Germination Behavior of Scirrhia acicola Conidia on Pine Needles

E. C. Setliff and R. F. Patton

Project Associate and Professor, respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison; and by U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Forest and Wood Products Disease Laboratory, Gulfport, Mississippi, through Cooperative Agreement No. 19-121 with the University of Wisconsin.

Accepted for publication 18 June 1974.

ABSTRACT

Suspensions of *Scirrhia acicola* conidia were sprayed onto secondary needles of Scotch pine (*Pinus sylvestris*) and longleaf pine (*P. palustris*). After incubation of tree seedlings in mist chambers, the fluorescent-brightener technique was used for direct observation of spores and germlings on the needle surface. Although conidia failed to germinate in many trials, when germination did occur, one-to-four germ tubes were produced from each spore. Germ tubes formed large coils or loops or grew in a random manner over the needle surface. Occasionally germ tubes grew into the stomatal pit. The possibility of attraction of a germ tube by an individual stoma is discussed. Germinated conidia on Scotch pine needles from heavily infected trees in a Christmas tree plantation were similar in appearance to those in the artificial inoculations.

Phytopathology 64:1462-1464

Additional key words: Brown spot, needle blight, *Lecanosticta acicola*.

Brown-spot needle disease caused by *Scirrhia acicola* (Dearn.) Siggers is the most important disease of longleaf pine (*Pinus palustris* Mill.). Its severity in young plantations is a limiting factor in the establishment of longleaf pine in the southeastern USA. Brown-spot disease was found in Scotch pine (*Pinus sylvestris* L.) in Wisconsin in 1962 (9), and since 1966 has constituted a serious problem to the Christmas tree industry (5).

Several reports have been published on the etiology and control of brown-spot disease (2,10,11,13). Very little is known, however, about the germination of spores on the needle surface, the mode of infection, and the expression of resistance in host plants. This paper deals with the behavior of germinating spores on the secondary needles of Scotch and longleaf pine.

MATERIALS AND METHODS.—One-year-old greenhouse-grown seedlings of Scotch pine (cultivars Austrian Hills, French, and Spanish) and longleaf pine were inoculated by atomizing a water suspension containing approximately 400,000 conidia per ml onto the secondary needles. Conidia were obtained from *S. acicola* (isolate ECS-758, obtained from Scotch pine at Brodhead, Wisconsin) cultures grown in an air conditioned laboratory in petri dishes, usually for about 3 weeks, on 2% malt extract agar. Inoculated seedlings were then maintained for 4-10 days in mist chambers so that a film or droplets of water were constantly on the needles. Conidia also were atomized into petri dishes containing 2% water agar or 2% malt extract agar to determine the germination percentage.

For direct observation of conidia on needle surfaces, we utilized the fluorescent brightener technique described earlier (7). Needles for study were cut from seedlings and immersed for about 1 hour in an aqueous brightener solution (0.4 ml/50 ml H_2O) containing Calcofluor White ST ®(American Cyanamid Co., Bound Brook, N.J.). Needles then were examined with a Leitz Ortholux incident-light fluorescence microscope. Light from a

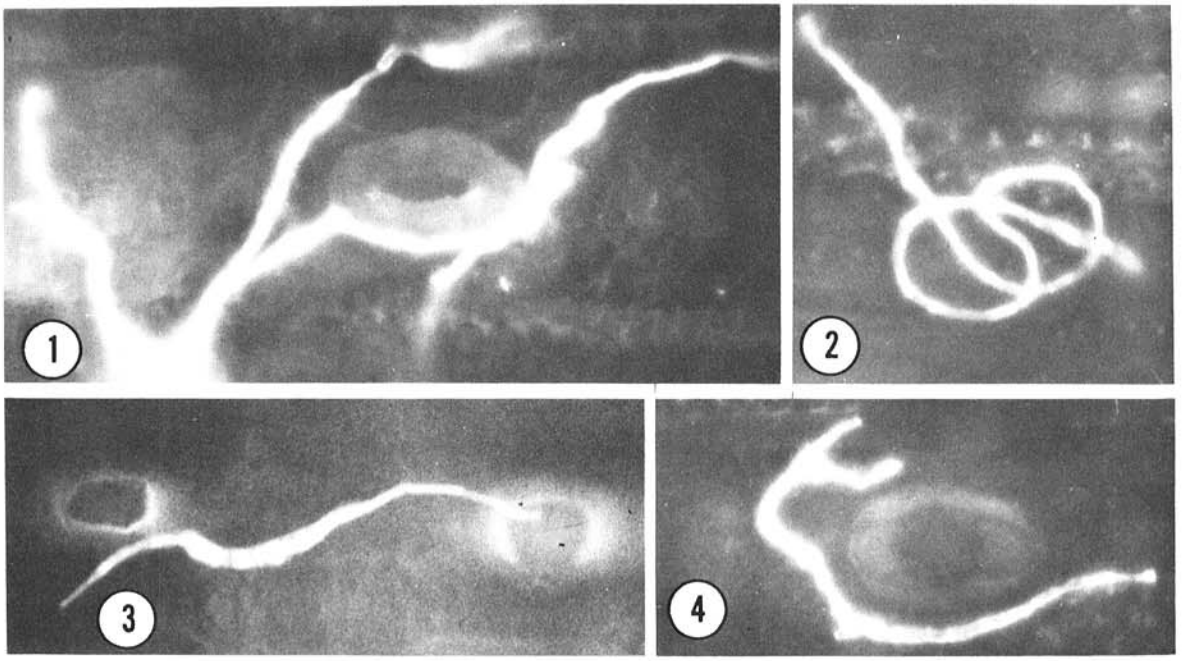

Fig. 1-4. Fluorescence photomicrographs of germinated conidia of *Scirrhia acicola* on needles of Scotch pine. **1)** More or less random growth of germ tubes from several conidia, with no positive attraction evidenced by the stoma. **2)** Typical coiled or looping growth of a single germ tube from a terminal cell of a conidium. **3)** Penetration of a stoma by one germ tube of a conidium and growth of the germ tube from the other terminal cell of the conidium away from a second stoma. **4)** Random growth of a germ tube beside a stoma.

Xenon XBO 150 W/1 lamp was passed through a UG-1 excitation filter, and fluorescence was observed through a K430 or K460 barrier filter. Black and white photographs were taken on Kodak Panatomic-X film.

RESULTS.—The amount of spore germination encountered on needles and even on water agar, malt agar, or PDA has been frustratingly variable. On agar plates, germination checks for a large number of inoculation trials have generally ranged from about 20% to almost 50% in 48 hours at about 20-24 C. In a few instances, however, germination of up to 90% occurred on agar plates. On pine needles, however, often no germination at all was observed, and in other trials germination ranged from about 2-30%. In one more recent trial, however, germination on needles of six inoculated trees ranged from 51-89% under essentially the same conditions as had been used numerous times before.

The type of germination was essentially the same on pine needles as on agar. No differences were found in spore germination and germ tube behavior between the two species of pine and among the three cultivars of Scotch pine. Usually the first, and often the only, germ tube grew from a terminal cell. From some three- or four-celled conidia, however, a germ tube grew from every cell. Germ tube growth was often extensive and germ tubes of up to 280 μm in length were measured on the needle surface. For the most part, germ tubes grew in what seemed to be a nondirected manner, sometimes generally parallel to the epidermal ridges and stomatal rows, sometimes at more or less right angles to these, or irregularly in a more or less random or haphazard manner (Fig. 1). Often germ tubes formed large coils or loops similar to those commonly formed by spores that germinated on water agar (Fig. 2). Sometimes spores presented a combination of spirally-curving or looping growth with later random growth over the needle surface. Germ tubes were narrow, septate, straight to gently curving, or sometimes sharply coiled.

Penetration of the outer stomatal pit by a germ tube was fairly common (Fig. 3). Where this did occur the more-or-less straight growth of a germ tube directly to a stoma suggested there might have been some positive attraction of the germ tube to the stoma. Much more often, however, germ tubes grew immediately beside, around, or even across, a stoma without entering (Fig. 4). In still other instances, germ tubes were seen to have grown in one direction, usually either at right angles or parallel to the epidermal ridges, often past one stoma, and then to have sharply changed direction before growing directly toward and into a stoma.

Although spore germination is the first step in the infection process, infection does not always follow spore germination. Most of the inoculated trees used for observations of spore germination behavior have never developed symptoms. Many of the observations described above were made on seedlings that did subsequently develop symptoms, however, and no differences have been observed in the manner of spore germination between trees on which symptoms did or did not develop. In one experiment, symptoms developed on a young seedling 17 days after inoculation and continued to appear for several weeks.

DISCUSSION.—A consistently successful and reliable technique for artificially inoculating pines with *S. acicola* has not been reported. As interest in disease resistance for control increases, more attention is being directed toward expanding our knowledge of the infection process, conditions influencing infection, and the nature of disease resistance.

Infection by this fungus apparently takes place through the stomata as indicated by direct observation of germinated spores on needles in this study, and as indicated by plastic film impressions made by Snow (12), and examinations of stripped epidermal segments (6). Although Wolf and Barbour (13) stated that the mycelium was first localized in the substomatal chambers, penetration of hyphae through the stomata was not reported.

The orientation of fungal germ tubes to stomata is of interest in relation to stomatal penetration. Parris and Killebrew (6) reported that, although the germ tube is not at first particularly oriented towards stomata of loblolly pine needles, it does occur later (4, and Abstracts, Southwide Forest Disease Workshop, 1970). Peterson (8) reported that for a similar and related fungus, *Dothistroma pini* Hulbary, more than 80% of the germ tubes were positively directed toward stomata of Austrian (*P. nigra* Arnold) and ponderosa (*P. ponderosa* Laws.) pines, and that they often changed their direction of growth toward stomata, with changes of 90° common. In contrast, however, Ivory (3) reported for his variety of the fungus [*D. septosporum* var. *keniense* (Ivory) Morelet] that growth of germ tubes on needles of Monterey pine (*P. radiata* D. Don) was random on needle surfaces and within stomatal pits. This report also confirmed Gadgil's (1) observations on random growth of hyphae of *D. pini* [= *D. septosporum* var. *pini* (Hulbary) Morelet] on Monterey pine needles. In the present work, the occasional entries of germ tubes into stomata might be interpreted, and were at first, as chance or random occurrences. The generally straight and undeviating growth of a germ tube toward and into an individual stoma, however, suggested some sort of stomatal attraction. These observations suggest that there may be some sort of stimulus that attracts a germ tube to a given stoma, but that this is on an individual stoma basis and not a general phenomenon expressed by all (or even most) of the stomata on a needle. Thus, somewhat conflicting observations have been reported regarding orientation of germ tube growth for both the brown spot fungus and its close relative, *D. pini*. The phenomenon is an important consideration because efficiency of a fungus that infects through stomata might well be involved. It seems possible that whether or not positive attraction to a stoma occurs might be influenced by micro-environmental factors on the needle surface or within the needle at the time germination occurs. If such factors can be identified, we will have a better understanding of the infection process and, consequently, greater possibilities for disease control.

LITERATURE CITED

1. GADGIL, P. D. 1967. Infection of Pinus radiata needles by Dothistroma pini. N. Z. J. Bot. 5:498-503.
2. HEDGCOCK, G. G. 1929. Septoria acicola and the brown-spot disease of pine needles. Phytopathology 19:993-999.
3. IVORY, M. H. 1972. Infection of Pinus radiata foliage by Scirrhia pini. Trans. Br. Mycol. Soc. 59:365-375.
4. KILLEBREW, J. F. 1968. The fungal flora of the loblolly pine needle and its relation to infection by the brown spot pathogen, Scirrhia acicola (Dearn.) Siggers. M.S. Thesis, Mississippi State Univ. 61 p.
5. NICHOLLS, T. H., D. D. SKILLING, and G. W. HUDLER. 1973. Scirrhia acicola in Scotch pine Christmas tree plantations. Plant Dis. Rep. 57:55-59.
6. PARRIS, G. K., and J. F. KILLEBREW. 1969. Germination and entrance of the brown spot disease fungus into the loblolly pine needle, and the possible relationships of associated extraneous fungi to infection. Phytopathology 59:117 (Abstr.).
7. PATTON, R. F., and D. W. JOHNSON. 1970. Mode of penetration of needles of eastern white pine by Cronartium ribicola. Phytopathology 60:977-982.
8. PETERSON, G. W. 1969. Growth of germ tubes of Dothistroma pini conidia positively directed toward stomata of Austrian and ponderosa pine needles. Phytopathology 59:1044 (Abstr.).
9. PREY, A. J., and F. S. MORSE. 1971. Brown spot needle blight of Scotch pine Christmas trees in Wisconsin. Plant Dis. Rep. 55:648-649.
10. SIGGERS, P. V. 1932. The brown-spot needle blight of longleaf pine seedlings. J. For. 30:579-593.
11. SIGGERS, P. V. 1944. The brown spot needle blight of pine seedlings. U.S. Dep. Agric. Tech. Bull. 870. 36 p.
12. SNOW, G. A. 1961. Artifical inoculation of longleaf pine with Scirrhia acicola. Phytopathology 51:186-188.
13. WOLF, F. A., and W. J. BARBOUR. 1941. Brown-spot needle disease of pines. Phytopathology 31:61-74.