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The Potential of Chemicals and Pythium Species to Control Marasmius Fairy Ring

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THE POTENTIAL OF CHEMICALS AND PYTHIUM SPECIES
TO CONTROL MARASMIUS FAIRY RING

by

Peter Bent Adams

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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IN
BIOLOGICAL SCIENCES

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DOCTOR OF PHILOSOPHY THESIS

OF

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1964

ABSTRACT

Fairy rings caused by Marasmius oreades Fr. and other fungi cause considerable damage to turfgrasses. The recommended control measures for this disease are either soil fumigation or digging up the affected turf and replacing it with noninfested soil. Both methods require subsequent re-seeding or resodding of the area. Results of two years' experimentation indicate that chemical control of fairy ring disease is not practical at the present. Hence, the possibilities of biological control were explored.

This investigation has shown that Pythium artotrogus and other Pythium species will inhibit the mycelial growth of M. oreades and Agaricus bisporus. Pythium artotrogus was more antagonistic to A. bisporus than was other Pythium species tested. Pythium ultimum and an isolate of P. irregulare were more antagonistic to M. oreades than was P. artotrogus. Inhibition of M. oreades mycelium was shown to be due to toxic metabolites of P. artotrogus which diffuse through an agar gel. Fresh culture filtrates of P. artotrogus completely inhibited the growth of the agaric hyphal tips within twenty minutes. The biological activity of the filtrate was not reduced when kept at 7° C for prolonged periods, but it was shown to be thermolabile.

Studies on the effect of temperature on mycelial growth showed that M. oreades and P. artotrogus had the same minimum temperature requirement. The optimum temperature for growth

was about 27° C for both fungi. Growth of P. artotrogus occurred at higher temperatures than M. oreades. At all temperatures P. artotrogus had a growth rate of two to ten times that of M. oreades.

Marasmius oreades grew over a pH range of about 3.0 to 7.5 with an optimum of about 5.5. Growth of P. artotrogus occurred from pH 2.5 to above 7.7 with an optimum at 5.5.

Pythium artotrogus was not parasitic to A. bisporus or M. oreades. However, this Pythium species was shown to parasitize the hyphae of P. aphanidermatum, P. debaryanum, P. irregulare, P. splendens and P. ultimum. When encountering susceptible hyphae, the hyphal tip of P. artotrogus coiled around the host hypha followed by the formation of an appressorium on the hyphal surface of the host. Following penetration, the infection hypha grew lengthwise in that of the host, degenerating the cellular contents as it advanced. Although not parasitic, the hyphae of P. artotrogus formed coils around the hyphae of P. arrhenomanes. Pythium artotrogus did not form coils and was not parasitic in its own or in the hyphae of P. graminicolum.

Of the many Pythium species that were shown to be antagonistic to A. bisporus and M. oreades, only P. artotrogus was shown to cause no injury to four species of Agrostis in laboratory pathogenicity tests.

The data indicate that P. artotrogus has the potential to be used as a biological control agent for fairy ring caused by M. oreades. However, since it was shown that

P. artotrogus would not grow among the mycelial mass of either A. bisporus or M. oreades, the antagonistic Pythium species will have to be applied to the turf on the outer edge and in advance of the fairy ring if it is to be a successful biological control agent.

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Marasmius fairy ring in bluegrass turf

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I. INTRODUCTION

Fairy ring is a disease which causes a considerable amount of damage to various grasses throughout the world. Any of a number of fungi may cause the injury, but Marasmius oreades Fr. is the most common one producing the disease in lawns and golf greens. The "ring" increases in size each year, thereby increasing the area of damaged turf. Fairy rings have been reported to be the most commonly encountered disease of lawn turf in North America. Marasmius fairy ring has been ranked as the sixth most severe disease of lawn grasses (17).

Control of this disease is usually difficult and expensive. Methods of control that involve the application of fungicides by drenching them into the turf have not proved to be satisfactory (3). Recommended control methods are either fumigation of the soil which also kills the vegetation, or the removal of the affected turf and replacing it with uncontaminated soil. After use of either method, reseeding or resodding of the area is required.

Recently Sinden and Schisler isolated a species of Pythium in Pennsylvania inhibitive for the growth and development of the commercial mushroom, Agaricus bisporus (Lange) Singer. The same fungus was later isolated by Sinden from mushroom beds in the Netherlands and England indicating that it is rather widespread in its occurrence in association with the commercial mushroom. The fungus was identified as Pythium artotrogus (Mont.) dBy. by Fergus (15). Species of

Pythium usually are not considered to be very antagonistic towards other fungi. However, several Pythium species have been shown to be parasitic on other Pythium species (13,14).

Agaricus bisporus (synonyms A. campestris Fr. and Psalliota campestris (L.) Fr.) has been reported as the cause of fairy ring in turf. Since Pythium artotrogus has been shown to inhibit the growth of this fungus in mushroom compost beds, it was decided to determine whether this Pythium species could be used for biological control of the fairy ring disease of turfgrasses. Marasmius oreades was selected for this study to represent a typical fairy ring fungus, since it is commonly encountered in turfgrass areas in Rhode Island. In order to determine whether P. artotrogus has the potential to be used for fairy ring control, the nature of the antagonism must first be determined. To accomplish this, certain experimentation was required. A study of the effect of temperature and hydrogen-ion concentration on the growth of both P. artotrogus and M. oreades was programmed. Experiments to determine the degree and type of antagonism exhibited by P. artotrogus towards A. bisporus, M. oreades and other Pythium species were designed. Pathogenicity tests on grass seeds and seedlings were planned with P. artotrogus and congeneric forms.

II. REVIEW OF LITERATURE

Fungus fairy rings are world-wide in distribution and may be found in lawns, golf courses, sports fields, pastures and other grass habitats. They are caused by a number of different basidiomycetes and a few ascomycetes. Couch (12) lists twenty genera of fungi, while Shantz and Piemeisel (31) list twenty-four different genera that cause fairy rings. Since they are such diverse fungi, they are found in association with nearly all the common grasses, especially those used for lawns and pastures.

The economic importance of this disease is very difficult to assess. People most concerned with controlling the disease, however, are those interested in maintaining good quality turf for recreational purposes. Gould (17) recently conducted a survey in which turfgrass pathologists from thirty localities throughout North America reported on the severity of various turfgrass diseases. The severity of the twenty-four diseases reported were separated into two categories on the basis of grasses maintained as lawns and those maintained as golf greens. In lawn turf, fairy ring was ranked as more severe than any other disease, while that caused by Marasmius spp. rated as sixth in severity. However, in golf putting green turf fairy ring was indexed as fourth and the Marasmius fairy ring was ranked eighth in severity.

The fairy ring syndrome is commonly characterized by a circular band of grass that is greener and faster growing

than the surrounding grass. Mushrooms or puffballs are frequently observed either in or at the advancing edge of the green ring. The diameter of the fairy ring varies greatly from a few to over two hundred feet. On the average, however, they range from three to twelve feet and increase in diameter each year.

Shantz and Piemeisel (31) classified fairy rings into the following three basic categories: type I, rings marked by a zone of dead grass; type II, rings marked only by a zone of stimulated grass; and type III, rings which produce no noticeable effect on the grass. In each of the three categories sporophores of the fungus may be present or absent depending on the season of the year and environmental factors. Marasmius oreades fairy ring belongs in the type I category.

A Marasmius oreades fairy ring in cross section, as drawn by Molliard (27), is illustrated in Figure 1. Zone I represents the central area inside the ring. Zone II represents an inner ring of stimulated grass that is greener than the grass in zone I. A band of dead grass is represented by zone III, in which the basidiocarps are produced. Zone IV represents an outer band of stimulated grass. The degree of stimulation is greater and the grass is greener in zone IV than in zone II. Zone V represents the surrounding unaffected normal grass. Molliard stated that in the spring and early summer, zones II, III and IV are a single wide ring of stimulated grass and that zone III develops in late summer and early fall. The mycelium of the fungus was found primarily

Figure 1. Cross section diagram of a fairy ring caused by Marasmius oreades. I. Center of fairy ring. II. Area of stimulated grass. III. Area of dead grass. IV. Area of stimulated grass. P - zone of low (7%) moisture, S - zone of higher (21%) moisture. V. Unaffected grass outside of fairy ring. Redrawn from Molliard (27).

in zones II, III and IV with the youngest mycelium in zone IV.

The stimulation of the grass was once thought to be due to atmospheric nitrogen being assimilated by the fungus and then released to the soil. Lawes et al. (22) showed that the fungus was able to utilize organic nitrogen from the soil and subsequently release "manure" for the grass. Bayliss-Elliott (5) stated that the luxurious growth in zone II was due to nitrogen release by decomposing plant roots and fungus mycelium. It was suggested that the grass in zone IV was stimulated as a result of the "...action of the fungus when it begins its attack on the grass," (5).

Albrecht et al. (2) have shown that the total nitrogen content of the soil in M. oreades fairy rings is two to three times greater than the nitrogen content of the surrounding soil. These authors also showed that the amino acid content of the soil was generally greater in fairy rings than in normal soil. They found that the ratio of a given amino acid between the two regions varied with the season of the year. The greatest difference occurred during the summer. In studies conducted with M. oreades grown on straw, Lindeberg (24) showed that the fungus would decompose 63 per cent of the lignin, 91 per cent of the cellulose and 83 per cent of the surfural-yielding substances of the substrate.

According to Molliard (27), wilting and death of the grass in zone III is due to a combination of a lack of water and an accumulation of ammonium compounds in the soil. In

the M. Oreades fairy ring studied by Molliard (Figure 1) the moisture content of the soil in zones I, IV-S and V was 21 per cent, while the moisture content of the soil in zone III was 5 per cent, and in zone IV-P was 7 per cent. The highest concentration of ammonium compounds, 80 mg. per 100 g. of dry soil, was found in zone III, while the amount of ammonium compounds was about 38 mg. per 100 g. of dry soil in zones I and V. Molliard stated that roots weakened under these conditions were susceptible to invasion by the fungus.

Bayliss-Elliott (4) reported that soil taken below the dead zone causes the root tips of healthy grass plants to turn brown or black within twenty-four hours. This same toxic reaction occurred after using a weak extract of the basidiocarps. The active principle was lost, however, when heated to 100° C. Further evidence showed that an active peptonizing enzyme, peptase, which digests vegetable fibrin, and a peptolytic enzyme, ereptase, which digests Witte peptone, were produced by M. oreades. Having thus weakened the plant, the fungus was credited with invasion of the grass roots causing the root tips to become stunted. The mycelium penetrated all of the soft parenchymatous parts of the root, leaving only the axial stele uninvaded. When the plant was almost dead, the mycelium invaded the leaves. Plants killed in this manner were:

Agrostis stolonifera, bentgrass

Anthoxanthum odoratum, sweet vernalgrass

Avena flavescens, yellow oats

Dactylis glomerata, orchardgrass

Holcus lanatus, velvetgrass

Lolium perenne, English ryegrass

Plantago lanceolata, buckhorn

Poa pratensis, Kentucky bluegrass

Trifolium repens, white clover

In studies conducted by Shantz and Piemeisel (31) on fairy rings caused by Agaricus tabularis it was concluded that death of the vegetation was caused solely by the lack of soil water in the dead zone and not by parasitism or toxic secretions of the fungus. The soil beneath this zone was found to be extremely difficult to wet, even when the equivalent of two inches of rain was applied to the surface.

Two distinct hypotheses have been formulated to explain the death of vegetation in this dead zone. Shantz and Piemeisel (31) believed that death occurred solely as a result of the lack of water in zone III (Figure 1). Bayliss-Elliott (4) on the other hand believed that the grasses died primarily as a result of fungus pathogenesis. Molliard (27) took a somewhat neutral stand by stating that the dead zone was due to a combination of lack of water and an accumulation of ammonium salts.

More recently Lebeau and Hawn (23) have shown that mycelium of M. oreades when grown in liquid culture produces hydrogen cyanide. These authors also demonstrated that hydrogen cyanide is present in the dead grass zone of Marasmius fairy rings, but not in the soil surrounding the rings.

Like Molliard, these authors take a neutral stand by stating that : "Very small quantities of HCN probably affect the growing tips of young grass roots and may initiate the disease. This view does not detract from the hypothesis that in the final stage of pathogenesis the plants die from drought caused by such a heavy growth of the fungus in the soil and the water cannot penetrate to the grass roots."

A fairy ring usually originates from a spore or from mycelial fragments which have been deposited in a favorable location by wind, or in some cases, by mowing machines. After a suitable period of growth, and when other factors are favorable, the resulting mycelial mass will produce fruiting structures. The mycelium continues to grow in an outward direction each year. As the fairy ring gets larger, the mycelium in the center of the ring apparently dies. Attempts at isolation from this zone fail to yield the fungus causing the fairy ring. Furthermore, one seldom finds a fairy ring inside another fairy ring except when the outer ring has an extremely large diameter. This outward growth phenomenon has been explained as being due to the fungus exhausting the available nutrients within the substrate. Bayliss-Elliott (4,5) observed that when two Marasmius fairy rings met, the mycelia at the points of contact appeared to die. She also showed that media previously used by M. oreades would not support normal growth even when fortified with fresh nutrients. From this evidence she concluded that the fungus produced toxic metabolites in the substrate which prevented

further growth of the mycelium. This evidence was used to explain the outward growth of the fungus.

The rate of advance of Marasmius fairy ring has been shown by Bayliss-Elliott (4) to range from a minimum of three inches to a maximum of 13.5 inches per year.

Wilkins and Patrick (37) considered M. oreades as a summer mushroom which produced basidiocarps from May through October. The sporophores developed more abundantly on a clay soil having a pH of 5.8 to 6.3 than on a sandy soil where the pH was 4.6 to 5.0. They were never observed on a soil composed predominately of chalk with a pH of 6.8 to 7.1.

While studying a Marasmius fairy ring on grass heath soils in England, Warcup (36) observed that the mycelium of M. oreades was not visible to the naked eye as it was in lawns. The mycelium was restricted to a zone near the surface, not exceeding a depth of four inches and with a lateral distribution of six to twenty-four inches. He also reported (36) that there was a pronounced change in the fungal flora of the soil in which M. oreades was present. Species of Absidia, Fusarium, Gliomastix, Penicillium, Phoma, Mortierella, Stysanus and Trichoderma were commonly found in normal soil, but were absent or only rarely found in the mycelial zone of Marasmius oreades. Penicillium nigricans and Cephalosporium sp. were common to both soils. Although rarely found in normal soil, Gymnoascus subumbrinus and Arachniotus sp. were commonly found in soils inhabited by M. oreades. Other fungi found associated with this fairy

ring fungus were Chaetomium spp., Sordaria sp., Thielavia sp. and Byssochlamys nivea.

The control of fairy ring has proved to be quite difficult, even with the extremely potent fungicides that are currently available. After her study of M. oreades fairy ring, Bayliss-Elliott (5) stated that "...if the grass in front of an advancing ring is manured with ordinary stable manure the rings die out....." No other reference has been found in the literature concerning the use of manure for the control of fairy ring. However, Buller (7) has reported that a mixture of one part horse manure to one part cow manure was used successfully for the cultivation of mushrooms of M. oreades. These seeming conflicting reports raise doubt on the value of manure as a treatment for the control of fairy ring.

Shantz and Piemeisel (31) stated that when methods involving digging up the soil are not practicable, then the application of a fungicide, such as Bordeaux Mixture, to the soil just above and a little in advance of the ring, "should" be effective in controlling the disease. From observations made in Rhode Island, Howard et al. (21) stated that organic cadmium fungicides appear to inhibit the formation of some mushrooms. Dolomitic limestone or magnesium salts also have been found to inhibit the development of basidiocarps of the cultivated mushroom (21).

Couch (12) has reported that appreciable reduction in the damage caused by fairy ring may be obtained by the

suppression of fungal growth. This was done by pouring phenyl mercury acetate or cadmium-containing fungicides mixed with a wetting agent into small holes four inches deep. These holes were twelve inches apart. The area was then drenched with water equivalent to at least one inch of rain. In order to obtain complete control of this disease, however, eradication of the fungus was required. This may be accomplished by removal of the soil and turf to a depth twelve inches and eighteen inches on either side of the stimulated zone. Fumigation of the soil with either formaldehyde or methyl bromide will also eradicate the fungus.

Alexander (3) tried to control a fairy ring caused by a species of Lepiota by use of a wetting agent applied in one-inch holes eighteen inches deep and six to eight inches apart. This treatment was followed by a solution of bichloride of mercury. An examination of the treated fairy ring a year later revealed that the diameter of the ring had increased thirty-two to thirty-four inches. Complete control of the fairy ring was obtained only when the soil was removed to a depth of twenty-eight inches and at least twelve inches in advance of symptoms. The hole was then filled with uncontaminated soil and the surface resodded.

In laboratory studies with M. oreades Lebeau and Hawn (23) found that the antibiotics Acti-dione, Rimocidin, endomycin and griseofulvin at 10 ppm. all inhibited mycelial growth.

Recently Fergus et al. (15) reported isolating a fungus

in Pennsylvania and in the Netherlands which completely inhibited the growth of the cultivated mushroom Agaricus bisporus. The antagonistic fungus was identified as Pythium artotrogus. A preliminary attempt was made by the authors to determine the nature of this antagonism. Cylinders filled with compost were inoculated with A. bisporus at one end and with P. artotrogus at the other end. Once the mycelia of the two fungi met, both organisms stopped growing. Pythium artotrogus is one of the few Pythium species in which no asexual spore (sporangial) stage has been demonstrated. This fungus belongs to a group of Pythium species which are characterized by echinulate oogonia. It was originally described by Montagne (6) as a pathogen on potato tubers and given the name Artotrogus hydnosporus. Smith (33) considered the spiny structures of this Pythium as the sexual stage of the potato blight fungus Phytophthora (Peronospora) infestans.

In his monograph of the genus Pythium, Middleton (26) reported that P. artotrogus has been isolated from the following plants in the localities cited:

Ananas comosus Merr., pineapple, Hawaii

Apium graveolens L. var. dulce, celery, England

Arabis alpina L., rockcress, The Netherlands

Brassica oleracea L. var. botrytis, cauliflower, Germany

Cajanus cajan Millsp., pigeonpea, Hawaii

Canavalia ensiformis DC., jackbean, Hawaii

Ipomoea batatas Lam., sweet potato, Hawaii

Lycopersicon esculentum Mill., tomato, Hungary

Nymphaea candida Presl., waterlily, Bohemia

Panicum purpurascens Raddi, Para grass, Hawaii

Phaseolus vulgaris L., garden bean, Hawaii

Pinus banksiana Lamb., Norway pine, United States

P. nigra var. austriaca Aschers. and Graebn.,
Austrian pine, The Netherlands

P. ponderosa Dougl., western yellow pine, United States

P. resinosa Ait., red pine, United States

Pseudotsuga taxifolia Britt., Douglas-fir, The
Netherlands

Raphanus sativus L., garden radish, Sweden

Saccharum officinarum L., sugar cane, Hawaii

Solanum tuberosum L., potato, England, France, Germany,
India

Spinacia oleracea L., spinach, The Netherlands

Vigna sinensis L., cowpea, Hawaii

Viola tricolor L., pansy, Norway

Matthews (25) isolated P. artotrogus from water in South Carolina.

Although P. artotrogus has been isolated from the tissues of many plants, this does not necessarily mean that the fungus is a pathogen to all of them. Clinton (9) found P. artotrogus on grape, but considered it a secondary invader following infection by another pathogen. Pythium artotrogus was isolated by Hartley (18) from damped-off Pinus resinosa and P. banksiana. The isolate was considered to be a potential parasite on pine seedlings, but not of any general importance.

Pythium species have not been considered to be

particularly antagonistic to other fungi. However, there are reports in the literature by Drechsler (13,14) concerning the parasitic and antagonistic activity of three Pythium species on other congeneric forms. It has been shown that P. oligandrum parasitizes P. arrhenomanes, P. butleri, P. debaryanum, P. graminicolum, P. helicoides, P. irregulare, P. mammillatum, P. oedochilum, P. palingenes, P. salpingophorum, P. splendens and P. ultimum. Pythium acanthicum was reported to parasitize P. anandrum, P. marsipium and P. mastophorum, while P. periplocum parasitized P. anandrum and P. marsipium. It is interesting to note that these three parasitic Pythium species all produce echinulate oogonia.

Drechsler (13,14) observed that the hyphae of the parasitic P. oligandrum first coiled around the hyphae of the host followed by penetration. Once penetration had occurred, the parasite grew lengthwise through the mycelium of the host causing degeneration of the protoplasm. Occasionally this internal mycelium branched and pushed its way out through the host's hyphal wall.

Drechsler (14) also reported that de Bary in 1881 had consistently noticed P. artotrogus in association with P. debaryanum on garden cress seedlings and that the former could not be cultured separately. De Bary further found P. artotrogus associated with P. debaryanum, P. vexans and Phytophthora infestans on potato. De Bary was reported to have offered two alternative explanations for this repeated association: a) P. artotrogus might require substances for

growth that were absent in his media but are present when grown in association with P. debaryanum; or b) P. artotrogus may subsist as a parasite on the congeneric species. De Bary's observations were later confirmed by Butler in 1902.

Clinton (9) found P. hydnosporum (a synonym for P. artotrogus) in association with Phytophthora infestans in rotten potato tubers, and with Phytophthora cactorum causing injury to pea roots kept in water. On grapes that had been injured by the black rot fungus and the grape berry moth, Pythium artotrogus was observed growing in the fruit tissue, in the "pycnia" of the fungus and in the larvae of the insect.

According to Drechsler (14), Sawada in 1927 found P. artotrogus (synonym P. hydnosporum) in old plant tissue that was infected with Phytophthora cactorum. In the same year Dissman (14) was reported to have found Pythium artotrogus on waterlily along with other Pythium species.

Drechsler (14) stated that many of the workers cited above identified P. artotrogus chiefly on the basis of spiny oogonia. Middleton (26) lists fourteen species of Pythium with echinulate oogonia. Since Drechsler found three Pythium species with echinulate oogonia (P. oligandrum, P. acanthicum and P. periplocum) to be parasitic on other congeneric forms, it was thought that there may have been some confusion as to which Pythium species was actually present.

III. GENERAL MATERIALS AND METHODS

The isolate of Marasmius oreades Fr. used in this investigation was obtained in September, 1963, by the author from a basidiocarp in a fairy ring on an athletic field at the University of Rhode Island. At that time this particular field contained many fairy rings all of which were caused by M. oreades. The turf was composed primarily of Kentucky bluegrass. The pH of the soil varied from 4.7 to 5.0, while the layer of thatch varied in pH between 5.0 and 6.0.

The isolates of Agaricus bisporus (Lange) Singer and Pythium artotrogus (Mont.) dBy. were kindly supplied by Edith M. Sigel of the Butler County Mushroom Farm, West Winfield, Pennsylvania. Other Pythium species used in this study along with their source are listed below.

<u>Pythium</u> sp.	<u>Source</u>	<u>Supplied by</u>
<u>P. aphanidermatum</u> (Edson) Fitzp.	Ryegrass	H. D. Wells, Tifton, Georgia
<u>P. arrhenomanes</u> Drechs.	*NTCC	A. F. Schmitthenner, Wooster, Ohio
<u>P. debaryanum</u> Hesse	*NTCC	A. F. Schmitthenner, Wooster, Ohio
<u>P. graminicolum</u> Subrm.	Alfalfa	A. F. Schmitthenner, Wooster, Ohio
<u>P. irregulare</u> Buisman	Alfalfa	A. F. Schmitthenner, Wooster, Ohio
<u>P. irregulare</u> Buisman	Soil	H. W. Klemmer, Honolulu, Hawaii
<u>P. splendens</u> Braun	Pineapple	H. W. Klemmer, Honolulu, Hawaii
<u>P. ultimum</u> Trow	---	H. B. Couch, Univ. Park, Pennsylvania

*National Type Culture Collection.

Cultures of Agaricus bisporus and Marasmius oreades were maintained on a 2 per cent glucose medium (32) composed of the following:

Glucose	20.0 g.
Potassium monobasic phosphate	1.5 g.
Peptone	1.0 g.
Magnesium sulfate	0.5 g.
Thiamine hydrochloride	2.0 mg.
Agar	20.0 g.
Distilled water to make 1000 ml.	
pH 5.5 after sterilization	

This medium was used because it provided for higher growth rates of the two basidiomycetes than potato-dextrose agar or potato-dextrose-yeast agar.

Cultures of Pythium species were maintained on potato-dextrose agar (PDA) composed of the following:

Dextrose	10.0 g.
Agar	20.0 g.
Broth from 200 g. of cooked potatoes	
Distilled water to make 1000 ml.	

Both media were sterilized by autoclaving for twenty minutes at a pressure of 15 psi and then aseptically dispensed as 20 to 25 ml. aliquots into sterile petri plates.

In studies to determine the effect of hydrogen-ion concentration on the linear growth of Pythium artotrogus and Marasmius oreades a modification of the 2 per cent glucose medium was employed. Two hundred ml. of one third molar phosphate buffer was substituted for 1.5 g. of

potassium monobasic phosphate. The phosphate buffer (38) was composed of various ratios of phosphoric acid, potassium monobasic phosphate, and potassium dibasic phosphate. The potassium monobasic phosphate was added to the medium prior to sterilization. Phosphoric acid and potassium dibasic phosphate were sterilized separately and added aseptically to the medium after autoclaving.

All petri plates were spawned with 5 mm. agar disks bearing mycelium of the fungus. Where possible the agar disks were taken from the edge of the fungal colony. This method provided a fairly constant amount of spawn and thereby reduced the amount of variation between replications in the experiments.

In order to study microscopically the antagonism by Pythium artotrogus to Agaricus bisporus, Marasmius oreades, and other Pythium species, the fungi were cultured on glass slides. The culture slides were prepared by flaming a one by three inch glass slide and placing it on a glass rod support in a sterile petri plate containing a few ml. of sterile water. Agar medium was then aseptically pipetted onto the glass slide so that a thin film of agar gel covered the surface of the slide. The culture slides were spawned with the desired fungi after the agar had solidified. When either A. bisporus or M. oreades was to be studied in association with a Pythium species, the basidiomycete was spawned ten to fifteen days prior to seeding the Pythium species. A phase contrast microscope and magnifications of 600 and 1500

diameters was used throughout this investigation.

Pathogenicity tests of Pythium species on Astoria Colonial bentgrass (Agrostis tenuis Sibth.), Seaside Creeping bentgrass (A. palustris Huds.), Velvet bentgrass (A. canina L.) and Redtop (A. alba L.) were conducted. The artificial light used in these laboratory pathogenicity tests was supplied by four 40-watt General Electric cool white fluorescent lamps. The light fixtures were suspended above the laboratory bench in such a manner that the distance between the light fixture and the bench surface could be varied and thereby vary the light intensity. In the pathogenicity tests on germinating seeds a light intensity of 100 foot candles was used, while in tests conducted on seedlings a light intensity of 200 foot candles was employed.

The seeds of Agrostis species are so small that a tiny glass dipper was constructed so that one full dipper contained an average of 145 seeds of A. palustris, 97 seeds of A. tenuis, 93 seeds of A. canina or 70 seeds of A. alba. Two full dippers of A. alba seed and one full dipper of seed of each of the other Agrostis species were used for each replicate. The seeds for each replicate were placed in an envelope made of folded filter paper. These envelopes were immersed in a 0.5 per cent solution of sodium hypochlorite for one minute followed by four rinses of one minute each in sterile distilled water. The seeds were then air-dried in the filter paper envelopes overnight at which time they were aseptically scattered over the surface of 1.5 per cent water agar gel in petri plates.

IV. CHEMICAL CONTROL OF MARASMIUS OREADES FAIRY RING

Fairy ring, as described in the review of literature, often causes severe damage to fine quality turf. Few field tests of fungicides have been conducted to determine the effectiveness of various products to control the causal agent of this disease. Howard et al. (21) reported observations that organic cadmium compounds and magnesium salts have an inhibitory effect on the development of mushroom sporophores. Appreciable reduction in the damage caused by fairy ring was reported by Couch (12) to be obtained by pouring solutions containing phenyl mercury acetate, or cadmium compounds mixed with a wetting agent into holes four inches deep made at twelve-inch intervals.

In an attempt to verify these findings and to test other chemicals, field experiments were carried out during the summer of 1962. Established M. oreades fairy rings located in sports turf on the campus of the University of Rhode Island were selected for the trials. The turf was composed primarily of Kentucky bluegrass. The fairy rings were divided into 36 two-square-foot plots with each plot separated from adjacent plots by a six-inch buffer zone. Eleven treatments and an untreated control were randomly replicated three times. Before the treatments were applied, the sod was perforated with the tines of a spading fork to a depth of about four inches. Applications were applied as a drench on

August 22, 1962, at the rate of two quarts of fungicide solution per plot.

Chemicals and Dosages Tested

<u>Chemical</u>	<u>Amount per quart of water</u>
Phenyl mercury acetate (10%), Vineland Chemical Company	0.4 ml.
Methyl arsine oxide, Vineland Chemical Company	188.0 mg.
SD-345 (2-Propene-1,1-diol diacetate), Shell Development Company	472.5 mg.
Dexon (p-Dimethylaminobenzenediazo sodium sulfonate 70%), Chemagro Corp.	1.4 g.
Cadmium chloride	355.0 mg.
Copper sulfate	355.0 mg.
Mercuric chloride	355.0 mg.
Dithane A-40 (Disodium ethylene bis-dithio- carbamate 97%), Rohm and Haas Co.	355.0 mg.
Magnesium sulfate	7.1 g.
H-3944 (3-oxy-4 phenyl-5-chloro-1,2 dithiole 50%), Hercules Powder Co.	1.4 g.
MF-89 (1,3-dichloro 5.5-dimethyl hydantoin 98%) Mallinckrodt Chemical Works	1.4 g.
Control	--

Since no sporophores developed in the treated areas or control during the season, the results were based on the amount of grass growth stimulation resulting from the influence of the fungus. Perforating the sod with a spading fork caused some injury to the grasses, as well as reducing the amount of stimulation of the turf.

Within six days after application, the following treatments caused moderate chemical injury to the turf: phenyl mercury acetate, methyl arsine oxide, cadmium chloride, copper sulfate, mercuric chloride, Dithane A-40 and magnesium sulfate. Severe injury was observed on the H-3944 and MF-89 plots. After thirty-one days only the phenyl mercury acetate, H-3944 and MF-89 plots exhibited residual chemical injury.

Reduction of the stimulated grass zone of the fairy ring was quite variable among replicate plots of the same treatment. For example, one or two of the replicates showed only slight turf stimulation, while on the third the turfgrass was stimulated as much as the control plots.

The three replicates of phenyl mercury acetate, Dithane A-40 and methyl arsine oxide, however, were fairly constant in their reduction of the stimulated zone. For this reason these fungicides were considered worthy of further testing.

During the summer of 1963 a more extensive field experiment was conducted in which seven treatments and a water control were randomly replicated three times. Each replicate consisted of a complete fairy ring having a diameter of from two to five feet. All fairy rings used in the test were located on turf consisting mostly of Kentucky bluegrass and were caused by Marasmius oreades.

Instead of applying the treatments as a drench, as in the previous year, the solutions were injected under pressure into the soil to a depth of twelve inches. Fungicide

injections were made with a tree fertilization probe or feeding gun attached by a hose to a Hardie "Town and Country" sprayer operated at a pressure of 125 psi. Injections were made along the middle of the green band of stimulated turf at intervals of about twelve inches. Each of the fairy rings in this test received four to five gallons of solution. On many of the fairy rings, especially those in which there was a dead zone, much resistance to penetration of the soil was encountered when pushing the probe down to a depth of twelve inches.

In evaluating treatments for the control of fairy ring it is difficult to compare one treatment with another unless one completely eliminates the fairy ring. For this reason data were recorded on; 1) the inside diameter of the green stimulated ring, 2) the width of the green stimulated band and 3) the number of sporophores that developed in each ring. Data were taken just prior to the application of the treatments on July 23, and again one and a half months later on September 5, 1963. The data noted for the three replicates of each treatment were combined and are presented in Table 1.

<u>Chemical tested</u>	<u>Amount per 10 gal.</u>
Control (water)	---
Methyl arsine oxide, 10%, Vineland Chemical Co.	38.0 ml.
Phenmad (Phenyl mercury acetate, 10%), Mallinckrodt Chemical Works.	30.0 ml.
Parzate liquid (Disodium ethylene bis-dithio- carbamate, 22%), E.I. duPont deNemours & Company	77.4 ml.

Table 1. The influence of fungicide treatment on M. oreades fairy ring, 1963

Treatment	*Ring inner diameter (inches)			*Width of green stimulated zones (inches)			*Number of sporophores		
	7/23	9/5	Diff.	7/23	9/5	Diff.	7/23	9/5	Diff.
Control	131	146	+15	29	34	+ 5	324	255	-69
Methyl arsine oxide	93	92	- 1	24	46	+22	265	267	+ 2
Phenmad	111	125	+14	26	43	+17	190	245	+55
Parzate liquid	124	118	- 6	22	36	+14	247	170	-77
Malachite green	123	133	+10	27	41	+15	328	238	-90
Calo-Clor	129	139	+10	23	37	+14	126	70	-56
Basic aluminum chloride	101	113	+12	25	40	+15	279	189	-90
Isothan	126	129	+ 3	22	38	+16	219	205	-14

*Sum of three replicates.

<u>Chemical tested</u> (cont'd)	<u>Amount per 10 gallons</u>
Malachite green	20.0 g.
Calo-Clor (Mercurous chloride, 60%, and mercuric chloride, 30%), Mallinckrodt Chemical Works	28.4 g.
Basic aluminum chloride	113.6 g.
Isothan (a mixture of 1 part 75% dialkyl dimethyl ammonium bromide to 1 part 75% alkyl isoquinolinium bromide), Onyx Chemical Company	15.0 ml.

Except for the water control, all treatments in addition contained 30 g. of urea and 30 ml. of Igepal GA-630 (a non-ionic wetting agent) per 10 gallons of water.

By injecting the chemical solutions directly into the soil, injury was much less severe than that experienced in the 1962 tests, when the treatments were applied as a surface drench. Severe injury was noted with Phenmad and a moderate amount with Calo-Clor, but only around the sites of injection where the solution was forced to the surface.

Table 1 shows that the inside diameter of the fairy rings decreased with treatments of methyl arsine oxide and Parzate liquid. Realizing that these figures represent the sum of the inner diameters of three replicated fairy rings and that the margins of the stimulated grass zones are not sharply defined, the small differences in diameter of the rings treated with methyl arsine oxide, Parzate liquid and Isothan may be due, in part, to error in measurement. With all the treatments, the width of the green stimulated band of growth was much greater than that of the control fairy

rings. This phenomenon was due most likely to the urea applied with the fungicide, or to the decomposition of the fungus mycelium. The three fairy ring replicates treated with phenyl mercury acetate had a total of 190 sporophores prior to fungicide application. At the conclusion of the field test these same fairy rings had a total of 245 sporophores, or an increase of 55 sporophores. The three control fairy rings had a total of 324 sporophores at the beginning of the test, but only 255 sporophores at the conclusion, or a decrease of 69 sporophores. The decrease in the total number of sporophores may be explained by the weekly mowings and continued mushroom production.

As stated above, there is difficulty in evaluating field tests for the control of fairy ring unless one or more treatments completely eliminate the fairy ring. This problem is immediately recognized when one observes the data in Table 1. However, the figures seem to indicate that methyl arsine oxide and Parzate liquid warrant further testing. Although phenyl mercury acetate does not appear to have been effective in this field test, further trials are suggested because it has been recommended for a number of years and some success reported.

V. THE INFLUENCE OF TEMPERATURE ON THE LINEAR GROWTH OF MARASMIUS OREADES AND PYTHIUM ARTOTROGUS

Pythium species are known to have relatively high growth rates for fungi. In studies of 73 Pythium species and varieties, Middleton (26) found that optimum growth rates varied from a minimum of 8 mm. per twenty-four hours to a maximum of 51 mm. per twenty-four hours. Basidiomycetes, in general, have much slower growth rates. It is to be expected, therefore, that P. artotrogus had a much greater growth rate than M. oreades. However, in order for P. artotrogus to be effective in halting the growth of M. oreades the optimum temperature range for growth of the two fungi should be somewhat similar.

The effect of temperature on the linear growth of M. oreades and P. artotrogus was determined by measuring at intervals the diameter of the fungal colony on 2 per cent glucose agar medium. Petri plate cultures of P. artotrogus were incubated at 5, 10, 15, 21, 25, 27, 30, 35 and 40 degrees C with five replications per temperature. The M. oreades cultures were incubated at the same temperatures, except 35° C and 40° C. A 5 mm. agar disk bearing mycelium of the test fungus was transferred to the center of each petri plate. The diameters of P. artotrogus colonies were measured after eighteen hours of incubation at each of the various temperatures. Subsequent measurements were recorded at six-hour intervals up to fifty-four hours. The diameters of the M. oreades colonies were measured at two-day intervals

up to eight days.

The results of the temperature study are shown in Figure 2. At 5° C no growth of either fungus occurred since the spawn consisted of a 5 mm. agar disk bearing mycelium of the fungus. The optimum temperature for mycelial growth of P. artotrogus was about 27° C with an average radial growth rate of 14.5 mm. per twenty-four hours. Marasmius oreades had a similar optimum temperature requirement for mycelial growth. However, its radial growth rate was 1.5 mm. per twenty-four hours.

Figure 2. Effect of temperature on linear growth of Pythium artotrogus and Marasmius oreades after 48 hours and 8 days respectively.

VI. THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON
LINEAR GROWTH OF MARASMIUS OREADES AND PYTHIUM
ARTOTROGUS

Like temperature, hydrogen-ion concentration has a pronounced effect on the growth rate of fungi. Fergus, et al. (15) cultured Agaricus bisporus and P. artotrogus on a mushroom-compost infusion agar which had an initial pH of 7.2. However, pH of soils inhabited by turfgrasses is usually 6.5 or lower. Thus, the acid soils of turfgrass areas may be a limiting factor in the effectiveness of P. artotrogus as an agent for the control of M. oreades.

In order to determine the influence of hydrogen-ion concentration on the linear growth of M. oreades and P. artotrogus, isolates of each were grown on 2 per cent glucose medium buffered at various H-ion concentrations. Plates of culture medium at each H-ion concentration were replicated five times for each fungus. Transfers were made by placing a 5 mm. agar disk bearing mycelium of the test fungus in the center of the petri plate. All plates were incubated at 25°C.

Increase in diameter of the fungal colony was used as the criterion of its growth. Measurements of P. artotrogus were recorded after the first twenty-four hours of incubation and repeated at six-hour intervals up to fifty-four hours. Measurements on M. oreades were recorded at two-day intervals up to ten days.

Colony diameters resulting from each H-ion concentration were averaged for the five replicates and the mean presented in Figure 3. The optimum pH for mycelial growth of P. artotrogus was about 5.6 with a minimum pH value of about 2.5. The optimum pH for mycelial growth of M. oreades was 5.5 with a minimum and maximum value of about 3.1 and 7.7, respectively.

Figure 3. Effect of hydrogen-ion concentration on linear growth of Pythium artotrogus and Marasmius oreades after 48 hours and 10 days respectively.

VII. THE ANTAGONISM OF PYTHIUM SPECIES TO AGARICUS
BISPORUS AND MARASMIUS OREADES

The purpose of this study was to determine the possible antagonistic relationship of P. artotrogus, and various other Pythium species, to Agaricus bisporus and Marasmius oreades. Information concerning the ability of various Pythium species to grow among and through the mycelium of the basidiomycetes was also obtained.

The following experiment was designed to provide this information. Six agar disks 5 mm. in diameter bearing mycelium of the basidiomycete were transferred to petri plates containing 2 per cent glucose agar medium. They were placed in a straight line equidistantly across the center dividing the plate into two halves, A and B (Figure 4). The plates were then incubated at 25° C for ten days by which time the mycelium had grown out from the agar disks forming a continuous band of mycelium across the plate. In tests conducted with A. bisporus this mycelial strip ranged from 28 mm. to 48 mm. in width, while with M. oreades the mycelial strip varied from 14 mm. to 26 mm. in width. Each of the plates was then spawned on side A with the Pythium species to be observed. There were three replicate plates for each Pythium species. Plates that were not seeded with Pythium species served as controls. The average ten-day growth of basidiomycete mycelium on side A and on side B of each plate was measured in mm. and recorded. After introducing Pythium the plates were incubated for another ten days when the growth

Figure 4. Diagram of method used to determine the degree of growth reduction of Agaricus bisporus and Marasmius oreades by Pythium species. M - mycelial band of either Agaricus bisporus or Marasmius oreades. P - Pythium species colony.

of the basidiomycete mycelium on sides A and B were measured again. It was also determined, by visual means, whether the Pythium species grew from side A through the basidiomycete mycelium to side B.

The amount of growth of the basidiomycete mycelium from the time of introduction of the Pythium species to the conclusion of the test was obtained for sides A and B in each of the three replicates. These values were then averaged and the per cent reduction in growth calculated. The per cent reduction of growth of the basidiomycete mycelium exposed to Pythium sp. is an indication of the degree of antagonism of the Pythium species.

The data presented in Table 2 show that P. artotrogus and P. ultimum greatly reduced the amount of growth of A. bisporus on side A, but very little on side B. It should also be noted that neither Pythium species was able to grow through the mycelium of A. bisporus.

When the antagonism of Pythium species was tested against M. oreades the results were different. Pythium artotrogus was antagonistic towards M. oreades mycelium, but did not penetrate through the mycelium. Pythium ultimum was more antagonistic to M. oreades and grew somewhat through the mycelium. However, the Hawaiian isolate of P. irregulare was the most antagonistic Pythium species tested. Furthermore, this Pythium species grew across the mycelial strip of M. oreades and reduced the amount of growth on side B by a greater degree than did P. artotrogus on side A.

Table 2. The degree of growth retardation of Agaricus bisporus and Marasmius oreades by Pythium species.

<u>Pythium</u> sp.	<u>Agaricus bisporus</u>			<u>Marasmius oreades</u>		
	Retardation of growth in %	<u>Pythium</u> growth into area B ^{1/}		Retardation of growth in %	<u>Pythium</u> growth into area B ^{1/}	
	<u>Side A</u>	<u>Side B</u>		<u>Side A</u>	<u>Side B</u>	
Control	0.0	0.0	---	0.0	0.0	---
<u>P. aphanidermatum</u>	50.9	14.3	Yes	40.2	13.0	Yes
<u>P. arrhenomanes</u>	0.0	15.7	No	42.7	9.3	Yes
<u>P. artotrogus</u>	72.4	15.7	No	59.8	0.8	No
<u>P. debaryanum</u>	46.8	6.8	No	48.7	22.5	Yes
<u>P. graminicolum</u>	49.1	40.5	Yes	11.8	9.3	No
<u>P. irregulare</u> (H) ^{2/}	57.1	6.8	No	88.9	69.2	Yes
<u>P. irregulare</u> (O) ^{2/}	43.0	14.1	No	34.2	13.0	Yes
<u>P. splendens</u>	61.3	21.0	Yes	48.7	9.8	Yes
<u>P. ultimum</u>	65.1	5.2	No	76.8	18.5	Yes ^{3/}

^{1/}See text figure 4.

^{2/}H and O refer to the Hawaiian and Ohio isolates, respectively.

^{3/}One of the three replicates of P. ultimum grew into area B.

VIII. THE NATURE OF ANTAGONISM OF PYTHIUM ARTOTROGUS
TO MARASMIUS OREADES

In order to determine if P. artotrogus has the potential to be used as a biological control agent for fairy ring caused by M. oreades, the nature of the antagonism between the two fungi needed to be determined.

The article by Fergus et al. (15) is the only report in the literature concerning the antagonism of a basidiomycete by P. artotrogus. These workers showed that growth of the commercial mushroom, A. bisporus, was completely inhibited by P. artotrogus. After an attempt to determine the nature of this antagonism, they stated that following mycelial contact neither organism grew. It was not determined whether P. artotrogus was parasitic on A. bisporus, or whether P. artotrogus produced metabolites which were toxic to its mycelium.

The nature of antagonism of P. artotrogus to M. oreades was determined in the present investigation by observing the two fungi growing in association with one another on slides coated with potato-dextrose agar. Observations were begun when the mycelia of the two fungi were about 5 mm. apart and continued at short intervals until the mycelia of the two fungi had been in contact with each other for several days. Since the agar medium rapidly dried out when left on the stage of the phase contrast microscope for more than an hour, many replacement culture slides were required.

Actively growing hyphae of both M. oreades and P. artotrogus on many occasions stopped growing after a cover glass had been placed on top of the mycelium. Hyphae of A. bisporus were never observed to continue growth after a cover glass had been placed on the mycelium.

The mycelium of M. oreades is typical of a basidiomycete in that the cells are binucleate and the hyphae produce clamp connections at the cross walls. The hyphal tip cells were found to be 2 to 3 u in diameter and ranged from less than 250 u to more than 600 u in length. Blunt, needle-shaped, colorless crystals were observed lying across the exterior of the hyphae. These crystals, which were about 1 u in width and 3 u to 7 u in length, were restricted to the older hyphal cells located about 1000 u back from the edge of the colony.

The mycelium of A. bisporus is not typical of a basidiomycete in that the hyphal cells are multinucleate and clamp connections are not present. The diameter of the hyphae varied from 5 u to 7 u, while the hyphal tip cells varied in length generally from 150 u to 180 u. Colorless, needle-shaped crystals less than 1 u in diameter and 7 u to 10 u long were observed lying on the exterior walls of the hyphae. These crystals were also observed to be restricted to the older hyphal cells, which were about 2000 u back from the edge of the colony.

The cellular contents of the hyphae of both A. bisporus and M. oreades were difficult to distinguish at magnifications up to 1500 diameters. Streaming of the protoplasm was

easily observed within the hyphae of P. artotrogus, but was not observed within the hyphae of A. bisporus or M. oreades. Therefore, hyphal growth in length was used as the criterion to judge if the mycelium of M. oreades was alive. The growth rate of hyphal tips of this fungus was observed to be about 2.5 μ in five minutes at room temperature. Since A. bisporus was never observed to grow when viewed through the microscope, it could not be determined with certainty whether or not the hyphae of this fungus was alive.

When the advancing edge of colonies of M. oreades and P. artotrogus approached each other, the hyphae of each fungus was growing at its normal rate. However, when the hyphal tips of these two fungi were adjacent to each other many hyphal tips of M. oreades stopped growing. As P. artotrogus continued its growth amongst the mycelium of M. oreades, more hyphal tips of the latter were inhibited.

The only change in the cellular contents of M. oreades that could be seen when the two fungi were in association with one another, was that the density of its protoplasm appeared to increase. Hyphae of P. artotrogus were observed to continue their growth for at least two hours. A few hyphal threads exhibited protoplasmic streaming after six to seven days of association with M. oreades. When the two fungi remained in close association for more than a day, the agar in the interhyphal zone began to turn brown, and the hyphae of P. artotrogus became very granular and vacuolated.

As the length of time during which the two fungi were in mutual association increased to four days, the hyphae of

M. oreades became difficult to identify. On these culture slides rectangular crystalline material forming a line or thread was frequently observed in the agar. These threads of crystalline material appeared to have originated from M. oreades hyphae which had been lysed.

Oogonia of P. artotrogus were produced in abundance in the interhyphal zone. The hyphae of P. artotrogus were never observed to enter and parasitize the hyphae of M. oreades.

Observations reported earlier in this paper (Table 2) indicate that P. graminicolum is not very antagonistic (or toxic) toward M. oreades. When this Pythium species was grown in association with M. oreades on culture slides, the hyphae of the latter remained distinct and did not lyse and form threads of crystalline material. The agar in the interhyphal zone did not become brown. Reproductive structures of P. graminicolum were not produced in the interhyphal zone.

Studies of the antagonism of P. artotrogus to A. bisporus were somewhat less productive. This was due to the lack of growth of A. bisporus after a cover glass was placed on the mycelium. The sequence of events in the antagonism of A. bisporus by P. artotrogus were, as near as could be determined, similar to the interaction between M. oreades and P. graminicolum. It could not be determined when the hyphae of A. bisporus stopped growing in relation to the position of the hyphae of P. artotrogus.

The results presented above indicate that the antagonistic substance of P. artotrogus is a toxic metabolite

produced by the fungus which diffused into the agar. In order to test this hypothesis, P. artotrogus was grown in potato-dextrose broth aerated by pumping sterile air through the liquid medium. After seven days of incubation at 27° C the culture medium was filtered through a sterile 0.45 μ Millipore filter. The filtrate was then added to sterile melted 2 per cent glucose agar medium. The concentration of the filtrate in the agar medium varied from 0.3 per cent to 50 per cent. One half of this filtrate medium was dispensed directly into sterile petri plates. The remaining half was first autoclaved and then dispensed into sterile petri plates. After the agar had solidified three replicate plates for each fungus at each filtrate concentration were spawned with either A. bisporus or M. oreades. Control plates consisted of 2 per cent glucose medium without P. artotrogus filtrate. After six days of incubation the diameters of the colonies were measured in millimeters and averaged. The unautoclaved 50 per cent filtrate concentration reduced the mycelial growth of A. bisporus by 22.1 per cent and M. oreades by 5 per cent. However, the autoclaved 50 per cent filtrate concentration reduced mycelial growth of A. bisporus by 10.2 per cent and M. oreades by 20.6 per cent. All other concentrations of the filtrate reduced the growth of the two fungi to a lesser degree.

In another experiment conducted to determine if metabolites of P. artotrogus were inhibitory to the two basidiomycetes, eight petri plates containing potato-dextrose agar

were seeded with P. artotrogus. Eight other petri plates were seeded with P. graminicolum, and a third group of eight petri plates were left uncontaminated. The petri plates were incubated at 25° C for seventeen days. Then the mycelium of the two Pythium species was removed from the agar-gel surface and the petri plates with the residual agar medium were sterilized by autoclaving. Of the eight petri plates in which P. artotrogus had been grown, four were spawned with A. bisporus and four were spawned with M. oreades. Four of the plates in which P. graminicolum had been grown were spawned with A. bisporus and the remaining four spawned with M. oreades. Of the eight uncontaminated plates four were spawned with A. bisporus and four with M. oreades to serve as controls. The petri plates were then incubated at 25° C for nine days after which time the diameters of the colonies were measured in millimeters.

The agar in which P. graminicolum had grown reduced the average amount of mycelial growth of A. bisporus by 42.1 per cent and M. oreades by 23.3 per cent. Agar in which P. artotrogus had been grown reduced the average mycelial growth of A. bisporus by 34.8 per cent and M. oreades by 69.4 per cent.

Since it was shown that Pythium species deposit metabolite (s) in a substrate that are toxic to A. bisporus and M. oreades, it was decided to determine what effect the cultural filtrate of P. artotrogus had on hyphal tip cells of M. oreades.

Five hundred ml. of potato-dextrose broth were seeded with P. artotrogus and incubated at 27° C. Aeration was supplied by pumping sterile air through the liquid medium. After twenty-four days of incubation, the culture medium was filtered through a sterile 0.45 u Millipore filter. In order to determine if the filtrate would affect growth of hyphal tip cells of M. oreades, potato-dextrose agar culture slides were prepared. The growth rate of an average hyphal tip under a standard set of conditions was determined prior to the application of the filtrate. After applying the filtrate to the mycelium, the rate of growth in successive five-minute periods was determined.

It was observed that following the application of the filtrate, the hyphal tip of M. oreades continued to grow at its normal rate of about 2.5 u per five minutes for the first ten minutes. Growth during the next five minutes was reduced to about 1.5 u. Growth was further reduced to about 1.0 u in the succeeding five minutes, after which time no further growth occurred.

A 10 ml. aliquot of the filtrate was then poured into a stoppered test tube and stored at 7° C. The remaining portion of the filtrate (about 450 ml.) was dried under vacuum at a temperature not exceeding 60° C. The dried filtrate was then redissolved in 25 ml. of distilled water and stored at 7° C.

After twenty-four days of storage at 7° C, the 10 ml. aliquot showed exactly the same degree of biological activity

toward M. oreades as did the fresh filtrate. However, the filtrate that had been dried in vacuo and resuspended did not alter the growth rate of M. oreades hyphal tips within forty minutes after application.

In Table 2 it was noted that P. artotrogus did not intermingle with and grow through a mycelial band of M. oreades. Therefore, it was decided to determine the nature of the effect of the cultural filtrate of M. oreades on the hyphal tips of P. artotrogus. Marasmius oreades was cultured in 2 per cent glucose broth at 27° C for 112 days. The culture medium was filtered through a sterile 0.45 u Millipore filter. The filtrate was tested for its growth retardation effect on the hyphal tips of P. artotrogus by the same method described above. The filtrate of M. oreades did not reduce the normal hyphal tip growth rate of about 13 u per minute within seventy minutes after application.

IX. ANTAGONISM OF VARIOUS PYTHIUM SPECIES BY PYTHIUM ARTOTROGUS

Pythium artotrogus has frequently been found associated with Phytophthora species and other Pythium species.

Drechsler (13,14) has shown that three Pythium species with echinulate oogonia are parasitic on congeneric forms. Since P. artotrogus also forms echinulate oogonia, it was decided to determine what effect this fungus has on the hyphae of other Pythium species.

In order to determine the effect of P. artotrogus on the various Pythium species, culture slides coated with potato-dextrose agar gel were seeded on opposite ends with the two species of Pythium to be studied. The slides were incubated at 25° C for two days at which time the mycelia of the two fungi was observed to have been in mutual contact for more than twenty-four hours.

Pythium artotrogus had no apparent effect on its own hyphae or the hyphae of P. graminicolum. Hyphae of P. artotrogus were observed to coil around the hyphae of P. aphanidermatum, P. arrhenomanes, P. debaryanum, P. splendens, P. ultimum and both isolates of P. irregulare. Coiling was at times rather extensive with many gyres, while at other times the coils consisted of only two or three gyres, as illustrated in Figure 5 A.

Pythium artotrogus hyphae were observed to enter and to parasitize the hyphae of P. aphanidermatum, P. debaryanum, P. splendens, P. ultimum and both isolates of P. irregulare.

Figure 5. Camera lucida drawing showing the relation of *Pythium artotrogus* to hypha of *P. ultimum* and *P. debaryanum*. A) *P. artotrogus* forming simple coils around the hyphae of *P. ultimum*. B) *P. artotrogus* penetrating and growing through the hyphae of *P. debaryanum*.

When encountering a susceptible hypha, the hyphal tip of P. artotrogus formed a swollen structure analogous to an appressorium (Figure 5 B). Penetration of the host was accomplished by means of a narrow infection peg originating from the appressorium. Once inside the host hypha, the invading hypha enlarged to almost its normal diameter and grew lengthwise, apparently causing degeneration of the host protoplasm. Often it then emerged through the enveloping host wall and grew out into the agar gels only to coil around or penetrate another host hypha (Figure 5 B). As many as four hyphae of P. artotrogus were seen lying parallel to one another within a host hypha. The degree of coiling and parasitism was reduced when the agar concentration of the gel was increased above 1.5 per cent.

The spiny oogonia of P. artotrogus developed within two days when this fungus was grown in association with P. debaryanum or P. splendens. Oogonia did not develop when P. artotrogus was cultured in association with the other Pythium species.

X. PATHOGENICITY OF PYTHIUM SPECIES TO FOUR AGROSTIS SPECIES

In a survey of turfgrass pathologists throughout North America, Gould (17) received reports that diseases caused by Pythium species were the sixth most injurious to golf putting green turf. Since several Pythium species are known to damage turfgrasses severely, it was necessary to determine whether P. artotrogus would injure grasses used for lawns and sports turf. The pathogenicity of the various Pythium species was tested in the laboratory on germinating seeds and seedlings of four Agrostis species.

Agrostis alba L. (redtop) is commonly grown in lawns, along highways and airports in the cool regions of the United States and Canada. Agrostis canina L. (Velvet bentgrass) may be found in meadows and golf putting greens from Newfoundland southward to Tennessee and as far west as Michigan. Agrostis palustris Huds. var. Seaside (creeping bentgrass) is used primarily for putting greens, fairways, and lawns. This species is largely restricted in the United States to the northeastern and northwestern states. Agrostis tenuis Sibth. var. Astoria (Colonial bentgrass) is used for lawns, golf putting greens, and tennis courts in the northeastern and northwestern portions of the United States (20).

A. Pythium injury to germinating Agrostis seeds.

In order to determine the degree of pathogenicity of the various Pythium species to germinating seeds of Agrostis species, seeds were germinated on a 1.5 per cent agar-water

gel in petri plates in the presence of the fungus. Each petri plate was spawned with three 5 mm. agar disks bearing actively growing mycelium placed equidistant on the gel surface. Petri plates of agar-water gel containing seed, but without the fungus, served as checks on germination. Each of the four Agrostis species was exposed to three replications of each Pythium species. The plates were incubated at room temperature (24° to 30° C) under fluorescent lights arranged so that the light intensity at the agar gel surface was 100 foot candles.

Counts were made of the number of seeds that germinated and of those that did not germinate after seven days of incubation. The per cent germination was determined for each replicate. An adjustment or correction factor to allow for nonviable seed was calculated by dividing the average per cent germination of the unexposed controls into the average per cent germination of the Pythium-exposed seed (Table 3). The average per cent seed germination of the unexposed controls were: A. alba, 41 per cent; A. canina, 40 per cent; A. palustris, 74 per cent; and A. tenuis, 73 per cent. In all cases the fungus covered the surface of the agar before germination of the seeds was observed. In some tests where a highly pathogenic Pythium species was involved, a few seeds lying adjacent to the inoculum germinated, while seeds at some distance from the inoculum which were covered with mycelium did not germinate.

The data indicating the relative pathogenicity of species

Table 3. Relative pathogenicity of Pythium species to germinating seeds of Agrostis species.

<u>Pythium</u> species	<u>Depression of seed</u>			
	<u>A. alba</u>	<u>A. canina</u>	<u>A. palustris</u>	<u>A. tenuis</u>
<u>P. aphanidermatum</u>	* 7.9	7.9	5.5	9.9
<u>P. arrhenomanes</u>	0.0	1.2	2.3	0.0
<u>P. artotrogus</u>	93.0	93.5	92.0	104.0
<u>P. debaryanum</u>	96.0	87.9	61.0	54.0
<u>P. graminicolum</u>	0.0	2.3	4.5	18.0
<u>P. irregulare</u> (H)**	84.7	58.8	46.0	66.9
<u>P. irregulare</u> (O)**	70.7	52.5	27.6	34.4
<u>P. splendens</u>	40.4	35.8	30.4	47.7
<u>P. ultimum</u>	58.6	39.4	59.0	80.0

*Per cent germination of Agrostis seed when compared to viable seed not exposed to the particular Pythium species.

** (H) and (O) refer to the Hawaiian isolate and the Ohio isolate, respectively.

of Pythium to germination of the viable seeds of the four Agrostis species are presented in Table 3.

B. Pythium injury to Agrostis seedlings.

In order to determine the degree of pathogenicity of the various Pythium species to seedlings of Agrostis species, seeds surface sterilized in sodium hypochlorite were aseptically placed on 1.5 per cent agar-water gel in petri plates. The petri plates were spawned nine days later with the various Pythium species by placing three 5 mm. agar disks bearing mycelium of the desired fungus equidistantly on the agar gel surface. Each of the four Agrostis species was exposed to three replicates of each Pythium species. Petri plates containing seedlings, but without the fungus, served as controls. All plates were incubated at 27° C under fluorescent lights arranged so that the light intensity at the agar gel surface was 200 foot candles. Results were recorded twenty-one days after introducing the fungus into the petri plates. In all cases, the Pythium species grew to cover the entire agar surface.

Injury to the seedlings was of two types: 1) leaf blighting, and 2) distortion and reduction in both length and number of root hairs. Moderate to severe blighting of the foliage of all four grasses was caused by P. arrhenomanes, while P. aphanidermatum caused moderate to severe blighting on A. alba, A. palustris and A. tenuis. None of the other Pythium species caused blighting of the Agrostis seedlings. No discolored or necrotic areas were observed in the roots of

any of the grasses. The Agrostis roots in the control plates produced an abundance of root hairs, nearly all of which were parallel to one another. Several of the Pythium species caused the root hairs to be reduced in number, as well as being bent and distorted. Table 4 shows the relative degree of injury to the Agrostis roots caused by the various Pythium species.

Pythium species	Degree of injury to Agrostis roots			
	Control	Pythium	Pythium	Pythium
1. P. mycelium	Control	Control	Control	Control
2. P. mycelium	Control	Control	Control	Control
3. P. mycelium	Control	Control	Control	Control
4. P. mycelium	Control	Control	Control	Control
5. P. mycelium	Control	Control	Control	Control
6. P. mycelium	Control	Control	Control	Control
7. P. mycelium	Control	Control	Control	Control
8. P. mycelium	Control	Control	Control	Control
9. P. mycelium	Control	Control	Control	Control
10. P. mycelium	Control	Control	Control	Control

1/2 inch of water in 10 minutes and 1/2 inch of water in 10 minutes.

1/2 inch of water in 10 minutes and 1/2 inch of water in 10 minutes.

Table 4. Relative pathogenicity of Pythium species to roots of Agrostis species.

<u>Pythium</u> species	Degree of injury to <u>Agrostis</u> seedling roots			
	<u>A. alba</u>	<u>A. canina</u>	<u>A. palustris</u>	<u>A. tenuis</u>
<u>P. aphanidermatum</u>	Medium ^{1/}	Slight	Severe	Severe
<u>P. arrhenomanes</u>	Medium	Severe	Severe	Severe
<u>P. artotrogus</u>	None	None	None	None
<u>P. debaryanum</u>	None	Slight	Slight	Medium
<u>P. graminicolum</u>	Slight	Slight	Slight	None
<u>P. irregulare</u> (H) ^{2/}	None	None	Slight	None
<u>P. irregulare</u> (O) ^{2/}	None	None	None	None
<u>P. splendens</u>	None	Slight	Slight	Slight
<u>P. ultimum</u>	Medium	Medium	Medium	Medium

^{1/}Estimated degree of distortion and reduction in both length and number of root hairs as compared to the controls.

^{2/}(H) and (O) refer to the Hawaiian and Ohio isolate, respectively.

XI. DISCUSSION

Evidence has been accumulated from the literature and by experimentation to advance knowledge of the Marasmius fairy ring disease of turfgrasses and its control. Both chemical and biological control of the causal fungus have been of interest. After two years of trial of chemical measures and the better defining of their shortcomings, the field of antagonistic fungi for biological control was explored. Soil inhabiting fungi were found that retard the growth of agaricaceous fungi and of these Pythium artotrogus was selected for detailed study. Hence, the antagonistic relationship between Pythium species and Marasmius oreades and the mechanism of the antibiosis have been examined.

Present chemical control measures for fairy ring do not appear to be effective or practical. The one exception to this statement may be fumigation of the soil with methyl bromide. However, this drastic treatment kills the grass and the area cannot be reseeded or resodded for seven to fourteen days (12).

In this study much difficulty was experienced in forcing the solution injection probe into the soil in the dead grass zone of the fairy ring. In adjacent healthy turf no such difficulty was encountered. Molliard (27) and others have shown that the soil beneath the dead grass zone is extremely dry and permeated by the fungus mycelium. Bayliss-Elliott (4) stated that this dry soil was extremely difficult to wet. The dryness of the soil in the dead zone and its resistance

to wetting were, no doubt, the reason for the difficulty encountered in pushing the probe down to a depth of twelve inches.

It is these same soil conditions which make chemical control of fairy ring ineffective. It was thought that the addition of a nonionic wetting agent to the treatments might overcome the resistance of the soil to wetting. The results have shown, however, that the addition of such a wetting agent to the fungicides does not completely solve the problem. Hence, other measures for ridding turfgrass sod of fairy ring fungi were sought.

Fergus et al. (15) reported that Pythium artotrogus completely inhibited the growth of Agaricus bisporus. Thus it was decided to determine if this, or other Pythium species, could be used as a biological control agent for Marasmius fairy ring.

There are many articles in the literature which suggest that various microorganisms have an antagonistic effect on species of Pythium. For example, Connell (10) reported that out of 5,638 isolates of bacteria tested, 202 were antagonistic to P. arrhenomanes. While investigating five soil types, Cooper and Chilton (11) tested over 8,000 isolates of actinomycetes for antagonism of P. arrhenomanes. They found that, depending on the type of soil, 18.5 per cent to 31.5 per cent of the isolates were at least slightly antagonistic. Butler (8) has reported that Rhizoctonia solani parasitizes some species of Pythium, but not others. Partial biological

control of an unidentified Pythium species was obtained by Wright (39) when the seed of white mustard was dusted with spores of various Penicillium and Trichoderma species.

However, after a thorough search of the literature only three reports which state that species of Pythium are antagonistic to other fungi have been found. Two of these were by Drechsler (13,14) and concerned three Pythium species parasitic on other Pythium species. The third report was the previously mentioned article by Fergus et al. (15) in which P. artotrogus was shown to inhibit the growth of Agaricus bisporus.

Considering these inconclusive findings, the observations on the antagonism of A. bisporus and M. oreades by Pythium species recorded herein are novel. Controlled experiments revealed that of nine Pythium isolates tested, eight reduced by almost one half or more the mycelial growth of A. bisporus. Species of Pythium were shown to be equally antagonistic to M. oreades, although the degree of antibiosis varied greatly among the Pythium species. Pythium artotrogus was shown to be less effective in reducing mycelial growth of M. oreades than that of A. bisporus. Pythium ultimum and the and the Hawaiian isolate of P. irregulare, however, were shown to be more antagonistic toward M. oreades. Yet, the Ohio isolate of P. irregulare was shown to be only moderately antagonistic to M. oreades.

While evaluating the pathogenicity of Pythium species on grasses, Sprague (35) noticed that various isolates of

P. debaryanum, P. ultimum and P. irregulare differed markedly in their relative pathogenicity. These results seem to indicate that at least some species of Pythium exist as physiological or pathogenic races, which can be distinguished on the basis of their ability to cause disease in plants or to retard the growth of other fungi. This being the case, other isolates of P. artotrogus should be tested to determine the degree of their antagonism toward M. oreades, and possibly to other fungi which cause the turfgrass fairy ring disease syndrome.

The older hyphal cells of A. bisporus were observed to have colorless, needle-shaped crystals lying on their exterior walls. Hein (19) reported finding similar crystals, identified as calcium oxalate, on the hyphae of this fungus. These crystals were reported to vary from 1 to 20 u in length, while the width varied from less than 1 u to 3 u. The older hyphal cells of M. oreades were also observed to have needle-shaped crystals lying on the exterior of the hyphae. These crystals were generally wider, shorter, and with blunt ends in contrast with the pointed ends of those of A. bisporus. This appears to be the first report of the existence of such crystals on the hyphae of M. oreades.

The hyphal tips of M. oreades did not all stop growing as soon as they encountered the hyphae of P. artotrogus. However, as the hyphae of P. artotrogus continued to grow amidst the mycelium M. oreades, more hyphal tips of the latter were inhibited. The two used culture filtrate agar

experiments caused the hyphae of M. oreades to develop at reduced growth rates. When the cultural filtrate of P. artotrogus was applied directly to the hyphal tips of M. oreades, twenty minutes were required before growth of the latter was completely inhibited. These experiments indicate that the toxic metabolite(s) of P. artotrogus is relatively slow acting.

The experiments in which M. oreades and P. artotrogus were grown on culture slides indicated that the antibiotic(s) of P. artotrogus does not diffuse in advance of the hyphae to any great extent. This may be due in part to the extremely high growth rate (as much as 17 u per minute) of this fungus on the substrates used in these studies. The toxic agent(s) must, however, be somewhat water soluble and diffuse through the agar, since growth of the hyphal tips of M. oreades was inhibited before their physical contact with the hyphae of P. artotrogus.

There were two striking differences between the antagonism to M. oreades and to A. bisporus by P. artotrogus. During the interaction between M. oreades and P. artotrogus the agar turned brown in the interhyphal zone and the hyphae of M. oreades appeared to be lysed leaving a residue of crystalline material. Lysis did not occur and the agar in the interhyphal zone did not turn brown when A. bisporus was antagonized by P. artotrogus. The antagonistic mechanism between P. artotrogus and each of the basidiomycetes is therefore different, at least in the final stages of the interaction.

The results of the used culture filtrate experiment in which A. bisporus and M. oreades were grown on a medium which had previously been used as a substrate for P. artotrogus, indicated that P. artotrogus was more antagonistic to M. oreades than to A. bisporus. However, the data in Table 2 indicate that P. artotrogus is more antagonistic to A. bisporus than to M. oreades. The latter situation is more likely the correct one, since the toxic metabolite(s) produced by P. artotrogus in the used culture filtrate experiment had been autoclaved.

The culture filtrate of P. artotrogus was shown to completely inhibit the growth of M. oreades within twenty minutes. Furthermore, the activity of the filtrate was not reduced when it was stored at 7° C. The biological activity of the filtrate was lost, however, when dried under vacuum at 60° C. It appears that the toxic agent(s) of the filtrate is thermolabile.

Drechsler (14) reported that P. oligandrum parasitized twelve species of Pythium including P. arrhenomanes, P. butleri (synonymous with P. aphanidermatum (26)), P. debaryanum, P. graminicolum, P. irregulare, P. splendens and P. ultimum. Of the species tested, P. artotrogus has been shown to parasitize all of these species, except P. graminicolum and P. arrhenomanes. Although not parasitic, the hyphae of P. artotrogus was observed to coil around the hyphae of P. arrhenomanes. Why the hyphae of P. artotrogus coiled around the hyphae of P. arrhenomanes, but failed to penetrate,

cannot be explained at this time. One could argue that P. artotrogus might have parasitized the hyphae of P. arrhenomanes if the culture slides had been incubated for more than forty-eight hours. This is a possibility, but it does not seem likely since spores of such slower growing fungi have been shown to germinate, grow extensively and produce appressoria on the surface of a leaf all within thirty-six hours (1).

Drechsler's (13,14) description, drawings and photographs of parasitic P. oligandrum in association with a susceptible Pythium species are very similar to those presented in this paper for P. artotrogus.

Butler (8) reported that Rhizoctonia solani parasitized several Pythium species. The hyphae of R. solani were observed to coil around the Pythium hyphae prior to penetration. He reported that coiling and penetration was the result of a thigmotropic response. Penetration of the hyphae was stated to be the result of physical pressure and not enzymatic decomposition of the wall material. Concerning the initiation of parasitism, Butler (8) stated: ".....hyphal coils and infection hyphae are formed by R. solani as a result of chemical stimuli produced on the surface of the hyphae of the host and parasite respectively. These substances seem to act in a complementary manner to determine the mode of infection. It could be further postulated that the relative amounts of each of the substances produced by the host and parasite determine the intensity of penetration and formation of parasitic coils."

In his paper, Drechsler (14) reviewed a number of articles in which P. artotrogus was reported to be closely associated with Phytophthora and other Pythium species. De Bary as early as 1881 was reported to have noticed this association and suggested that P. artotrogus may be a parasite on congeneric forms. Many of these earlier workers based their identification of P. artotrogus on the size and shape of the spiny oogonia. Since he found three species of Pythium, each producing spiny oogonia, to be parasitic on other Pythium species, Drechsler suggested that the fungi may have been improperly identified. The present investigation has shown that P. artotrogus is, as was suggested by de Bary, a parasite of Pythium species.

The effect of temperature on growth of P. artotrogus has been reported by Middleton (26) and Fergus et al. (15). These workers used corn meal agar as a substrate, while a 2 per cent glucose agar medium was used in the present investigation. Pythium artotrogus grew at all temperatures at which M. oreades grew, as well as at higher temperatures. The optimum temperature (25° C to 27° C) for both organisms was nearly identical. The growth rate of P. artotrogus was shown to be greater than that of M. oreades at all temperatures, and was greater by a factor of almost ten at the optimum temperature. Insofar as temperature requirements, P. artotrogus could qualify as a successful biological control agent for M. oreades.

The hydrogen-ion requirement was quite similar for both

P. artotrogus and M. oreades. The optimum pH for growth of both organisms was about 5.5. The range at which growth of P. artotrogus occurred was greater than that of M. oreades. The growth rate of P. artotrogus was greater than that of M. oreades at each pH value tested.

The isolate of M. oreades used in this investigation was obtained from an athletic field in which the pH of the soil ranged from 4.7 to 5.0. The mycelium of this fungus was found in abundance in the soil, as well as in the layer of thatch composed primarily of living and dead plant tissues. The pH of the layer of thatch varied from 5.0 to 6.0. The relatively high pH of the thatch layer was probably due to previous limestone applications on the foliage. Hence, present data indicate that hydrogen-ion concentration would rarely limit the effectiveness of P. artotrogus as a biological control agent for M. oreades. Similarly, Agaricus bisporus (campestris) has been shown (16) to tolerate a pH range from about 4.0 to 8.0 with an optimum of 6.0. Fergus et al. (15) reported that the growth of A. bisporus is completely inhibited by P. artotrogus in commercial compost beds.

Sprague (34) observed that P. arrhenomanes, P. debaryanum, P. irregulare and P. ultimum sometimes cause seed rot of species of Gramineae. The results of the pathogenicity tests conducted in the present study show that P. aphanidermatum, P. graminicolum and P. splendens can be added to this list. Pythium artotrogus, however, was shown to cause a negligible amount of seed rot of the four Agrostis species used in this study.

The results of the pathogenicity tests of Pythium species to seedlings of Agrostis species did not agree completely with the data in the literature. Pythium aphanidermatum has been reported to cause a blighting of the foliage of A. palustris (28,29), which agrees with the data presented in this study. Moore et al. (30) found that P. ultimum caused a blight of Highland Colonial bentgrass, but in the present study it did not blight the foliage of Astoria Colonial bentgrass.

Pythium artotrogus caused no significant injury to any of the Agrostis seedlings tested. Unlike other species of Pythium which were antagonistic to M. oreades, P. artotrogus could be used as a biological control agent without causing injury to Agrostis species.

Although Fergus et al. (15) reported that P. artotrogus completely inhibited the growth of A. bisporus in manure compost, its growth was retarded about 70 per cent and that of M. oreades about 60 per cent under the laboratory conditions used in this study. Studies showed that P. artotrogus would not grow completely through the mycelium of either A. bisporus or M. oreades. Bayliss-Elliott (4,5) pointed out that the only direction in which the mycelium of a fairy ring grows is in an outward direction. Thus, for P. artotrogus to be a successful biological control agent, the fungus will have to be applied to the turf in advance of the fairy ring symptoms.

Temperature and hydrogen-ion tolerance studies have

shown that the growth rate of P. artotrogus was greater than M. oreades at all temperatures and hydrogen-ion concentrations tested. This also supports the possible use of P. artotrogus as a biological inhibitor of M. oreades.

Although other Pythium species were shown to be more antagonistic to M. oreades, P. artotrogus was the only Pythium species tested that was not pathogenic to four bentgrass species, an important consideration when selecting a biological control agent.

These data indicate that P. artotrogus has the potential for use as a biological control agent for fairy ring disease caused by M. oreades.

XII. CONCLUSIONS

1. Direct application of chemicals for the control of the Marasmius fairy ring disease of turfgrass is not practical at present.
2. Although species of Pythium have not been considered as being antagonistic to other fungi, it has been shown that many species of Pythium are antagonistic to A. bisporus and to M. oreades. Pythium artotrogus was more antagonistic to A. bisporus than any of the other Pythium species tested. Pythium ultimum and the Hawaiian isolate of P. irregulare displayed greater antagonism towards M. oreades.
3. Pythium artotrogus evidently produces a toxic metabolite(s) which inhibits the growth of M. oreades. The toxic agent(s) is at least partially water soluble and will diffuse through agar, but it is thermolabile.
4. Pythium artotrogus is not endoparasitic on the hyphae of A. bisporus or M. oreades.
5. Pythium artotrogus was shown to be endoparasitic in the hyphae of P. aphanidermatum, P. debaryanum, P. irregulare, P. splendens and P. ultimum.
6. The hyphae of P. artotrogus were observed to coil around the hyphae of the host prior to penetration. The infection hyphae of P. artotrogus grew lengthwise within the host's hyphae causing degeneration of the protoplasm.
7. Temperature and hydrogen-ion tolerances of P. artotrogus and M. oreades are similar.
8. Pythium artotrogus was shown to cause no injury to

seeds or seedlings of four species of Agrostis.

XIII. SUMMARY

Fairy ring of turfgrasses, caused by Marasmius oreades and other fungi, have long been a problem to those interested in maintaining fine turf. Chemical control of Marasmius fairy ring has been shown to be ineffective at the present time, thus other types of control measures were sought.

This investigation has shown that many Pythium species inhibit the growth of A. bisporus and M. oreades. Pythium artotrogus was more antagonistic to A. bisporus than any of the other Pythium species tested. Pythium ultimum and an isolate of P. irregulare were more antagonistic to M. oreades than was P. artotrogus. Thus it appears that Pythium species may play an important role in the ecology of soil inhabiting fungi.

Pythium artotrogus was shown to inhibit mycelial growth of M. oreades by the production of a toxic metabolite(s) which diffuses through an agar medium. When a culture filtrate of P. artotrogus was applied directly to the hyphae of M. oreades, growth of the latter was inhibited completely within twenty minutes. The activity of the filtrate was not reduced when stored at 7° C, but proved to be thermolabile.

Pythium artotrogus was not endoparasitic in the hyphae of A. bisporus or M. oreades. It did parasitize the hyphae of P. aphanidermatum, P. debaryanum, P. irregulare, P. splendens and P. ultimum. The hyphae of P. artotrogus coiled around the hyphae of a susceptible host. The parasite formed an appressorium on the surface of the host hypha. Following

penetration, the infection hypha grew lengthwise within the hypha of the host, degenerating the protoplasm as it advanced. Although not parasitic, the hyphae of P. artotrogus coiled around the hyphae of P. arrhenomanes. Pythium artotrogus did not form coils and was not parasitic on its own or on P. graminicolum hyphae.

Studies on the effect of temperature on linear growth of M. oreades and P. artotrogus have shown that the optimum temperature for both organisms to be near 27° C. The minimum temperature at which growth occurred for both fungi was about 5° C. The maximum temperature at which growth of M. oreades occurred was above 30° C, while the maximum temperature for growth of P. artotrogus was between 34° and 40° C. At the various temperatures tested P. artotrogus had a growth rate of two to ten times greater than that of M. oreades.

Although many species of Pythium were shown to be strongly antagonistic to A. bisporus and to M. oreades, only P. artotrogus was shown, in laboratory tests, to be nonpathogenic to four Agrostis species. Thus it appears that P. artotrogus has the potential to be used as a biological control agent for M. oreades, the causal organism of Marasmius fairy ring.

XIV. LITERATURE CITED

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