Seasonal Availability of *Phaeocryptopus gaeumannii* Ascospores and Conditions That Influence Their Release

E. MICHAELS and G. A CHASTAGNER, Western Washington Research and Extension Center, Washington State University, Puyallup 98371

ABSTRACT

Michaels, E., and Chastagner, G. A. 1984. Seasonal availability of *Phaeocryptopus gaeumannii* ascospores and conditions that influence their release. Plant Disease 68:942-944.

Ascospores of *Phaeocryptopus gaeumannii* were released from pseudothecia on moistened needles from April through September, with maximum numbers released during June and July. Pseudothecia on 1-yr-old needles released 10 times more ascospores than did those on 2-yr-old needles. Moistened pseudothecia released 75% of available ascospores within 20 min and all within 4 hr. Ascospores were released at 5–30 C, with maximum release at 20 C. Compared with darkness, ultraviolet or fluorescent light sources enhanced ascospore release.

Infection of needles by *Phaeocryptopus gaeumannii* (Rohde) Petr., the ascomycete causing Swiss needle cast of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), has resulted in serious damage to plantations of trees in Europe, northeastern and north central United States, and New Zealand since its discovery in Switzerland in 1925 (1,6,8,10,12). Considered harmless in the natural range of Douglas-fir since its discovery there in 1938, this fungus has recently been observed to be injurious to Christmas tree plantations in western Washington and Oregon (7,11). Premature needle loss resulting from infection reduces tree quality at harvest, and the presence of infected needles accelerates dehydration and needle loss after harvest (4,11).

In other regions, ascospores are released from pseudothecia during spring and summer and current-year needles are infected during shoot elongation (1,5,6, 10,12). Disease in those regions has been controlled by use of climatically adapted strains of Douglas-fir and application of protectant fungicides during the infection period (1,6,10,12,13).

A study in western Washington during 1977 revealed two periods of ascospore release, spring and autumn, with peak spore release in late May and October (B. A. Fatuga, *unpublished*). Infection was believed to take place during January and August. The timing of the first infection period in August and the occurrence of a second major infection period associated with a second period of inoculum production in Washington differed from all previous accounts of the disease cycle. Identification of the periods of ascospore availability and of the conditions influencing their release from pseudothecia was necessary to design control measures appropriate for the Pacific Northwest.

Washington State University Scientific Paper No. 6482, College of Agriculture Research Center Project 0505.

Accepted for publication 16 June 1984 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Techniques for ascospore release. Needles with pseudothecia were brought into the laboratory and separated by year of origin. Ten needles were randomly selected, placed over a 1 × 2 cm aperture cut in a filter paper circle, and secured with tape so the undersurface of 10 1-cm segments of needles was exposed. The filter paper circle was placed in the cover of a 60 × 10 mm plastic petri plate, and the needles were misted with tap water until runoff. The cover was then replaced on the plate, which contained water agar. The 1 × 2 cm of agar directly beneath the exposed parts of the needles was marked on the bottom of the plate (we had determined previously that most ascospores released from pseudothecia fell perpendicularly onto the media). At the end of each experiment, plates were sprayed with rose bengal solution to stop fungal growth. Ascospores released onto the agar in the rectangle beneath the exposed parts of the needles were counted with the aid of a compound microscope.

The number of pseudothecia exposed to each plate was estimated by counting the number on one-eighth of the exposed needle area. This method did not establish how many pseudothecia were contributing to spore release.

Factors influencing inoculum availability. Three radiation regimes were used to determine if light influences spore release: an ultraviolet source (GE-F15T8 BLB, 15W black light), a fluorescent source (W-F15T12 CW, 15W cool white), and darkness. One-year-old needles collected during June and July from five trees near Rochester, WA, were brought into the laboratory and plates were prepared up to the misting step under laboratory lights. Needles were misted with tap water (20 C) while under one of the light regimes, placed over the agar, and incubated at 20 C in darkness or 15 cm beneath a light source for 24 hr. (The petri plate covers were known to permit transmission of a large fraction of radiation of wavelengths 310–410 nm emitted by the ultraviolet source.) Five plates per treatment were used and the experiment was repeated four times.

One-year-old needles were used to determine cardinal temperatures for spore release. Eight temperatures—0, 5, 10, 15, 20, 25, 30, and 35 C—were tested. The agar plates, misting water, and infected needles were allowed to equilibrate at desired temperatures for 24 hr. The plates were then prepared, misted, and replaced in incubators under

ultraviolet radiation for 24 hr. The number of spores released was counted. Each treatment was replicated five times and the experiment was repeated once.

The dynamics of ascospore release after a single misting of pseudothecia was investigated. Plates prepared as described were incubated under ultraviolet radiation at 20 C. At the end of a designated time interval, the plate covers with moistened 1-yr-old needles were moved to fresh agar-filled bottoms to avoid recounting previously released spores. Three sets of time intervals were used: hourly for 12 hr, every 30 min for 5 hr, and every 10 min for 2 hr. Spores on five plates per test were counted and the experiment was repeated three times.

To determine if rewetting pseudothecia after initial ascospore release would result in release of additional ascospores, needles were misted at 4-hr intervals for 48 hr. Spores on five plates were counted in each of three repetitions of the experiment. After each 4-hr interval, pseudothecia were scraped from needles from randomly selected plates, crushed, and examined under the microscope for ascospores.

The ability of pseudothecia on 1- and 2-yr-old needles to release ascospores was studied during a 17-mo period. Five trees in a plantation near Rochester, WA, were marked and needles were collected from them weekly from May through September 1981, then monthly through September 1982. Three plates of 1- and 2-yr-old needles were made from each of the five trees. Plates were placed under ultraviolet radiation at 20 C for approximately 12 hr. Spores released onto plates were counted. To determine if ascospores were present but not being released, pseudothecia from randomly selected needles not affixed to plates were examined for ascospores.

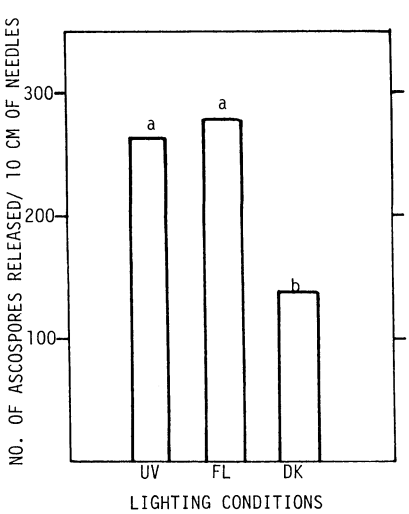

Fig. 1. Influence of light on release of ascospores from pseudothecia. Each bar represents an average spore deposition in 20 plates. Bars with the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Plates were incubated at 20 C for 24 hr. UV = ultraviolet, FL = fluorescent, DK = darkness.

RESULTS

Pseudothecia incubated in light released significantly more ascospores than did those incubated in darkness (Fig. 1). No significant difference in spore release was observed between ultraviolet and fluorescent lighting.

Ascospores were released at temperatures of 5–30 C and in greatest abundance at 20 C (Fig. 2). Intermediate numbers were released at 5, 10, 15, and 25 C, few were released at 30 C, and none were released at 0 and 35 C.

Ascospores were released rapidly from moistened pseudothecia. Approximately 75% of the ascospores were released within 20 min and all were released within 4 hr. Periodic mistings for 48 hr did not result in release of additional ascospores. Microscopic examination of previously misted pseudothecia revealed that apparently mature and immature ascospores remained within asci.

According to field collections, pseudothecia were first visible on infected current-year needles in late October 1981 and apparently mature ascospores were first observed inside pseudothecia during late March. One-year-old needles had the potential to release ascospores from April through September and released about 10 times as many ascospores from May through August as did 2-yr-old needles (Fig. 3). Microscopic examination of pseudothecia indicated that the majority on older needles were empty. The greatest numbers of ascospores were released from pseudothecia on both 1- and 2-yr-old needles during June and July.

DISCUSSION

When moistened, pseudothecia of *P. gaeumannii* rapidly released ascospores, the number depending on time of year, light, and temperature. Misting was essential for spore release. Peaks in the number of *P. gaeumannii* spores trapped in the field have occurred during periods of frequent rainfall (6). Maximum ascospore release occurs in the light when stomata, the site of infection, are most likely to be open. Spore germination rates are greatest at the same temperature (20 C) that favors spore release (E. Michaels, *unpublished*).

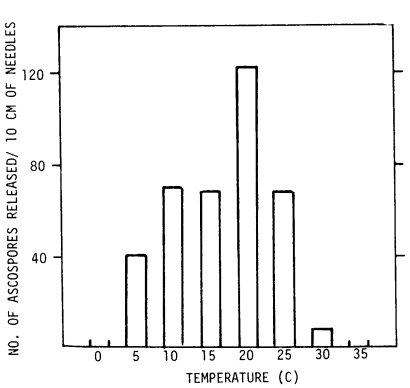

Fig. 2. Influence of temperature on release of ascospores from pseudothecia. Each bar represents average spore deposition in 10 plates.

Additional spore release could not be demonstrated with repeated mistings even though examination of pseudothecia revealed apparently mature ascospores within asci. Spores within pseudothecia of related fungi such as *Venturia inaequalis* (Cke.) Wint. may be replenished within hours after rewetting (2,9). *P. gaeumannii* may require a longer recharge period or different temperature and light combinations for spore maturation than we used.

Environmental conditions that allow ascospore release can be expected to occur frequently throughout the year in western Washington. Pseudothecia had the potential to release ascospores from April through September, with no release observed during the rest of the year. This agrees with information from other regions (5–7,9,10,13) but differs from previous studies in the Pacific Northwest (B. A. Fatuga, *unpublished*).

Fungicide control experiments in Washington and Oregon have shown that infection takes place during shoot elongation following bud break in late May and June (3,6). The late autumn and winter infection periods previously reported (B. A. Fatuga, *unpublished*) were not found to occur. Lack of

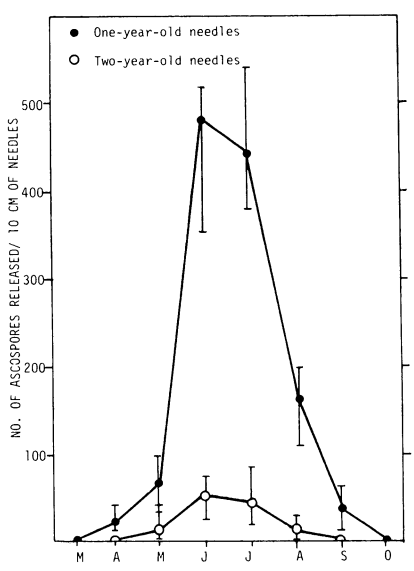

Fig. 3. Availability of ascospores during the 17-mo period from May 1981 through September 1982. Each dot represents average spore deposition in 15 plates incubated for 12 hr at 20 C. Needles were collected from five trees near Rochester, WA. Data are based on collections during the first week of each month; data from May through September are an average of collections from 1981 and 1982. Vertical lines represent the range of spore release.

inoculum from October through March would also suggest that a late autumn and winter infection period does not exist. Our data show that inoculum is potentially available during a longer period of time than that during which infection takes place. Previous investigations have indicated that needle susceptibility to infection decreases after needles are 6–8 wk old (10). Although critical studies have not been done in western Washington and Oregon, environmental conditions favoring infection would also not be expected to be limited to the time period during which needles become infected. This indicates that changes in host susceptibility determine the infection period. Further investigations of factors controlling the infection process and the mechanisms of resistance found in mature needles will lead to a better understanding of the relationship between host and pathogen.

ACKNOWLEDGMENTS

Partial funding for this study was provided by the Northwest Christmas Tree Association, the Puget Sound Christmas Tree Association, and the Gile Albion Lenore McGowan Memorial Fund. We thank James Hadfield, Steven Keyes, the Hofert Company, and Dianne Doonan for their technical assistance.

LITERATURE CITED

1. Boyce, J. S. 1940. A needle cast disease of Douglas-fir associated with *Adelopus gaumanni*. Phytopathology 30:649-659.
2. Brook, P. J. 1969. Effects of light, temperature, and moisture on release of ascospores by *Venturia inaequalis* (Cke.) Wint. N.Z. J. Agric. Res. 12:214-227.
3. Chastagner, G. A, and Byther, R. S. 1983. Infection period of *Phaeocryptopus gaeumannii* on Douglas-fir needles in western Washington. Plant Dis. 67:811-813.
4. Chastagner, G. A, Byther, R. S., MacDonald, J. D., and Michaels, E. 1984. Impact of Swiss needle cast on postharvest hydration and needle retention of Douglas-fir Christmas trees. Plant Dis. 68:192-195.
5. Chen, R. C. 1972. *Adelopus* needle cast disease of Douglas-fir in central New York. Natl. Univ. Taiwan Exp. For. Tech. Bull. 103. 51 pp.
6. Ford, K. F., and Morton, H. L. 1971. Etiology, impact and distribution of Swiss needle cast on Douglas-fir in Michigan. (Abstr.) Phytopathology 61:1023.
7. Hadfield, J., and Douglass, B. S. 1982. Protection of Douglas-fir Christmas trees from Swiss needle cast in Oregon. Am. Christmas Tree J. 26(2):31-33.
8. Hahn, G. C. 1941. New reports on *Adelopus gaumanni* on Douglas-fir in the United States. Plant Dis. Rep. 25:115-117.
9. Hirst, J. M., and Stedman, O. J. 1962. The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.) II. Observations on the liberation of ascospores. Ann. Appl. Biol. 50:525-550.
10. Hood, I. A., and Kershaw, D. J. 1975. Distribution and infection period of *Phaeocryptopus gaeumannii* in New Zealand. N.Z. J. For. Sci. 5:201-208.
11. Michaels, E., and Chastagner, G. A. 1984. Distribution, severity, and impact of Swiss needle cast in Douglas-fir Christmas trees in western Washington and Oregon. Plant Dis. 68:939-942.
12. Peace, T. R. 1962. Pathology of Trees and Shrubs with Special Reference to Britain. Oxford University Press, London. 723 pp.
13. Skilling, D. D. 1981. Control of Swiss needle cast in Douglas-fir Christmas trees. Am. Christmas Tree J. 25(3):34-35, 37.