Mode of Infection and Early Colonization of Slash Pine Seedlings by *Cronartium quercuum* f. sp. *fusiforme*

Thomas Miller, Robert F. Patton, and Harry R. Powers, Jr.

The first and third authors are plant pathologists, USDA-Forest Service, School of Forest Resources and Conservation, University of Florida, Gainesville 32611 and Forestry Sciences Laboratory, Athens, GA 30602. The second author is professor of Plant Pathology and Forestry, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Research support for R. F. Patton was by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by National Science Foundation Grant GB-3297.

Mention of a trade name is solely to identify materials used and does not constitute endorsement by the U.S. Department of Agriculture.

Accepted for publication 9 June 1980.

ABSTRACT

MILLER, T., R. F. PATTON, and H. R. POWERS, Jr. 1980. Mode of infection and early colonization of slash pine seedlings by *Cronartium quercuum* f. sp. *fusiforme*. Phytopathology 70:1206-1208.

After artificial inoculation of slash pine seedlings with basidiospores of *Cronartium quercuum* f. sp. *fusiforme*, the fungus produced an infection peg which penetrated through the cuticle and cell walls of the epidermal cells of hypocotyls, cotyledons, stems, and primary and secondary needles. In some instances an appressorium was formed. Within infected cells, the fungus developed a vesiclelike structure, or an irregularly oblong primary hypha usually single-celled, but occasionally several-septate. From these structures, a hypha penetrated the cell wall and developed a largely intercellular mycelium, from which typical haustoria were produced in some cells.

Additional key words: fusiform rust, *Pinus elliottii* var. *elliottii*.

The development of slash pines (*Pinus elliottii* Engelm. var. *elliottii*) resistant to fusiform rust, which is caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, is a basic objective of forest disease and tree improvement research in the southeastern USA. Although varying levels of resistance have been demonstrated in certain half-sib families through progeny testing and artificial inoculation of seedlings, the nature of the genetic control of this resistance has not been determined. One of the initial steps in investigating resistance is to determine the mode of penetration of the fungus into susceptible organs of slash pine. Although the anatomical responses of resistant and susceptible slash pine seedlings to infection by *C. quercuum* f. sp. *fusiforme* and the characteristics of gall development have been described (3–5,7,8), the early events during basidiospore germination and penetration of slash pine tissue have received only scant attention (7,10).

This report describes the mode of penetration of hypocotyls, cotyledons, stems, and primary and secondary needles by *C. quercuum* f. sp. *fusiforme*.

MATERIALS AND METHODS

In one series of experiments, spore-cast inoculations were initiated from telia-bearing northern red oak (*Quercus rubra* L.) leaves which were supported for 48 hr over 4- to 6-wk-old slash pine seedlings in an iceless refrigerator equipped with a humidifier and maintained at 20 C. One week after inoculation, cotyledons and primary needles were collected and soaked about 20 min in a fluorescent brightener solution which is a derivative of di-amino stilbene disulfonic acid formulated as a 12% solution in 42% aqueous Cellosolve (The American Cyanamid Co., Bound Brook, NJ 08805) (11). The needles were cut into segments ~5 mm long, fixed in formalin-acetic acid-alcohol, embedded in paraffin and sectioned at 12 μm. Serial sections on slides were hydrated to 35% alcohol and placed in one of two mounting media—buffered glycerine or Elvanol which is semipermanent and has low fluorescence (12). Sections were examined by fluorescence, phase-contrast, and bright-field microscopy.

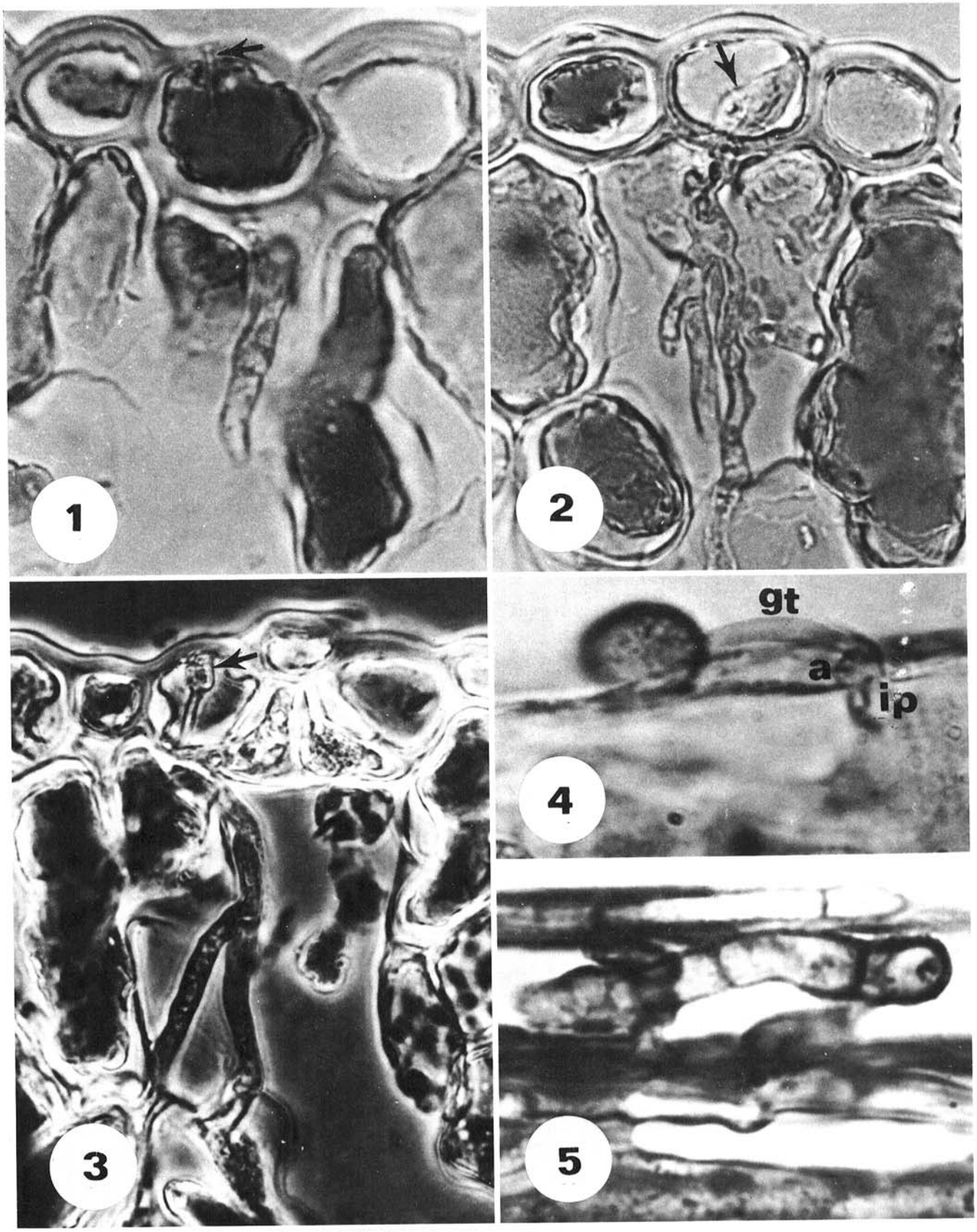
In a second experiment, more than 1,800 slash pine seedlings were inoculated by transferring precast basidiospores from Millipore filter disks onto specific locations on hypocotyls, cotyledons, stems, and primary and secondary needles (6). The inoculated organs were collected for histological examination at 1-wk intervals for 8 wk beginning 1 wk after inoculation. They were processed by standard histological techniques, serially-sectioned longitudinally at 15 μm, and stained with periodic acid-Schiff's reagent (2).

RESULTS

Infection after spore-cast inoculations. The fungus directly penetrated epidermal cells of cotyledons and primary needles. A fine infection peg or hypha from the appressed germ tube grew through the cuticle and epidermal cell wall (Fig. 1). Within the cell, the fungus developed a swollen, vesiclelike structure (Fig. 2) similar to that described by Allen (1) and Nusbaum (9) as a primary hypha in *Malva* sp. and *Althaea rosea* (L.) Car. infected with *Puccinia malvacearum* Bert. and in *Malus sylvestris* (L.) Mill. (*Pyrus malus* L.) infected with *Gymnosporangium juniperi-virginianae* Schw. Several sections were observed in which the germ tube was visible at the point of direct penetration of the epidermal cell wall. A number of similar penetrations were observed in which the germ tube apparently had broken away from the very thin penetration hypha. No evidence of an appressorium was seen on any of these sections. The fluorescent brightener was not visible in the infection peg or any other structure of the fungus subsequently developing within the cell or tissue, but these elements of the fungus were easily observed by phase-contrast microscopy. The subsidiary cells of the stomata seemed to be particularly susceptible to penetration by the fungus (Fig. 3). One such cell contained two separate infection vesicles, both of which could be traced to the needle surface by infection pegs through the cell wall.

In a few sections, fluorescent germ tubes had grown into stomatal antechambers, but no penetrations between the guard cells and into the mesophyll were observed.

The colonization of tissue adjoining the initially infected epidermal cells was initiated by a hypha that developed from the vesicle or primary hypha and penetrated the epidermal cell wall. It emerged into an intercellular space and developed a mycelium in



Figs. 1–5. Penetration and development of infection structures by *Cronartium quercuum* f. sp. *fusiforme* in needles and stems of slash pine seedlings. **1,** Infection peg penetrating epidermal cell wall (×1250). **2,** Vesiclelike structure in an epidermal cell (×1250). **3,** Vesiclelike structure in a subsidiary cell of a stoma (×1250). **4,** Germinated basidiospore, germ tube (gt), and appressorium (a) with an infection peg (ip) penetrating an epidermal cell wall (×1200). **5,** Septate primary hypha in an epidermal cell (×1200).

the mesophyll tissue. In cotyledonary or primary needle tissue that received the sporecast inoculations, the fungus branched. Hyphae were both inter- and intracellular and spread in all directions from the point of infection. In some mesophyll cells, and even in one section in a cell of the endodermis, typical haustoria were produced. Also, haustoria sometimes were produced in the cells of the epidermis from the developing mycelium in the mesophyll of needles and cotyledons.

Infection after direct application of precast basidiospores. On hypocotyls, cotyledons, and stems, the germ tubes from basidiospores grew a short distance along the surface of the different organs and penetrated directly by means of a narrow infection peg through a cell wall. In some instances, a well-defined appressorium formed at the point of penetration (Fig. 4). Penetration from other germ tubes occurred without the formation of a definite appressorium. In the latter case, however, there was usually a slight swelling of the germ tube near the point of penetration. In these sections also, no germ tubes were observed in the stomata of hypocotyls, cotyledons, or stems.

Although infected, no actual penetrations were observed in primary needles.

After penetration by the infection peg, an irregularly oblong primary hypha developed in the affected epidermal cell. In hypocotyls and stems, the primary hyphae usually were single-celled, but multi-septate specimens also were observed (Fig. 5). In cotyledons, the primary hyphae usually were smaller than those observed in hypocotyls and stems and occasionally they were branched. Hyphal branches developed from the primary hyphae. They penetrated the interior walls of the affected epidermal cells and spread into the underlying tissues as described above. Descriptions of the subsequent patterns of colonization by the fungus in the different organs are published (7).

On secondary needles, direct penetration of epidermal cells also was observed, but usually it occurred in the specialized cells associated with the stomata. Such penetrations resulted from basidiospores in the stomatal antechambers. Infection pegs from the germ tubes of basidiospores penetrated directly into subsidiary cells or the guard cells. Subsequent development in the infected cells and colonization of the needles were then similar to the invasion of primary needles and other tissues as described above.

DISCUSSION

The mode of infection of slash pine by *C. quercuum* f. sp. *fusiforme* was by direct penetration through the cell walls of all organs in which penetrations were observed. No evidence was seen of indirect penetration through the stomata and between the guard cells, as occurs in the infection of white pine needles by *Cronartium ribicola* J. C. Fisher ex Rabh. (11). The vesiclelike primary hyphae seen in the epidermal cells infected by *C. quercuum* f. sp. *fusiforme*, however, are similar to the substomatal vesicles formed by *C. ribicola*.

The inoculation of secondary needles with precast basidiospores produced the first report of infection of secondary needles by *C. quercuum* f. sp. *fusiforme* (7). The inoculation technique used may have forced basidiospores into the stomata which would not have occurred under natural conditions. However, the opening between the subsidiary cells and the depth of the stomatal antechamber is large enough to permit passage of basidiospores, unless their entry is prevented by physical forces operative at and around the stomata. The openings of the stomatal pores at the surface of the needles generally were circular to elliptical with average dimensions of 21.7 × 16.9 μm. The average depth of the stomatal antechambers, from the lip of the subsidiary cells to the guard cells, was 16.5 μm. The basidiospores of *C. quercuum* f. sp. *fusiforme* average about 6 × 10 μm. It remains to be determined whether secondary needles can be infected in nature.

Since resistance to infection and disease development is a major consideration in the development of long-term control of both the fusiform rust of southern pines and the white pine blister rust, it is interesting that the closely-related fungal pathogens that cause these diseases exhibit dissimilar modes of infection. The knowledge of the sequence of events involved in penetration and infection of slash pines by *C. quercuum* f. sp. *fusiforme* is indispensable basic information required for any investigation of the mechanisms of resistance and for the identification of slash pines with increased levels of resistance.

LITERATURE CITED

1. ALLEN, R. F. 1935. A cytological study of *Puccinia malvacearum* from the sporidium to the teliospore. J. Agric. Res. 51:801-818.
2. FARRIS, S. H. 1966. A staining method for mycelium of *Rhabdocline* in Douglas-fir needles. Can. J. Bot. 44:1106-1107.
3. JEWELL, F. F., R. P. TRUE, and S. L. MALLETT. 1962. Histology of *Cronartium fusiforme* in slash pine seedlings. Phytopathology 52:850-858.
4. JEWELL, F. F., and G. A. SNOW. 1972. Anatomical resistance to gall-rust infections in slash pine. Plant Dis. Rep. 56:531-534.
5. JEWELL, F. F. 1975. Characteristics of *Cronartium fusiforme* in slash pine cotyledons. Plant Dis. Rep. 59:407-410.
6. MILLER, T. 1970. Inoculation of slash pine seedlings with stored basidiospores of *Cronartium fusiforme*. Phytopathology 60:1773-1774.
7. MILLER, T., and E. B. COWLING. 1977. Infection and colonization of different organs of slash pine seedlings by *Cronartium fusiforme*. Phytopathology 67:179-186.
8. MILLER, T., E. B. COWLING, H. R. POWERS, Jr., and T. E. BLALOCK. 1976. Types of resistance and compatibility in slash pine seedlings infected by *Cronartium fusiforme*. Phytopathology 66:1229-1235.
9. NUSBAUM, C. J. 1935. A cytological study of the resistance of apple varieties to *Gymnosporangium juniperi-virginianae*. J. Agric. Res. 51:573-596.
10. PATTON, R. F., and D. W. JOHNSON. 1966. Penetration of pine needles by *Cronartium* basidiospores labeled with a fluorescent brightener. (Abstr.) Phytopathology 56:894.
11. PATTON, R. F., and D. W. JOHNSON. 1970. Mode of penetration of needles of eastern white pine by *Cronartium ribicola*. Phytopathology 60:977-982.
12. RODRIGUEZ, J., and R. DEINHARDT. 1960. Preparation of a semipermanent mounting medium for fluorescent antibody studies. Virology 12:316-317.