

Parasitic Aspects of a Fairy Ring Fungus, *Marasmius oreades*

T. H. Filer, Jr.

Former Research Assistant, Department of Plant Pathology, Washington State University, Pullman; now Plant Pathologist, Southern Hardwoods Laboratory, Southern Forest Experiment Station, Forest Service, USDA, Stoneville, Mississippi.

Portion of a Ph.D. thesis, Washington State University.

Scientific Paper No. 2636, Washington Agricultural Experiment Stations, Pullman, Project No. 1322.

Accepted for publication 2 April 1965.

ABSTRACT

Marasmius oreades parasitizes *Poa pratensis*, *Festuca rubra*, and *Agrostis tenuis*. The fungus penetrates the root directly in all three species and does

not require natural openings or wounds. The mycelium ramifies in the cortical cells and destroys the cell contents.

Many hymenomycetous fungi are associated with the disease "Fairy Ring" of turfgrasses. *Marasmius oreades* (Bolt. ex Fr.) Fr. is one of the more common species. Withering (6) was the first to associate this fungus with the disease and to report that it killed grass. Ballion (1) and Bayliss (2, 3) attributed the death of vegetation to parasitism by the fungus. Shantz and Piemeisel (5) reported that death of vegetation was due to the impermeable nature of the infested soil resulting from physical changes brought about by the fungus. They suggested that the parasitic attack was secondary. Since controversies still exist as to the parasitic nature of the fungus, the purpose of this investigation was to study the possible parasitism of the fungus on three turfgrass species.

Examination of hand and microtome sections from grass roots growing in *M. oreades*-infested soil was made to determine if the fungus could be observed. Although it was possible to demonstrate the presence of hyphae in the roots of field-grown grass species, it was impossible to prove by this method that the hyphae present were produced by this fungus.

The pathological histology of the host was studied in the laboratory using surface-sterilized seedlings in vitro with pure cultures of *M. oreades*.

MATERIALS AND METHODS.—Four isolates of *Marasmius oreades* were used through this study. Isolate 1 was obtained from sporophores collected near Tacoma, Wash., by C. J. Gould, Western Washington Experiment Station, Puyallup.

Of approximately 200 isolations made from sporophores of *M. oreades* collected in Everett, Olympia, Seattle, and Tacoma, Wash., only two were retained (isolates 2 and 3). These two were cultured from stipes and differed in their growth rates and cultural characteristics. Isolates 1 and 3 were similar; 75% of the isolations made from sporophores in Washington resembled isolates 1 and 3.

Isolate 4, cultured from a stipe, was obtained from A. H. McCain, University of California, Berkeley. The California isolate was similar to isolate 2.

Mycelium of *M. oreades* is snow white. The culture characteristics varied, depending on the particular isolate involved and the media used. Generally, cultures of isolates 1 and 3 showed a white, cottony appearance caused by numerous aerial hyphae, whereas isolates 2 and 4 showed a white, resupinate growth with sparse aerial mycelium. At 25 C the California isolate grew

approximately four times as fast as the Washington isolates.

Host species used in this study were *Poa pratensis* L., *Festuca rubra* L., and *Agrostis tenuis* Sibth. Seeds were surface sterilized with 50% ethyl alcohol for 30 sec, followed by soaking in 5.28% sodium hypochlorite (Clorox) for 10 min, and then placed on moist filter papers in sterilized petri plates. Nine days after germination, five seedlings were transferred to each of 225 test tubes (29 × 200 mm) containing 6 g of Perlite saturated with 20 ml of modified Murray's medium as described by Zscheile (7).

Forty-eight hr later the tubes were inoculated with 9-mm discs of inoculum taken from petri plate cultures of *M. oreades*. Three tubes of each grass species were inoculated with each fungus isolate.

Initial studies of seedlings inoculated in vitro had indicated that the time from inoculation to infection was less than 7 days. Starting 2 days after inoculations, a set of tubes containing each host-fungus combination was removed at 24-hr intervals for 5 days. A sample of each root was used to reisolate the fungus and another portion of the same root was retained for sectioning. Root segments, 0.5 cm long, were fixed and killed in formalin, acetic acid, and ethyl alcohol solution. The material was taken through a tertiary butyl alcohol series and embedded in paraffin as described by Sass (4).

Longitudinal and cross sections of embedded material were cut 8 μ thick with a rotary microtome. The best differential staining was obtained with the safranin-fast green combination (4).

In addition to microtome sections, freehand sections were made of different-aged seedlings grown in the laboratory and grass plants taken from a fairy ring. Freehand sections were made for microscopic examination of large quantities of roots suspected of having mycelia present.

RESULTS.—All isolates of *M. oreades* were reisolated from roots of *P. pratensis*, *F. rubra*, and *A. tenuis*. Microtome sections showed hyphae in the grass roots 3 days after the inoculum was placed in the tubes adjacent to the roots. The time required for the fungus to invade the roots was limited by the rate of growth of the fungus which was 1.5 mm/24 hr.

After 30 days, hyphae were found in all tissues of the roots except the phloem and xylem. The hyphae ramified through cells with no consistent pattern of growth

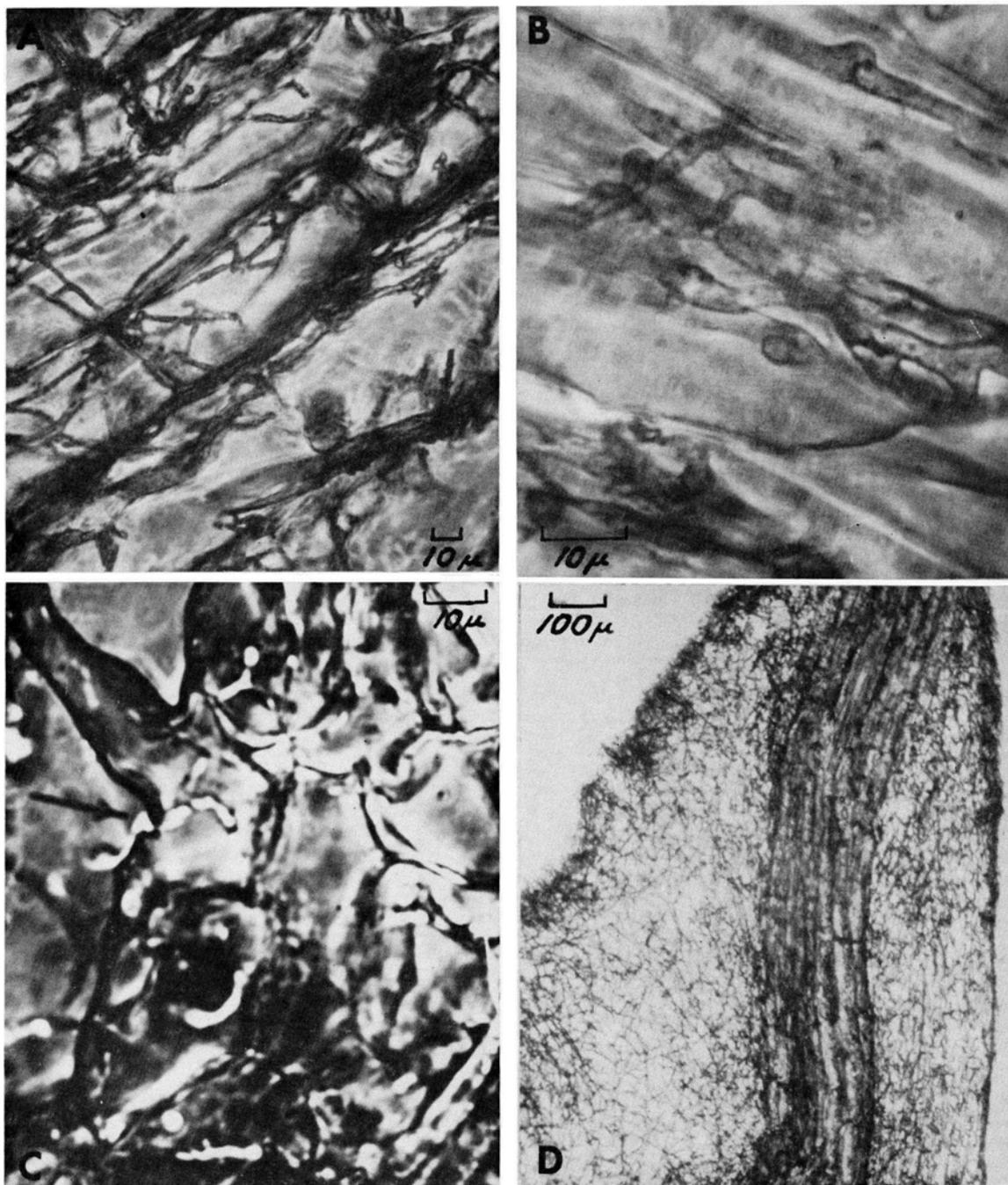


Fig. 1. **A)** The hyphae of *Marasmius oreades* (isolate 3) in cortical tissue of *Agrostis tenuis*. **B)** Clamp connections on hyphae of *Marasmius oreades* (isolate 4) in cortical tissue of *Poa pratensis*. **C)** Penetration of an epidermal cell near a root hair of *Agrostis tenuis* by hypha of *Marasmius oreades* (isolate 4). **D)** Mantle of mycelium covering root of *Agrostis tenuis* 3 months after inoculation.

(Fig. 1-A). Hyphal morphology changed somewhat after hyphae entered plant tissue. The hyphae increased in diameter and branched more than hyphae grown on artificial media. The diameter of the hyphae ranged from 1.6 to 3.2 μ . Although clamp connections were observed in the plant tissues (Fig. 1-B), they were produced less frequently in the host than on artificial media.

M. oreades can penetrate the roots of *Agrostis*, *Festuca*, and *Poa* directly, and does not require a natural opening or wound (Fig. 1-C). In sections of infected roots, hyphae were observed entering the epidermal cells directly.

In roots of all three grass species, the hyphae were found penetrating cells in which the nuclei appeared intact. In contrast, the nucleus of the host cell from

which the hyphae subsequently emerged was disrupted.

The roots of grass taken from infested field soil contained basidiomycetous hyphae that were similar to those of *M. oreades* observed in roots of grass seedlings grown in vitro. In the field or laboratory, the fungus formed a dense mantle of mycelium on the outside of the root as shown in Fig. 1-D.

DISCUSSION.—Grass seedlings grown in large test tubes with pure cultures of the fungus showed fungal infection in the roots. The seedlings received sufficient moisture to eliminate the possibility that moisture deficiency was necessary for fungal infection. Roots of the seedlings were killed or severely damaged by the fungus.

Comparison of the staining characteristics of sectioned healthy and diseased root tissue indicates that the fungus can penetrate living cells. Sections of inoculated grass roots showed that mycelium had penetrated living cells, and judging by their staining properties, invaded cells were apparently killed by the fungus.

Microtome sections of infected grass roots growing in fairy rings in the field combined with supporting

evidence from microtome sections of grass roots grown with the fungus in vitro showed that the fungus had penetrated the roots. The mycelium ramified in the cortical cells and destroyed the cell contents. By comparing healthy with diseased roots, it appeared that the fungus induced increased branching of roots, but the new roots were also destroyed.

LITERATURE CITED

1. BALLION, P. 1906. Recherches sur les cercles mycéliens (ronds de fees). Actes Soc. Linn. Bordeaux 61:62-88.
2. BAYLISS, J. S. 1911. Observations on *Marasmius oreades* and *Clitocybe gigantea*, as parasitic fungi causing "fairy ring". J. Econ. Biol. 6:111-132.
3. BAYLISS, J. S. 1926. Concerning "Fairy rings" in pastures. Ann. Appl. Biol. 13:277-288.
4. SASS, J. E. 1958. Botanical microtechnique. 3rd ed. Iowa State Coll. Press, Ames. 228 p.
5. SHANTZ, H., and R. PIEMEISEL. 1917. Fungus fairy rings in eastern Colorado and their effect on vegetation. J. Agr. Res. 11:191-246.
6. WITHERING, W. 1796. An arrangement of British plants. Vol. 4. 3rd ed. G. G. and J. Robinson, London.
7. ZSCHELE, F. P. 1951. Nutrient studies with the wheat bunt fungus *Tilletia caries*. Phytopathology 41:1115-1124.