoc by Rees (1871), and in a nutrient solution by Moeller (1887). Bonnier (1889) reported the germination of ascospores of Xanthoria parietina on bark fragments that had been inoculated with Protococcus.

Little subsequent work on lichen spores was reported until a series of papers by Werner was published in the 1920's. Kilian and Werner (1924) and Werner (1925) obtained pure cultures of the mycobionts of Cladonia squamosa and Xanthoria parietina from germinated ascospores. Pure cultures were obtained by affixing a moistened

ascocarp to the lid of a Petri dish and allowing spores to be discharged onto the surface of the culture medium in the dish (Werner, 1927). This method has been used by many workers in subsequent investigations. Werner (1930) suggested that in eastern France, the ascospores of Xanthoria parietina were mature only in the springtime.

Hilitzer (1926) reported observations on the discharge and the germination of spores of Solorina saccata.

Thomas (1939) studied some of the effects of temperature and of moisture on the discharge and the germination of lichen ascospores. He reported that ascospore discharge was not hindered by exposure of apothecia to excess moisture. Spore discharge in Xanthoria parietina was found to occur equally well between 3 and 27 C, but discharge at 30 C was very weak. The optimum temperature for spore germination was found to be around 24 C. Spore germination at 27 C was very rapid, but few spores germinated.

The investigations of Xanthoria parietina by Tobler (1944) indicated that active substances such as vitamin B could be conducive to lichen spore germination. He suggested that contaminating microorganisms might also supply substances necessary for effective germination.

Am Ende (1950) studied the effect of carbon source on both spore germination and fungal growth in Xanthoria parietina. Pectin, erytrit, and glycerin exerted a positive influence on germination.

Glycol exerted no influence on germination, although it did inhibit subsequent growth of the mycobiont.

Observations on spore discharge and germination in various groups of lichens have been reported. Werner (1956) published observations on the morphology of spore germination in species of Verrucaria and Pertusaria. Kofler (1957) reported observations on ascospore discharge and germination in members of the Umbilicareaceae. Her observations indicated that spore discharge was influenced more by weather conditions than by a seasonal cycle. Henriksson (1958) reported that moistened apothecia of Collema tenax discharged spores as the apothecia began to dry. She observed 90% spore germination within four days on a medium made with a solution in which algae had been grown.

Kofler and Bouzon (1960) published an investigation on factors influencing the discharge and germination of various lichen ascospores. Spores of Xanthoria parietina were found to germinate more quickly on media with glucose than on media without glucose. Also, germination was more rapid on media solidified with Bacto-Agar than on media solidified with Purified Agar. Light conditions had no apparent affect on spore germination. Meteorological conditions such as temperature and moisture conditions were considered to play a significant role in the subsequent discharge of ascospores.

Ahmadjian (1961) reported the discharge and germination of spores of various lichenized fungi. He stated that discharge commenced when moistened apothecia began to dry. Of the 26 species observed, spores of 16 species germinated on soil extract-nutrient agar. Cultural characteristics of the mycobionts were described.

Scott (1959, 1964) obtained successful germination of Peltigera ascospores only in an extract of a pure culture of Nostoc. Spore rupture occurred when spores were inoculated on agar media that had not been washed with pyridine. Scott suggested that the mycobiont of Peltigera may require the presence of living Nostoc in order to survive in nature.

Tomasselli (1956) observed the germination of ascospores of Xanthoria parietina, and subsequently cultured the mycobiont. Nicoli et al. (1964) observed the germination of spores of several lichens, including Xanthoria parietina, on Sabouraud's medium. Werner (1968) reviewed much of the literature on mycobiont culture.

Recent studies explored the affects of air pollutants on lichen ascospores. Dust particles from industrial plants were found to inhibit spore germination of Physcia pulverulenta. Spores of Lecanora hageri and Xanthoria parietina were found to be more resistant to the dust (Kofler et al., 1968). Sporulation of Xanthoria parietina was inhibited by placing thalli in sealed jars containing an atmosphere of 10 vpm sulfur dioxide for 10 days (Pyatt, 1969a).

Bailey (1966, 1968) reported that moistened apothecia of various lichens discharged spores when placed in an atmosphere of 100% RH under constant illumination. Garrett (1968) reported similar observations of ascospore discharge by lichens incubated in the dark. After seven days, water films containing ascospores were observed, and germination was noted. Spores of Xanthoria parietina were not found to germinate in the water film (Bailey, 1966). Bailey and Garrett (1968) described the results of further investigations on lichen spore discharge. Apothecia were placed on moist filter paper to induce discharge. Spore discharge in Lecanora conizaeoides was found to occur both in the light and in the dark, and occurred more readily at 6 C than at 20 C. Spores of Xanthoria parietina were discharged horizontally up to 12 mm from the apothecia. In the 17 species observed, discharge usually began two to four hours after being placed in a moist atmosphere, and continued for four to seven days.

Pyatt (1968a, b) described the germination of ascospores of Pertusaria and Ochrolechia. He also noted that 4% of the discharged ascospores of Pertusaria pertusa were accompanied by algal cells (Pyatt, 1973b).

Pyatt (1968c) investigated conditions influencing lichen ascospore discharge and germination. Xanthoria parietina was found to discharge spores in continuous light, continuous dark, and 12 hours light-12 hours dark. When apothecia were placed on various media,

discharge occurred for longer periods of time on water agar than on nutrient agar. The optimum pH for discharge of X. parietina spores was found to be 5, but this varied with the place of collection of thalli. Glass slides inoculated with spores were suspended 3 mm above buffer solutions to determine the pH range of germination. Ascospore germination occurred over the range of pH 3 through 9, but was optimal when spores were placed over a buffer of pH 3.

Pyatt (1969b) conducted a monthly investigation of spore discharge and germination in various lichens. Thalli were collected, and portions bearing ascocarps were placed in water in Petri dishes. Spores caught on glass slides were counted after three days, and observed for germination in the water film after seven days. Spores of Xanthoria parietina were discharged every month, but germination in the water film was only observed in October.

Roussard (1969) considered the relationship between the time of septation and the germination of ascospores of various lichenized fungi.

In many studies of lichen spore germination, agar plates have been placed above moistened apothecia. Discharged ascospores landed on the agar surface which served as the germination medium. This method did not allow for a random distribution of spores from more than one apothecium or more than one thallus. Kofler (1970) described a method of spore collection and storage which allows mixing

of spores from various sources. Ascospores from more than one apothecium were discharged onto Parafilm, collected and mixed in a water droplet, dried, and stored. Spores of Xanthoria parietina and Physcia pulverulenta remained viable for at least one month when collected and stored in this way. Various spore densities of X. parietina were inoculated on an agar surface and observed for germination. Density of spores had no apparent effect upon germination. Spores of X. parietina sank to the bottom and failed to germinate when placed in a 1 cm high layer of liquid.

Garrett (1971) investigated the effects of relative humidity and of water films on the spore discharge of various lichens. When apothecia of Xanthoria parietina were held in place with petroleum jelly, discharge occurred at 100% RH but not at 90% RH. When apothecia were placed on moist filter paper, discharge occurred at both 100% and 90% RH. Discharge of spores also occurred when apothecia were under the influence of "dew," a thin film of moisture.

Pyatt (1973a) reviewed some of the work on discharge and germination of lichen ascospores, including some of his own previously unpublished data. Ascospore discharge was found to begin one to two hours after moistening of apothecia. Discharge of spores by some lichens, including Xanthoria parietina, was found to peak once after being moistened, and then again, after being placed in a dry atmosphere. X. parietina ascospores were discharged horizontally up to

23 mm from the apothecia, and were usually found deposited in groups of eight. Observations on the germination of X. parietina ascospores were also made. The percent germination was found to be slightly higher on water agar than on lichen extract agars made from Parmelia and Ochrolechia thalli. The germination of ascospores of other lichens on bark extract agar was also noted.

II. MATERIALS AND METHODS

Source and Handling of Thalli

Lichen thalli used in field observations of spore discharge were epiphytic on five Oregon ash (Fraxinus latifolia Berth.). Trees were located on the Oregon State University Hyslop Farm, two miles north of Corvallis. Originally, one thallus with noticeable apothecia was chosen on each tree. As studies proceeded, one more thallus per tree was chosen for additional observations. All thalli observed were located on branches one to two meters above the ground.

Lichen material used in laboratory tests was collected from bigleaf maple (Acer macrophyllum Pursh), Oregon ash (Fraxinus latifolia), apple (Malus sp.), elm (Ulmus americana L.), walnut (Juglans sp.), and azalea (Rhododendron sp.) in the Willamette Valley. Twigs and bark covered with lichen thalli were collected during dry periods of the day and stored in paper bags at room temperature until use. No thallus stored more than one week was used in discharge or germination studies.

Discharge Studies

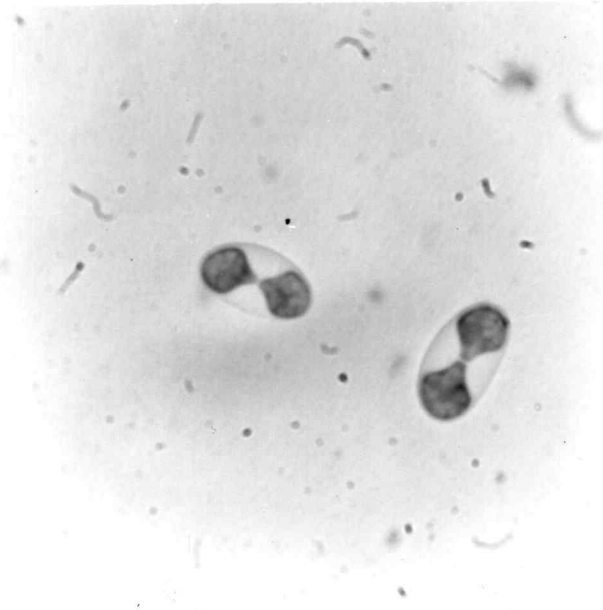
In all cases, spore discharge was assessed by microscopic examination of a substrate upon which ascospores had impinged.

Slides or coverslips which had been placed 5 to 10 mm above apothecia were observed at a magnification of 125X. Ascospores were usually stained with 0.5% trypan blue in lactophenol. This technique resulted in stained protoplasts and revealed the polarilocular nature of the ascospores (Figure 1). Polarilocular spores could then be clearly differentiated from other airborne spores.

The occurrence of spore discharge in the field was studied for one week in each season. Number 27 alligator clips wired to branchlets were used to support glass slides over lichen thalli. In fall, winter, and spring, a 5 mm overhang of cellophane tape skirted the glass slides to prevent rain drops from running over the lower surface of the slide and removing discharged spores. Daily weather data, including temperature, relative humidity, and precipitation, were obtained from a weather station located on the Hyslop Farm.

Discharge studies in the laboratory were conducted with apothecia that had been excised from lichen thalli. Apothecia were placed in a Petri dish on glass fiber filter paper moistened with 5 ml of sterile, distilled water. Lucite rings or bent glass rods were used to suspend coverslips above the apothecia. The plates were then incubated for 48 hr in the dark at 15 C, at which time spore discharge was assessed. This basic procedure was varied depending on the environmental factor being studied.

Figure 1. Ascospores of Xanthoria polycarpa stained with 0.5% trypan blue. 1000X.



The time of spore discharge in the laboratory was investigated by counting discharged ascospores at 12 or 14 hr intervals for 14 days. The coverslips were removed for counting and replaced by clean ones. The filter paper began to dry noticeably after one week. A total of 12 apothecia were observed in each run.

The horizontal distance of ascospore discharge was observed by placing apothecia on wet filter paper draped over upright supports (Figure 2). This allowed horizontal discharge to occur. Spores landed on 7.6 cm sections of clear plastic rulers marked at 1 mm intervals. Each apothecium was centered 4 to 5 mm above the surface of the ruler. The ruler itself was observed microscopically. The distance traveled by discharged spores and the apparent number of spores per projectile were recorded. Twelve apothecia were observed during each of two runs.

The temperature of spore discharge was investigated by incubating apothecia at 5, 10, 15, 20, 25, and 30 C. The spores discharged from 15 apothecia were counted at each temperature.

The effect of light was studied by exposing 12 apothecia to two 4-watt fluorescent bulbs at a distance of six inches throughout the 48 hr of incubation. The same number of apothecia were incubated in a dark box. The number of ascospores discharged was then counted.

In order to investigate the effects of relative humidity on discharge, apothecia were placed in screw-capped glass jars rather than

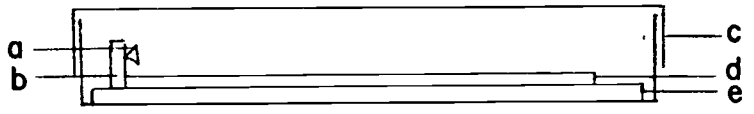


Figure 2. Apparatus for measuring the distance of spore discharge. (a) apothecium, (b) upright covered with filter paper, (c) Petri dish, (d) clear plastic ruler marked at 1 mm intervals, (e) wet glass fiber filter paper.

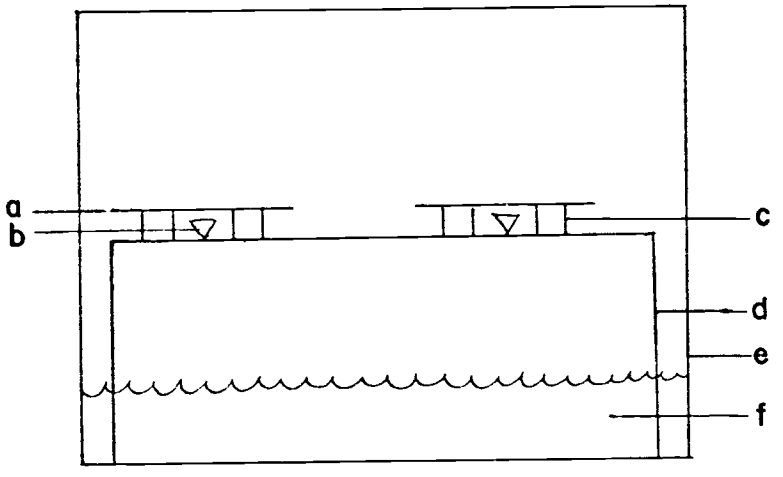


Figure 3. Apparatus for maintaining apothecia at various relative humidities. (a) glass coverslip, (b) apothecium, (c) Lucite ring, (d) wire mesh platform, (e) screw-capped glass jar, (f) saturated salt solution.

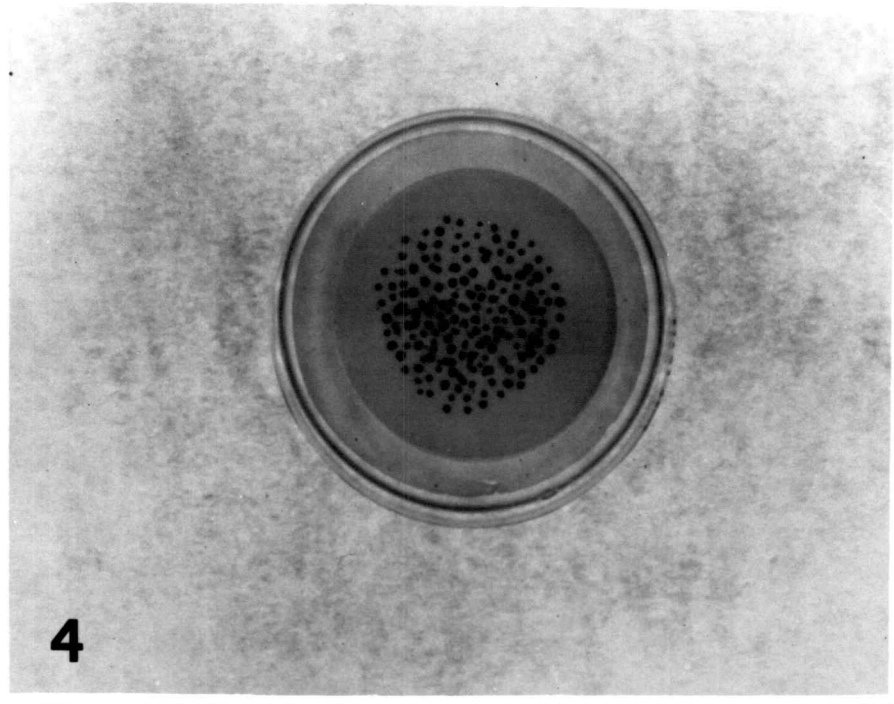
Petri dishes (Figure 3). Saturated salt solutions and water were used to produce specific relative humidities within the jars at 15 C (Winston and Bates, 1960). Fifty ml of one of the following solutions was placed in each jar: H_2O - 100% RH, KH_2PO_4 - 99% RH, KCl - 86.5% RH, $NaNO_3$ - 76.5% RH, and "dry" - no liquid. Three apothecia per jar were placed on wire mesh platforms that raised them above the level of the liquid. The wire mesh platforms also supported the Lucite rings upon which coverslips were placed. Six apothecia were observed at each relative humidity during each of two runs.

Germination Studies

Two techniques of spore collection were used in germination experiments. Both methods were adapted from the technique described by Kofler (1970). Excised apothecia were placed in Petri dishes on filter paper moistened with 5 ml of sterile, distilled water. In early experiments, Parafilm was fitted into small Petri dish lids and placed over the apothecia. Spores were discharged onto the hydrophobic Parafilm, and subsequently collected for inoculation. In most of the experiments conducted, shallow (10 mm) Petri dishes were used (Figure 4). The plates were treated with Siliclad, a silica compound, which makes glassware hydrophobic. The hydrophobic inner lid of the treated Petri dish was the surface upon which spores were discharged

Figure 4. Shallow Petri dish set up for collection of spores from apothecia.

Figure 5. Ascospores discharged onto surface of Petri dish lid.



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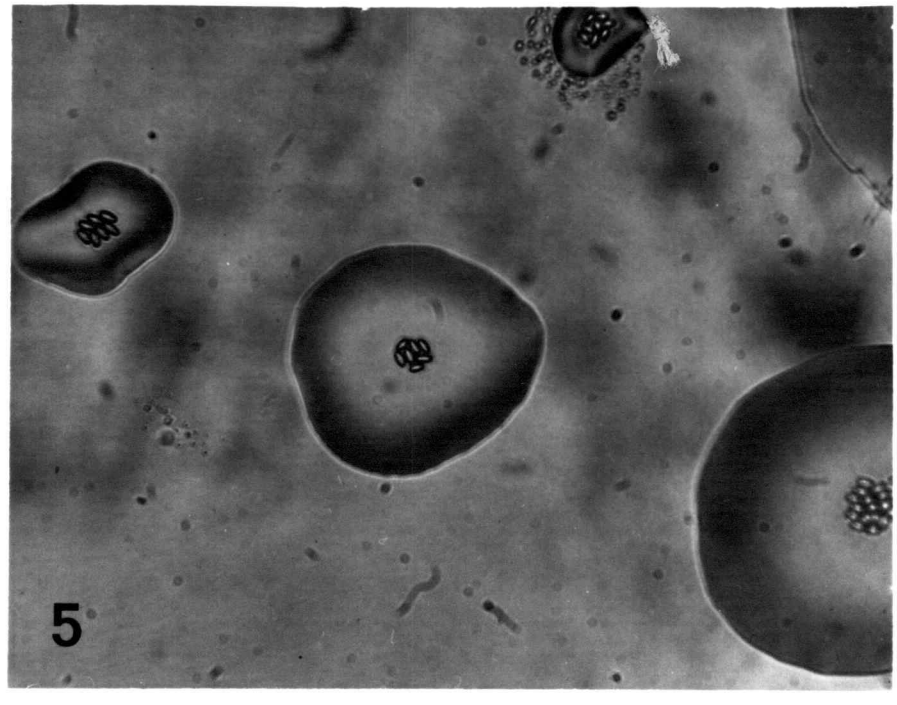

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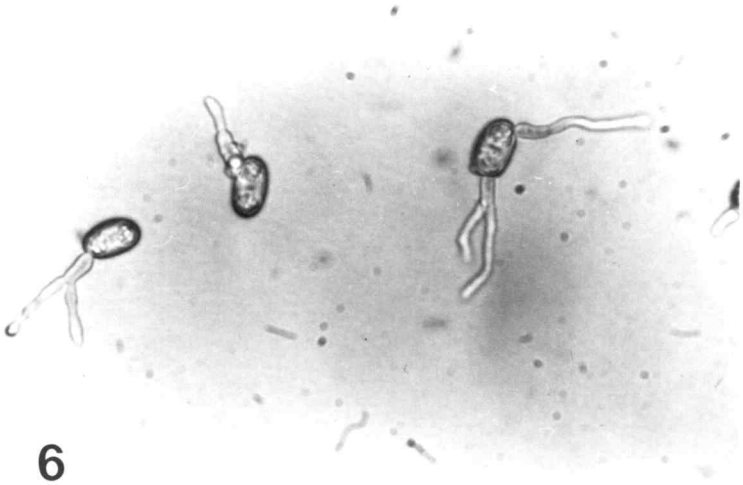
and from which spores were collected for inoculation (Figure 5). The second method of spore collection was preferred, since the plates could easily be sterilized by autoclaving. Ascospores from more than one thallus were used in order to minimize variation in response of spores from different apothecia.

Apothecia were incubated at 15 C for 48 hr in the dark. Once discharged on a hydrophobic surface, the ascospores were collected by running a droplet of sterile, distilled water over the surface. This droplet was then increased in volume by addition of more water and served as the inoculum. The concentration of spores was determined by use of a hemacytometer. Spore concentrations ranged from 25,000 to 50,000 spores per ml. Although varying the spore concentration in this range had no apparent effect on spore germination, an attempt was made to maintain similar concentrations of spores. The spores were inoculated by placing droplets of the spore suspension on the appropriate medium using a Pasteur pipette.

The percentage of germinated ascospores was determined by microscopic examination seven days after inoculation. A spore was considered to have germinated if a germ tube equal in length to the spore width was evident. Preliminary studies indicated that most ascospores germinated within three to four days of inoculation (Figure 6). After seven to eight days of incubation, individual spores were difficult to distinguish due to hyphal growth and branching. Therefore,

Figure 6. Ascospores germinating on the surface of water agar, five days after inoculation. 400X.

Figure 7. Ascospores germinating on the surface of water agar, seven days after inoculation. 125X.



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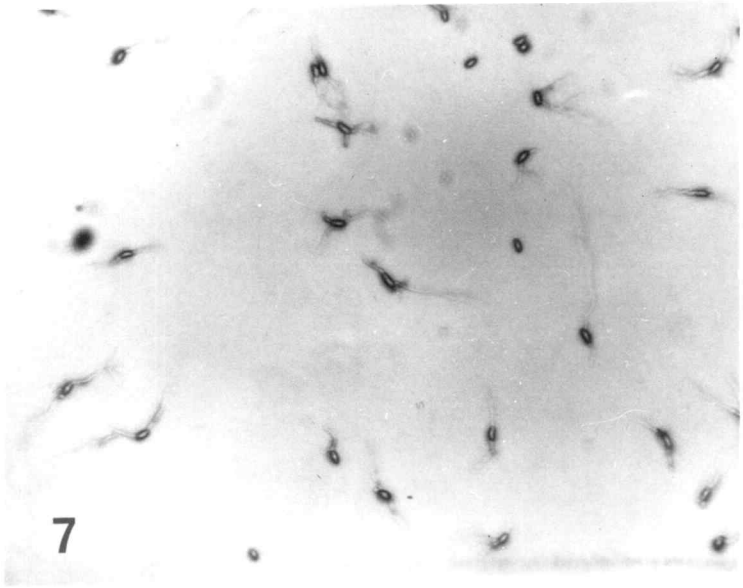

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the relative numbers of germinated and ungerminated spores were determined after an incubation period of seven days (Figure 7).

The basic procedure used in germination studies was to inoculate ascospores on water agar medium made with 2% Difco Noble Agar, pH 5.5-6.0. Plates were then incubated for seven days at 15 C in the dark. Spore germination was assessed by microscopic examination. This procedure was varied depending on the factor being investigated.

The time of ascospore germination in the laboratory was investigated by counting germinated spores at 12 and 24 hr intervals for 10 days. Five hundred spores per plate on each of four plates were monitored at specific time intervals after inoculation. The same plates were observed throughout the test period.

The temperature of spore germination was investigated by incubating inoculated plates at 5, 10, 15, 20, 25, and 30 C. One hundred spores per plate on each of six plates per temperature were monitored 7 and 14 days after inoculation. The effect of alternating temperatures on germination was also studied. Plates were incubated for a 12 hr period at 15 C, followed by a 12 hr period at 30 C. This temperature schedule was followed for a period of one week. One half of the plates receiving alternate temperature treatments were first incubated at 15 C, the other half at 30 C. Four hundred spores per plate on each of five plates per treatment were monitored after seven days of incubation.

Light effects on germination were studied by exposing five inoculated plates to two 4-watt fluorescent bulbs throughout the seven day incubation period. An equal number of plates were incubated in a dark box. In one trial 400 spores per plate were evaluated. In the second trial, 200 spores per plate were observed for germination.

The effect of pH on spore germination was investigated. Five to 10 ml of McIlvaine's Buffer, a citrate-phosphate buffer, was added to 100 ml of water agar to obtain media with pH's of 3, 4, 5, 6, 7, and 8 (Machlis and Torrey, 1956). Four hundred spores on each of five plates at each pH were assessed for germination. Relative numbers of germinated and ungerminated spores on non-buffered water agar, pH 5.5-6.0, were also recorded.

Relative humidity studies were conducted using the jars and saturated solutions described in discharge methods. Water droplets containing spores were inoculated onto glass coverslips. Droplets were allowed to evaporate before being inverted in jars with various relative humidities. Six coverslips with spores were examined at each relative humidity. In some cases, droplets suspended in a 100% RH atmosphere were not allowed to evaporate, and therefore were exposed to free water throughout the time of incubation. Agar blocks were also inoculated with spores, and incubated in an atmosphere of 100% RH.

Ascospore germination on water agar, in tubes of water, and in water droplets was compared. Three plates of water agar and three plates of sterile, distilled water were inoculated. Water droplets containing spores were inoculated on coverslips, inverted, and incubated at 100% RH to prevent evaporation. After seven days, 100 spores from each vessel were observed.

Germination on washed and unwashed Noble Agar was compared. Agar was washed by placing it in a cloth bag and immersing it in distilled water for two days (Tuite, 1969). A total of 400 spores on each of five plates per treatment were assessed for germination.

Germination on water agar, malt-yeast extract agar, bark extract agar, and lichen extract agar was also investigated. The bark extract agar was made by chopping up 5 g of the bark of Acer macrophyllum. This was boiled in water for one minute, and filtered through four layers of cheesecloth. The resulting extract was added to water and agar to yield 500 ml of medium. The lichen extract agar was made similarly, except 5 g of the thalli of Xanthoria polycarpa were used. The malt yeast agar was made with 20 g of malt, and 2 g of yeast extract per liter. Two hundred spores per plate on a total of five plates per medium were observed.

All of the above laboratory experiments were repeated at least once.

IV. RESULTS

Spore Discharge

Ascospore discharge was studied in the field and in the laboratory. In both cases, the magnitude of spore discharge varied with different apothecia. Correlation techniques were used to evaluate the seasonal discharge data. Nonparametric statistical methods were used to evaluate much of the laboratory data on spore discharge.

Seasonal Discharge Studies

Field observations of ascospore discharge were made during summer, fall, winter, and spring. Five thalli were monitored for one 7-day period in each season. If no spore discharge occurred during the seven day period, thalli were wetted and observed for one more day. Additional observations were made on a rainy day in August, 1975.

The same five thalli were used for summer and fall studies. These thalli failed to discharge spores when subsequently tested. Therefore, five more thalli were chosen for winter, spring, and August, 1975 observations.

Table 1 indicates the results of field observations of lichen thalli. Spore discharge was observed in all seasons of the year.

Table 1. Number of ascospores discharged in fall, winter, spring, and summer, with precipitation and temperature values indicated.

Season	Temperature (C)		Precipitation	Number of
	Min	Max	(mm)	spores*
Summer	16.1	34.4	0.8	0
July 29-Aug. 4	13.3	33.9	0	0
1974	14.4	35.6	0	0
	12.2	35.6	0	0
	10.6	32.8	0	0
	11.1	33.3	0	0
	11.7	32.8	0	0
	10.6	31.7	wet**	4145
Fall	10.0	13.9	9.4	7620
Oct. 27-Nov. 3	4.4	12.2	9.9	9404
1974	4.4	15.0	0	1168
	4.4	11.1	5.1	2426
	1.7	15.0	2.0	1142
	1.7	13.9	0	4
	-0.6	9.4	0	10
Winter	0	11.7	0	0
Feb. 24-Mar. 3	0.6	12.8	0	0
	3.9	11.7	4.1	779
1975	6.1	12.8	18.3	55688
	9.4	16.1	1.0	14576
	8.9	12.8	4.1	9896
	5.6	13.3	3.8	4098
Spring	4.4	12.8	5.1	1862
Apr. 1-Apr. 25	-1.1	12.2	trace	127
1975	2.8	13.9	0	0
	3.9	17.8	0	0
	5.0	11.7	11.9	496
	5.6	14.4	6.6	362
	3.3	11.1	8.4	5757
Aug. 17, 1975	13.9	18.3	13.0	13688

*Each value represents the total number of spores discharged from five thalli in a 24 hr period.

**Thalli wetted with water from spray atomizer.

Discharge was sparse in the summer, and was observed only when thalli were wetted or when heavy rains occurred. Abundant discharge occurred in the fall, winter, and spring. Periods of discharge appeared to be associated with periods of rainfall. A correlation coefficient of 0.695, significant at the .01 level, was obtained when rainfall and discharge were compared.

Time of Discharge

Ascospore discharge observed in the laboratory at 12 hr intervals exhibited no striking time pattern. Some apothecia discharged most of their spores within the first two days of incubation. Others maintained low levels of spore discharge throughout much of the observation period. Some apothecia did not discharge spores until late in the observation period, while other apothecia did not discharge any spores at all. After 17 days of observation, apothecia were observed microscopically for presence of ascospores. Fertile asci were found in all cases.

Distance of Discharge and Number of Spores per Projectile

Horizontally discharged ascospores were collected on a marked ruler, and the distance of spore discharge was recorded (Table 2). Seventy-seven percent of the ascospores discharged were found

Table 2. Horizontal distance of ascospore discharge.

Distance from apothecia (mm)	Number of spores*
1	417
2	1345
3	2112
4	2736
5	3744
6	4907
7	5408
8	5360
9	6452
10	6873
11	6607
12	6244
13	5259
14	3531
15	2067
16	1918
17	1885
18	1499
19	1370
20	443
21	93
22	56
23	61
24	24

*Spore values totaled from 23 apothecia.

between 5 and 14 mm away from the apothecia. The most frequent distance traveled by ascospores was 10 mm. Ascospores were found up to 24 mm away from apothecia.

Some ascospores were grouped together on the slides. The number of spores per group was counted to get some indication of the number of spores per projectile (Table 3). Most ascospores were found to occur in groups of eight.

Light

Results of studies on the effects of light on discharge were evaluated with Wilcoxson's two sample sum of ranks test (Steel and Torrie, 1968). Table 4 indicates the total number of spores discharged from apothecia incubated in the light and from apothecia incubated in the dark. The test indicated that either set of values could have been a random sample from the same population; therefore, no obvious effect of light on discharge was indicated.

Relative Humidity

When apothecia were exposed to atmospheres of various relative humidities, no discharge occurred below 100% RH. Discharge did occur at 100% RH and when apothecia were placed on moist filter paper. Table 5 indicates totals of spores discharged at various relative humidities. Analysis with Wilcoxson's test does not indicate

Table 3. Percentage of discharged ascospores occurring in groups of various numbers.

Number of spores/ group	Percentage occurrence*
1	0.10
2	0.06
3	0.10
4	0.19
5	0.34
6	0.30
7	0.77
8	88.70
9	0.08
10	0
11	0.03
12	0.35
13	0.02
14	0.04
15	0.04
16	7.60
17	0
18	0
19	0
20	0
21	0.79
22	0
23	0
24	0.14
25	0
>25	0.34

*Percentages obtained from a total of 69,446 spores discharged from 23 apothecia.

Table 4. Number of ascospores discharged in the light and in the dark.*

Trial	Dark	Light
1	2377	6231
2	3232	6770

*Each value represents the total number of spores discharged from 12 apothecia.

Table 5. Number of ascospores discharged at various relative humidities.*

Trial	Relative humidity (%)					
	wet**	100	99	86.5	76.5	dry***
1	2056	1142	0	0	0	0
2	2423	3051	0	0	0	0

*Each value represents the total number of spores discharged from six apothecia.

**Wet - apothecia were placed on moist filter paper.

***Dry - no liquid was in container.

differences between spore discharge values at 100% RH and "wet," thalli which were placed on moist filter paper.

Temperature

Results of studies on the effect of temperature on discharge are indicated in Table 6. No discharge was recorded at 25 or 30 C. The numbers of spores discharged at 5, 10, 15, and 20 C were compared using the Kruskal-Wallis test (Kruskal and Wallis, 1952). Results indicate that all of the numbers are not random samples from the same population, and therefore indicate that at least one set of values is different from the rest. This is confirmed by Wilcoxson's test performed on data from two temperatures at a time, which indicate that 5, 10, and 15 C values are similar, but different than 20 C values. Wilcoxson's tests performed on data from another trial of this study, however, indicate no differences between values at 5, 10, 15, and 20 C.

Table 6. Number of ascospores discharged at various temperatures.*

Trial	Temperature (C)					
	5	10	15	20	25	30
1	3658	5777	5824	3522	0	0
2	5032	5653	9261	919	0	0

*Each value represents the total number of spores discharged from 15 apothecia.

Spore Germination

Ascospore germination was examined in the laboratory. Analysis of variance techniques were used to evaluate germination data. Values were analyzed at the .05 level of significance. The Studentized Range Test was used to compare means (Snedecor and Cochran, 1968). In each set of means compared, means labeled with the same letter were not found to be significantly different.

Time of Germination

Ascospores inoculated on water agar were observed periodically for germination. Figures in Table 7 indicate the percentage of spores that had germinated after given periods of incubation at 15 C. In both trials a large proportion of the spores were found to germinate between 12 and 48 hr after inoculation. No significant difference exists between germination values at seven days and 11 days after inoculation. No significant difference exists between germination values at 3-1/2 days and seven days after inoculation.

Temperature

Plates inoculated with ascospores were incubated at 5, 10, 15, 20, 25, and 30 C. The percentage of spores that had germinated by one and two weeks after inoculation is indicated in Table 8. At both

Table 7. Time of ascospore germination after inoculation. *

Hours after inoculation	Trial 1		Trial 2	
	Mean % germination	Mean number germinated spores / plate	Mean % germination	Mean number germinated spores / plate
12	0	0 A	0	0 A
24	14.1	70.5 B	16.2	81.2 B
36	66.2	331.0 C	74.2	371.2 C
48	85.0	424.8 D	86.7	433.6 D
60	88.5	442.5 E	91.5	457.4 E
72	91.3	456.3 F	93.2	465.8 F
84	91.7	458.3 FG	93.7	486.4 FG
96	92.8	463.8 FGH	94.2	470.8 FGH
108	92.8	464.0 FGH	93.9	469.4 FG
120	92.9	464.5 FGH	93.9	469.4 FG
132	93.7	468.3 GH	94.2	470.8 FGH
144	93.3	466.5 FGH	94.5	472.6 GH
156	94.1	470.5 H	94.6	473.0 GH
168	93.7	468.3 GH	94.6	472.8 GH
192	93.1	465.5 FGH	94.5	472.4 GH
216	93.0	465.0 FGH	94.5	472.6 GH
240	92.8	464.0 FGH	95.0	474.8 H
264	93.3	466.5 FGH	95.1	475.4 H

*Five hundred ascospores per plate were counted. Trial 1 values are means from four plates. Trial 2 values are means from five plates. Values followed by the same letter within any trial are not significantly different (P = .05) by the studentized range test (Snedecor and Cochran, 1967).

one and two weeks after inoculation, the highest percentage of germinated ascospores was found on plates incubated at 10, 15, and 20 C. Ascospore germination at 5 C was also high, but less than optimum. Spore germination at 25 C was low. No spore germination was observed at 30 C. Significant differences were not found between germination values at one week and two weeks with one exception (5 C).

Table 8. Ascospore germination at various temperatures.*

Trial	Temperature (C)											
	5		10		15		20		25		30	
	1 wk	2 wk	1 wk	2 wk	1 wk	2 wk	1 wk	2 wk	1 wk	2 wk	1 wk	2 wk
1	88.0	89.0	91.8	89.7	94.7	95.8	94.0	93.2	33.7	35.7	0	0
	C	CD	CDE	CDE	DE	E	CDE	CDE	B	B	A	A
2	79.0	84.7	88.0	88.7	87.2	90.7	88.8	90.7	25.0	26.5	0	0
	C	D	DE	E	DE	E	E	E	B	B	A	A

*Each value represents the mean from six replicate plates.

When ascospores were subjected to an alternating temperature regime of 15 and 30 C, little or no germination was observed (Table 9).

Light

Ascospores inoculated on water agar were exposed to light or to total darkness throughout the incubation period. Figures in Table 10 indicate the percentage of spores that had germinated after seven

Table 9. Ascospore germination at constant and alternating temperatures.*

	Temperature (C)			
	Constant		Alternating	
	15	30	15-30	30-15
	Trial 1			
Mean % germination	93.7	0	0	0
Mean number germinated spores/plate	378.6	0	0	0
	Trial 2			
Mean % germination	94.1	0	0.5	2.2
Mean number germinated spores/plate	383.0	0	2.0	8.2

*Each value represents the mean obtained from counting 400 spores on each of five replicate plates.

Table 10. Ascospore germination in the light and in the dark.*

	Light	Dark
	Trial 1	
Mean % germination	94.7	93.9
Mean number germinated spores/plate	379.6 A	375.6 A
	Trial 2	
Mean % germination	89.0	87.9
Mean number germinated spores/plate	178.0 A	175.8 A

*Each value represents the mean obtained from five replicate plates. Four hundred spores per plate were counted in Trial 1. Two hundred spores per plate were counted in Trial 2.

days of incubation. No significant difference was found in germination values between spores exposed to the light and spores incubated in the dark.

Hydrogen Ion Concentration

A citrate-phosphate buffer was used to adjust the pH of water agar. Plates of pH 3, 4, 5, 6, 7, and 8, and plates without buffer, pH 5.5, were inoculated with ascospores. Table 11 summarizes the results of this study. The highest percentage of germinated spores was found to occur at pH 5, pH 6, and in the control plates with no buffer. In one experiment, germination of spores at pH 7 was equally high, but when repeated, the percentage of germinated spores at pH 7 was significantly lower. At pH 8 the percentage of ascospores germinated was lower than optimum, but still greater than 75%. Germination at pH 3 was very poor.

Relative Humidity

When inoculum was allowed to evaporate on coverslips and then placed in atmospheres of various relative humidities, no germination was observed (Table 12). Ascospores suspended in a water droplet and incubated at 100% RH germinated to a minor extent. Ascospores inoculated on agar blocks and incubated at 100% RH showed a high percentage of germination.

Table 11. Ascospore germination on media with various hydrogen ion concentrations.*

	pH						Control (no buffer)
	3	4	5	6	7	8	5.5
		Trial 1					
Mean % germination	0.16	89.4	94.5	96.3	95.1	92.4	95.9
Mean number germinated spores/plate	0.65 A	357.6 B	379.6 D	385.2 D	380.4 D	369.6 C	383.6 D
		Trial 2					
Mean % germination	0	85.6	93.5	93.4	89.9	86.8	93.6
Mean number germinated spores/plate	0 A	342.2 B	373.8 D	373.4 D	359.4 C	347.2 B	374.4 D

*Each value represents the mean obtained from counting 400 spores on each of five replicate plates.

Table 12. Percentage of spores germinating at various relative humidities.*

Trial	Relative humidity** (%)					100% RH***	
	100	99	86.5	76.5	dry	Agar block	Water droplet
1	0	0	0	0	0	-	-
2	0	0	0	0	0	94.5	3.5

*Each value represents the mean obtained from six replicates.

**Spores were dried on coverslips and incubated over saturated salt solutions at the indicated relative humidity. "Dry" jar had no liquid.

***Agar blocks and water droplets were incubated at 100% RH.

Media

Ascospores incubated on water agar were found to have a much higher percentage of germinated spores than ascospores incubated in tubes of water or in hanging water droplets (Table 13).

Table 13. Ascospore germination in water droplets, in water columns, and on water agar.*

Trial	Water droplet	Water column	Water agar
1	17.7	15.3	93.2
2	20.5	19.0	89.5

*Values represent the percentage of spores germinating out of 200 spores per vessel. Trial 1 values are means from a total of three replicates. Trial 2 values are the totals from one observation in each category.

No difference in percentage of germination was found between ascospores inoculated on washed or unwashed Noble agar medium (Table 14).

The percentages of spores germinating on water agar and lichen extract agar were equally high (Table 15). Germination values on malt-yeast extract agar were significantly lower. The percentage of ascospores germinating on bark extract agar was the lowest on all of the solid media tested, but was still greater than 50%.

Table 14. Ascospore germination on washed and unwashed Noble agar.*

	Washed	Unwashed
Trial 1		
Mean percent germination	94.8	94.7
Mean number germinated spores/plate	379.4 A	379.6 A
Trial 2		
Mean percent germination	94.1	94.1
Mean number germinated spores/plate	376.4 A	376.4 A

*Each value represents the mean obtained by counting 400 spores on each of five replicate plates.

Table 15. Ascospore germination on various agar media.*

	Bark extract agar	Malt yeast agar	Lichen extract agar	Water agar
	Trial 1			
Mean percent germination	66.7	88.6	93.6	94.1
Mean number germinated spores/plate	135.4 A	179.2 B	187.2 C	188.2 C
	Trial 2			
Mean percent germination	74.3	82.5	90.0	89.2
Mean number germinated spores/plate	148.6 A	165.0 B	178.4 C	180.0 C

*Each value represents the mean obtained by counting 200 spores on each of five replicate plates. The pH of bark extract agar was 5, malt yeast agar 6, lichen extract agar 6, and water agar 5.5.

V. DISCUSSION AND CONCLUSIONS

Seasonal Discharge

Summers in the Willamette Valley are characteristically dry, with hot days and cool nights. Much of the rest of the year is cool and damp, with abundant rainfall.

Field studies show that ascospore discharge can occur during any season of the year. Discharge in the summer, however, was rare. Discharge in the fall, winter, and spring was common.

Early observations by Werner (1930) suggested that lichens discharged spores primarily in the springtime. Work by Kofler (1957) indicated that discharge was influenced by daily weather conditions. Results found here agree with those of Kofler (1957). The pattern of spore discharge seems to reflect daily precipitation patterns rather than a seasonal cycle.

The only time discharge was observed in the summer was when thalli were wetted, either artificially or due to natural rainfall. The discharge that occurred on August 18 indicates that thalli do have the ability to discharge spores in the summer if moisture conditions are favorable. The weather that day was atypically cool and rainy. Thalli also discharged spores in the summer when they were sprayed with water. The high daytime temperatures and the lower nighttime

temperatures of this day were more typical of summer. The occurrence of discharge in this case suggests that the high daytime temperatures of summer do not seriously limit the occurrence of discharge in the summer. The lack of rainfall seems to be much more important in this respect.

The production of spores was not monitored. It is possible that weather conditions favorable to discharge could have occurred, but no spores were discharged due to lack of spore production. The presence of spores can be confirmed by microscopic examination of sliced or crushed apothecia. Since this is a destructive technique, apothecia would no longer be available for further observation. Although sporulation was not monitored in this study, observation of a sample of the apothecia for sporulation would have been useful.

Time

In laboratory investigations, all apothecia did not show the same time pattern of ascospore discharge. The most common pattern exhibited was peak discharge within the first two days of incubation. However, when large numbers of apothecia were set up for spore discharge and collection, 48 hr was sufficient time in which to collect enough ascospores for germination studies.

Pyatt (1973a) found that spore discharge in X. parietina peaked after thalli were moistened, and then again upon drying. This

phenomenon may explain discharge that occurred near the end of the incubation period when filter paper was drying out. Variability in patterns of discharge may also reflect the past history of individual apothecia as well as genetic variability.

Ascospores germinated readily within several days of inoculation on water agar. Apparently the spores do not go through a period of constitutional dormancy, that is, a state of dormancy due to innate properties of the spore.

Distance

Discharged ascospores were found from 1 to 24 mm away from apothecia. The most frequent distance of discharge was 10 mm. Spores were commonly found to occur in groups of eight, indicating that the spores of a single ascus often function as one projectile. Pyatt (1973a) found that the spores of X. parietina were discharged up to 23 mm from the apothecia, and usually occurred in groups of eight.

Light

Ascospore discharge can occur when apothecia are incubated in the dark or in the light. Germination also occurs equally well in the light as in the dark. Investigations of discharge and germination under various alternating light and dark periods should be conducted

in order to explore more fully the effects of light on discharge and germination.

Temperature

Results of studies indicate that discharge and germination are both favored by low temperatures. Although ascospore discharge occurred at 5, 10, 15, and 20 C, no discharge was observed at 25 or 30 C. A similar pattern was noted in germination studies. The percentage of germinated spores was high at 5, 10, 15, and 20 C, and low at 25 C. No germination was observed at 30 C. Spores exposed to alternating 15 C and 30 C temperatures also had a very low percentage of germinated spores. These results coincide fairly well with those of Thomas (1939) who examined discharge and germination in X. parietina. He found frequent discharge between 3 and 27 C, but little at 30 C. He also found that few spores germinated at 27 C.

The high for daytime summer temperatures in the Willamette Valley is typically greater than 25 C. Nighttime temperatures, however, are much cooler. When thalli were moistened in the summer, discharge was observed during a period with a temperature maximum of 31.7 C and a minimum of 10.6 C. This suggests that summer temperatures do not prevent spore discharge. However, laboratory results from alternating temperature studies suggest that high summer temperatures may not be conducive to spore germination.

Hydrogen Ion Concentration

The highest percentage of spores germinated at pH 5 and pH 6. Spores germinated well on media between pH 4 and pH 8, but germination at pH 3 was poor. Spore germination values were comparable on buffered and unbuffered media of similar pH. No effect due to the presence of the buffer was indicated. Experiments using another buffer would be necessary to determine if germination can occur at a pH greater than pH 8. It should be noted that fungal response to pH can vary with the buffer used and with the nutrients supplied in the media. In this study, the effects of various buffers and of different media were not explored.

The ability of ascospores to germinate over a pH range of 4 through 8 suggests that in most natural situations, pH would probably not limit germination. However, the increased occurrence of acid precipitation, especially in industrial areas, could conceivably hamper germination.

Pyatt (1968c) found that the optimum pH for spore germination in X. parietina was pH 3. His technique was different than that used in this study. He suspended spores on glass slides above a liquid buffer, rather than placing ascospores on media of various pH's. The differences in pH optima found may be due to natural differences between the two species or to differences resulting from the techniques used.

Relative Humidity

Results of relative humidity studies reaffirm the importance of moisture to the discharge of ascospores. Discharge was only observed when apothecia were placed in an atmosphere of 100% RH or on moist filter paper. No discharge occurred when dry apothecia were placed in an atmosphere of 99% RH or less. Apparently, 100% RH or a source of free water is necessary for spore discharge to occur. Although relative humidity experiments were conducted in a water bath, there is a possibility that condensation occurred at 100% RH. However, no evidence of free water was noted.

The results obtained agree with the findings of Garrett (1971). He found that dry apothecia of X. parietina discharged spores when placed in an atmosphere of 100% RH, but not at 90% RH. Apothecia placed on moist filter paper discharged spores at both 90% and 100% RH, as did apothecia covered with a film of moisture.

Ahmadjian (1961) suggested that discharge begins when moistened apothecia dry. Work by others (Bailey, 1966, 1968; Bailey and Garrett, 1968; Garrett, 1968, 1971) suggests that ascospores are discharged when apothecia are moistened. Pyatt (1973a) noted that discharge can occur when apothecia are moistened and again when they dry. All spore collection methods used, as well as results from relative humidity studies, indicate that spores of

X. polycarpa are discharged when apothecia are moistened. Discharge upon drying has not been investigated.

Ascospores dried onto coverslips were placed in atmospheres of various relative humidities. No germination occurred at any of the relative humidities tested, including 100% RH. Ascospores did germinate when inoculated onto blocks of agar media and in water. This suggests that ascospores must have free water available or be able to obtain water from a substrate in order to germinate. Spores inoculated on agar were suspended in a water droplet, but spores discharged directly onto agar also germinate.

Media

Although the percentage of ascospores germinating on water agar was very high, the percentage of spores germinating in water was low. Kofler (1970) suggested that the failure of spores of X. parietina to germinate in liquid could be due to a lack of oxygen, since spores sink to the bottom in a column of liquid.

In this laboratory, ascospores were inoculated into tubes of water and onto coverslips. When the coverslips were inverted, spores sank to the bottom of the hanging droplets. Presumably, spores at the bottom of these droplets were not deprived of oxygen, since they were very near the air-water interface. The percentage of spores germinating in the droplets was similar to the percentage of

spores germinating at the bottom of a tube of water. This suggests that lack of oxygen is not responsible for the lower percentage of spore germination in water than on water agar.

The high percentage of germination on agar could be due to the presence of a nutrient or growth factor in the agar. Noble agar was used routinely due to its higher purity. When the percentage of germinated spores on washed and unwashed Noble agar was compared, no difference was found. If something in the agar was responsible for the increase in germination, it was not eliminated by washing. More experimentation is necessary before the high percentage of germination on water agar as compared to the low percentage of germination in water can be adequately explained.

The germination of ascospores on various solidified media indicates that nutrition can have an effect on the percentage of spore germination. Although germination on all four media tested was greater than 50%, differences in the percentages of germinated spores were apparent. The relatively low percentage of spore germination on bark extract agar was not expected, since bark is the substrate upon which thalli of X. polycarpa are found. Germination on lichen extract agar was high, suggesting that spores landing on thalli of X. polycarpa in nature would exhibit a high percentage of germination. Germination on nutrient poor water agar was also high, while germination on nutrient rich malt-yeast extract agar was significantly

lower. It may be misleading to draw conclusions on the germination of spores on natural substrates based on results obtained from using extracts. Components of the original substrate could be lost or altered during media preparation. Results do indicate, however, that ascospore germination can be affected by the substrate.

General Conclusions

Results of field and laboratory studies indicate that ascospore discharge and germination in X. polycarpa are favored by the cool, damp, and rainy climate characteristic of western Oregon for much of the year. Discharge and germination readily occur at low temperatures and are favored by the presence of moisture. The discharge of ascospores up to 24 mm away probably allows for further dissemination by wind or water. The spores germinate readily under a variety of pH and substrate conditions. It is hoped that these investigations will stimulate further studies on the fate of ascospores in nature, and the role they play in the life cycle of the lichen.

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