THESIS

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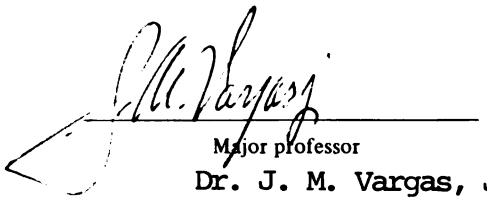
THE ISOLATION, CHARACTERIZATION AND CONTROL OF RHIZOCTONIA CEREALIS CAUSING YELLOW PATCH ON POA PRATENSIS L. IN MICHIGAN

presented by

Cynthia L. Brown

has been accepted towards fulfillment
of the requirements for

M.S. degree in Plant Pathology



Major professor
Dr. J. M. Vargas, Jr.

Date June 5, 1984

THE ISOLATION, CHARACTERIZATION AND CONTROL OF *RHIZOCTONIA CEREALIS* CAUSING YELLOW PATCH ON *POA PRATENSIS* L. IN MICHIGAN

By

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ABSTRACT

THE ISOLATION, CHARACTERIZATION AND CONTROL OF *RHIZOCTONIA CEREALIS* CAUSING YELLOW PATCH ON *POA PRATENSIS* L. IN MICHIGAN

By

Cynthia L. Brown

A new disease was recognized on *Poa pratensis* in Michigan in 1981 and 1982. Symptoms developed during the spring and fall, appearing in patches or "frog-eye" patterns. A *Rhizoctonia*-like fungus was consistently isolated from diseased foliar and crown tissues. The pathogenicity of these fungi was demonstrated in growth chamber and field studies. The fungi were characterized based on nuclear state, growth rate and cultural characteristics. The isolates were binucleate and produced buff pigmented mycelium and yellow to brown sclerotia. Hyphal anastamosis testing done with *Ceratobasidium* anastamosis group (CAG) testers revealed the isolates to anastamose only with the tester from CAG1, the group comprised of *R. cerealis*. The fungi were identified as *R. cerealis*, reported to cause yellow patch of turfgrasses.

Fungicides were screened for eventual use in a yellow patch management program. Iprodione, triadimefon, chlorothalonil, propiconazol and benomyl warrant further testing in field control studies.

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INTRODUCTION AND LITERATURE REVIEW

Kentucky bluegrass (*Poa pratensis* L.) is the most important and widely utilized of the cool season turfgrass species (2). It is native to Eurasia and has become widely distributed throughout the cool temperate and transitional climates of the world (2). It is a perennial grass propagated primarily by seed; however, with vigorous rhizome development and an extensive root system, it is widely used in commercial sod production (2). When properly maintained, Kentucky bluegrass can form a high quality turf for lawns, athletic fields, golf courses and other general purpose turf areas.

Considerable variability in leaf texture and color, growth habit, shoot density, rhizome development and particularly disease resistance exists among cultivars of Kentucky bluegrass (2). Some diseases that may cause severe problems on Kentucky bluegrass include rust, stripe smut, *Fusarium blight*, the snow molds, *Pythium* blight, dollar spot and brown patch. These diseases are of fungal etiology and can generally be managed by early diagnosis, the implementation of wise cultural practices and carefully timed fungicide applications.

A new problem was recognized through the mid to late seventies when turfgrass researchers reported disease symptoms on Kentucky bluegrass, bentgrass (*Agrostis* spp.), and zoysiagrass (*Zoysia* spp.) (11,30,31). The symptoms appeared during periods of cool, wet weather and were described as patches of foliar blighted turf or chlorotic "frog-eyes".

The "frog-eyes" closely resembled those associated with the disease Fusarium blight which led to the suggestion that possibly a "cool weather Fusarium" was responsible (Vargas, personal communication). Fungi that resembled Rhizoctonia solani Kuhn in hyphal and cultural appearance were isolated from the chlorotic "frog-eyes" or foliar blight lesions (11,30,31). R. solani, the cause of brown patch of turfgrasses, is usually associated with periods of humid weather and air temperatures in the mid-eighties (10,36). The organism inciting the newly recognized disease problem was termed the "cool weather Rhizoctonia" (31).

In 1977 Sanders et al. (31) reported preliminary results of studies on the "cool weather Rhizoctonia" turfgrass pathogens. The majority of the isolates were binucleate and believed to be species of Ceratobasidium. The optimum temperature range for in vitro growth of the binucleate isolates was 21-23°C. Greenhouse pathogenicity tests conducted at 10, 17-20, 21-26 and 32-38°C demonstrated that the binucleate isolates were highly virulent on bentgrass over a wide range of temperatures. Nearly 100% foliar blighting occurred within the range of 10-26°C. In contrast, an isolate of R. solani causing brown patch on bentgrass was found to have an optimal in vitro growth temperature of 28°C and caused no apparent symptoms on bentgrass below 17°C. These growth and pathogenicity differences between the binucleate and multinucleate Rhizoctonia spp. are in agreement with the temperatures at which the respective disease symptoms occurred in the field.

The characteristics considered most critical for the accurate identification of R. solani are multinucleate hyphal cells with prominent septal pore apparatus and the production of the Thanatephorus cucumeris sexual (perfect) state (13). In 1967 Parmeter et al. (26) reported the

finding of R. solani-like fungi isolated in the United States from a number of hosts including strawberry, potato and Bermudagrass. Comparative studies found that isolates with hyphal and cultural characteristics of R. solani could be separated into two distinct groups. One group had multinucleate hyphal cells and produced a T. cucumeris sexual state, and the other group had binucleate hyphal cells and produced a Ceratobasidium sexual state. The perfect state of the binucleate isoltes could be assigned to Ceratobasidium Rogers on the basis of fruiting habit, basidial morphology and repetitive spore germination as discussed by Talbot (33). On the average, the binucleate isolates had smaller hyphal diameters and slower growth rates than the multinucleate isolates.

The identification of Rhizoctonia species is complicated by the fact that many of the Rhizoctonia fungi possess asexual structures that are morphologically similar (34). Incorrect identification of some fungi as R. solani has undoubtedly occurred in the past because of the heavy reliance on features that are highly variable and not unique to R. solani (31). Such characteristics as branching patterns, dolipore septation and shades of brown mycelial pigmentation occur in numerous Rhizoctonia species (34). R. cerealis was described by van der Hoeven (4) as being distinguishable from R. solani by its slower radial growth rate, smaller hyphal diameters and binucleate hyphal cells. Of the four Rhizoctonia spp. recognized as pathogens on cultivated turfgrasses, R. solani and R. zeae Voorhees are multinucleate, R. oryzae Ryker and Gooch has four nuclei per cell and R. cerealis is binucleate (32).

Burpee suggested that host specificity and anastamosis reaction may be of taxonomic value in identifying R. cerealis (6). In 1980, Burpee

et al. (8) established seven Ceratobasidium anastamosis groups (CAG) based on hyphal anastamosis. Pairings were made among isolates of C. cornigerum (Bourd) Rogers, C. anceps and related binucleate fungi having Rhizoctonia imperfect states. Seven CAG were established and the only CAG to exhibit any homogeneity with regards to the host of the isolates was CAG1. The isolates in CAG1 were isolated from hosts of the Gramineae and included the "cool weather Rhizoctonia" turfgrass pathogens. Burpee (6) identified the "cool weather Rhizoctonia" turfgrass pathogens as R. cerealis van der Hoeven. R. cerealis was originally described in 1977 as the causal agent of sharp eyespot lesions on wheat in the Netherlands (4). Identification was based on hyphal anastamosis between the isolates of CAG1 and the R. cerealis type culture. The R. cerealis type culture failed to anastamose with CAG2 through CAG7 (6). Anastamosis has been useful in the identification of other fungi since hyphal anastamosis is considered evidence that two mycelia represent the same species (12). The R. cerealis isolates collected to date have been host specific to the Gramineae and make up the CAG1 which is distinct from R. solani and other Rhizoctonia spp. (6). Lipps and Herr (21) suggest that growth rate may facilitate in the separation of R. cerealis from other binucleate isolates.

For the disease formerly known as cool weather brown patch caused by the "cool weather Rhizoctonia" Burpee (6) proposed assigning the name "yellow patch" to the disease of cultivated turfgrasses caused by R. cerealis. Symptoms have been observed primarily on creeping bentgrass and Kentucky bluegrass (9), however, there have also been reports of symptoms occurring on tall fescue, zoysiagrass, Bermudagrass and St. Augustinegrass (6,17). Greenhouse host range studies indicated that

perennial ryegrass (Lolium perenne) and tall fescue (Festuca arundinacea) were susceptible and may be potential hosts (7). Koch's postulates have been satisfied on a turfgrass host primarily for the foliar blight symptom and have been performed in the greenhouse and the growth chamber.

No fungicides are currently labelled for management of R. cerealis on turfgrasses (17). Sanders et al. (31) reported significant radial growth inhibition of the "cool weather Rhizoctonia" in vitro with chloroneb, chlorothalonil, benomyl or iprodione. Martin (24) examined the sensitivity of binucleate R. solani-like fungi isolated from tall fescue to benomyl, carboxin and PCNB. In vitro radial growth inhibition was greatest on the benomyl amended PDA. Van der Hoeven et al. (35) reported radial growth inhibition of several isolates of R. cerealis from wheat on benomyl amended PDA. There are no reports of any in vivo greenhouse or field fungicide evaluation studies.

During 1981 and 1982 Kentucky bluegrass exhibited foliar blight and/or crown rot in many locations in Michigan. R. solani-like fungi were consistently isolated from the affected tissues. Macro symptoms in the field appeared as "frog-eyes" or patches of dead grass or round patches of foliar blighted turf. The symptoms appeared in the spring and fall which are seasons of the year normally associated with cooler environmental conditions.

The objectives of the following investigations were to 1) describe the symptomatology, 2) determine if the etiology of this new disease in Michigan is R. cerealis, and 3) screen potential antifungal control chemicals.

SECTION I

ETIOLOGY OF THE COOL SEASON BLIGHTING OF *POA PRATENSIS* L. IN MICHIGAN

Considerable confusion and speculation followed the recognition in the late seventies of unusual symptoms occurring during cool weather on Michigan home lawns. Symptoms of the disease were associated with Kentucky bluegrass sod during "cooler" environmental conditions; however, the etiology of the problem remained unknown.

The objectives of these investigations were to 1) determine the causal agent of the symptoms and 2) provide a useful diagnostic description of the symptoms incited by the organism.

Materials and Methods

Diagnostic Description of Symptoms. Kentucky bluegrass plants affected with this disease were observed *in situ* for macroscopic symptoms at several locations. Photographs were taken and samples of diseased turfgrass were brought to the laboratory for microscopic observation. Numerous samples were also received by Michigan State University during the periods of spring and fall, 1981 and 1982. Leaf blades, crowns and roots were removed from samples and closely examined for any lesions and microorganisms with the aid of a binocular stereo microscope and a compound microscope.

Association and Isolation of the Causal Organism. Diseased leaf and crown tissues were washed in tap water, surface disinfected in 10.0% sodium hypochlorite solution for 1-2 minutes, rinsed in sterile deionized water and placed in petri plates containing either potato dextrose agar (PDA, Gibco Laboratories, Madison, Wisconsin) or water agar (Bacto-Agar, Difco Laboratories, Detroit, Michigan). The plates were incubated in the laboratory under a 12 hour light/dark cycle at 21±1°C and examined periodically for the growth of potential pathogens. Pure culture isolates of fungi were obtained by transferring hyphal tips to PDA and water agar slants and stored at 4°C.

Pathogenicity. To determine the pathogenicity of the isolated fungi, inoculations were performed in a growth chamber. Field inoculation studies were conducted in an attempt to duplicate disease symptoms under natural conditions. All inoculum was prepared by autoclaving 100 ml of whole wheat seeds and 75 ml of distilled water in a 500 ml Erlenmeyer flask for one hour on two consecutive days. Colonized agar disks from pure cultures of each isolate were transferred to the flasks and incubated at 21±1°C for 14 days. Inoculum was removed from the flasks and stored in plastic bags at 4°C until use.

Growth chamber inoculations: Inoculations were made by placing three wheat seeds infested with an isolate at the base of the leaf blades of six week old 'Touchdown' Kentucky bluegrass plants grown in the greenhouse in three inch plastic pots. The plants were fertilized twice with a moderate rate of complete soluble fertilizer and trimmed to a height of 2 1/2-3" every 10 days. Autoclaved, non-fungus-infested wheat seeds were used to inoculate the control plants. Each treatment was replicated four times. After inoculation the plants were misted with

sterile distilled water and placed in plastic bags in temperature controlled growth chambers. The temperature and relative humidity inside the bags were maintained at 20±2°C and 100%, respectively. The photoperiod in the growth chamber was 12 hours. The pots were misted once daily with sterile deionized water to maintain leaf wetness. Four days after the inoculations were made the pots were removed from the plastic bags, disease severity evaluations were made and attempts were made to reisolate the fungi from the necrotic tissues. The inoculation procedure was repeated one time.

Field inoculations: Field inoculations were made on a block area of two year old seeded 'Fylking' Kentucky bluegrass located at the Michigan State University Hancock Turfgrass Research Center in East Lansing, Michigan. Ten pieces of infested wheat seed were placed at the base of the plants within the thatch layer at each inoculation site. Placement of the inoculum was standardized within each 4 X 4' block by using a piece of poster board from which a square had been cut out of the center (Figure 1). The inoculum was placed at each of the four corners of the center square. One square was used for the four replications of each isolate. Inoculations were done on September 14, 1982, using Michigan isolates 1 through 5 and autoclaved, non-fungus-infested wheat seeds to inoculate the control block. The inoculation area was fertilized twice with urea during the summer of 1982. One pound of nitrogen per thousand square feet was applied in June and another pound at the end of July. Following the inoculations the area was irrigated only as necessary to prevent wilting. No cover was placed over the inoculated area. Thirty days following inoculation disease symptom ratings were made. Disease symptom ratings were made by examining two variables: the width of the

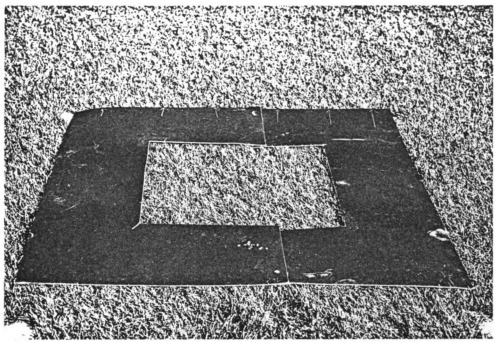


Figure 1. The placement of inoculum was standardized in the field inoculations by using a piece of poster board. Within each block the wheat seed was placed at each of the four corners of the inside square.

affected patch and the severity of the damage within the patch as described on page 21. Attempts were made to isolate the fungi from necrotic tissues.

Environmental Conditions Associated with Natural Symptom Development. Measurements of the daily rainfall and air temperature were recorded throughout the spring of 1982 at the MSU Hancock Turfgrass Research Center. The amount of rainfall was measured by a rain gauge and the average daily air temperature was calculated as an average of twenty-four hourly readings recorded by a hygrothermograph.

Results and Discussion

Diagnostic Description of Symptoms. Two distinct symptoms atypical for that time of the year were observed on Kentucky bluegrass in Michigan. The first symptom was noted to occur on sodded turfs. The plants were killed in either a patch or "frog-eye" pattern (Figure 2) and under microscopic examination exhibited a reddish-brown crown and root necrosis. Dark colored sclerotia were abundant on the necrotic tissues (Figure 3). Dark colored fungal mycelium was also found to be present on sheath and crown tissues, growing along the junctures between epidermal cells. Plants showing no apparent symptoms exhibited neither the rot or the presence of the fungal structures. Plants with reddened leaf blades were often present in the outer margins of the diseased patches (Figure 4). No wilting stage was associated with symptom development. Little recovery occurred during the season of injury. These symptoms are similar to those of yellow patch as described by Joyner (17) and cold temperature brown patch as described by Hirrell and Shurtleff (15).

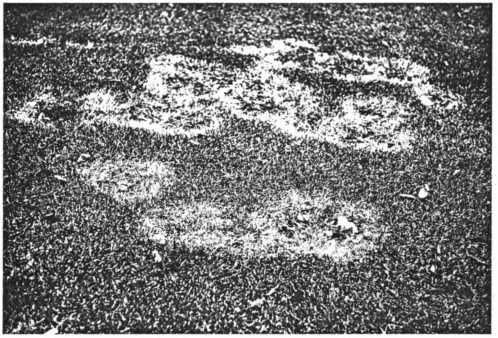
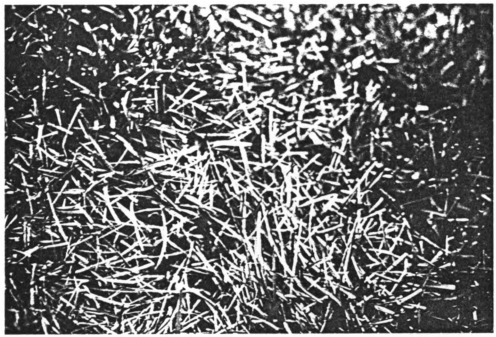


Figure 2. The patch and "frog-eye" symptoms associated with the cool season blighting of Kentucky bluegrass in Michigan.

3.

4.



Figures 3 and 4. Features associated with the diseased Kentucky bluegrass. (3) Dark fungal mycelium and sclerotia are found on the crown, sheath and rhizome tissues of the diseased plants. (4) Plants with reddened leaf blades are present at the margins of the diseased patches.

The second symptom is a blighting which affects foliar tissues. It has been reported infrequently in Michigan, occurring primarily on seeded turfstands. The blighting occurs in round patches 15-100 cm in diameter and causes the turf to become tan to bleached in color (Figure 5). The discoloration of the turf results from the extensive foliar blighting. Foliar lesions ranged in size from 0.5 to 2.5 cm in length, and usually encompassed the entire width of the blade. The affected area on the leaf was straw to ash brown in color and often surrounded by a darker colored border. The crown and root tissues of these plants appeared healthy and within one month the foliar lesions had been mowed off, leaving little indication of the earlier present necrotic symptom. These symptoms are similar to those of yellow patch as described by Burpee (6) and those caused by the "cool weather Rhizoctonia" as described by Sanders et al. (31) and Dale (11).

Early observations suggested an apparent difference in the severity and occurrence of symptoms on sodded versus seeded lawns. Five of the six early reports of this disease problem were on sodded lawns less than five years old. Upon consideration of these two methods of lawn establishment two major factors are noted which may be contributing to the variation. First, the turfgrass species and cultivars used in sodded and seeded lawns in Michigan are usually different. The infected sod has been reported to be Kentucky bluegrass blends of three or four of the new "improved" cultivars such as 'Touchdown', 'Cheri', 'Adelphi', 'Victa', and 'Baron'. In contrast, seed purchased by the homeowner for the planting of a lawn usually contains the seeds of common or older Kentucky bluegrass cultivars, fine leaf fescue or ryegrass, but little or none of the "improved" Kentucky bluegrass cultivars. The fact that the cultivars and

Figure 5. The field symptom of the less severe cool season foliar blight.

plant species which are used on sod and seed are different suggests the role genetic factors may play in disease development.

A factor which may contribute to the increased susceptibility of some sod plantings to this disease is the adverse conditions under which sod is sometimes established. Proper site preparation is critical for the successful establishment of any turf area (3,28) and it is not unusual to visit a site where the topsoil was removed and sod laid directly onto the clay subsoil. This type of soil compacts easily and is not favorable to root development (3,28). Sodded turf is often associated with rapid thatch accumulation. The disadvantages of a thick thatch are numerous and include decreased heat, cold and drought tolerance and increased insect and disease problems (2). The fact that disease symptoms have not been reported to occur before the sod has been cut and removed from the farm and that a sod farmer may sell his sod to several different customers and only one customer's lawn develops disease symptoms suggests that establishment conditions may play a role in predisposing potentially susceptible plants to this disease.

Association and Isolation of the Causal Organism. Several isolates of a Rhizoctonia sp. were obtained from the infected tissues as listed in Table 1. It was noted that the isolation of the Rhizoctonia sp. was more difficult from crown than from foliar tissues. Water agar supported more rapid growth of the Rhizoctonia sp. and facilitated in the separation of the Rhizoctonia sp. from the other fungi present on the plant tissues. Tentative identification of the fungi as Rhizoctonia sp. was based exclusively on the hyphal morphological and cultural characteristics as described for R. solani by Parmeter (27). These characteristics include 1) branching near the distal septum of cells in young vegetative hyphae,

Table 1. *Rhizoctonia* sp. associated with the cool season blighting of *Poa pratentsis* L. in Michigan

Isolate Designation	Geographical Location	Plant Tissue
1	Lansing, Michigan	Foliage
2	Detroit, Michigan	Crown
3	Lansing, Michigan	Crown
4	Flint, Michigan	Foliage
5	Pontiac, Michigan	Foliage
6	Lansing, Michigan	Foliage

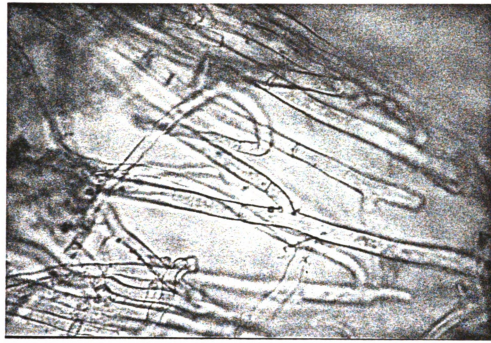
2) constriction of the branch and the formation of a septum in the branch near the point of origin and 3) some shade of brown pigmentation in culture (Figures 6a and b).

Pathogenicity.

Growth chamber inoculations: Michigan isolates 1 through 6 caused disease symptoms in all of the performed inoculations. The fungi had grown out from the wheat seeds within 24 hours and within 48 hours watersoaking of the leaf tissues had occurred. Leaves became matted together with mycelium and the watersoaked areas turned tan to golden-orange in color. Lesions were present on 40-50% of the leaf blades after four days in all four of the inoculation pots tested with each isolate. No lesions were present on any of the control plants. Ten blades with necrotic lesions were removed from the inoculation pots of each isolate and surface disinfected for 2 minutes in 10.0% sodium hypochlorite solution. The blades were rinsed in sterile deionized water and plated out on PDA. A Rhizoctonia sp. was isolated from 100% of the necrotic blades. A Rhizoctonia sp. was not isolated from ten blades from the control plants.

Field inoculations: Several of the isolates tested caused symptom development in the field inoculations. The infected plants occurred in round patches ranging in diameter from 1-14 cm. Symptom severity within each patch varied from a few foliar lesions to nearly 100% foliar blight as shown in Figure 7. Foliar symptoms included bleached lesions with dark borders and reddened leaf blades. Some plants exhibited stem discoloration and a limited number of plants were killed; the crown and root systems heavily rotted. No dark mycelium or sclerotia were present on any of the necrotic tissues. The control inoculations showed no

a.



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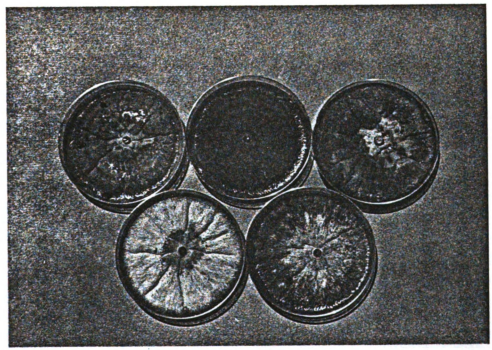

Figure 5. The cool season pathogens exhibited hyphal morphological and cultural characteristics commonly associated with Rhizoctonia solani. These include typical hyphal branching and septation (a) and shades of brown mycelial pigmentation (b).

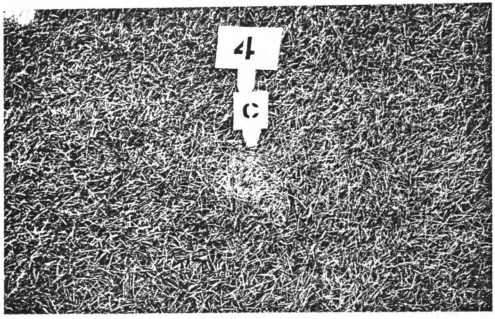

Figure 7. Blighting caused by Michigan Rhizoctonia isolate 2 in the field inoculations.

evidence of foliar lesions or leaf reddening. The data from the field inoculations are presented in Table 2.

A number of blighted plants were removed from the patches. From these plants ten necrotic blades and four crowns were washed in tap water for four hours, surface disinfected in 10% sodium hypochlorite for 1-2 minutes, rinsed in sterile deionized water and plated out on PDA. A Rhizoctonia sp. was isolated from 100% of the leaf tissue tested. No Rhizoctonia sp. was isolated from the crowns. An additional attempt to isolate a Rhizoctonia sp. from three partially rotted crowns failed. No Rhizoctonia sp. was isolated from blades or crowns of plants from within the control block.

The preceding growth chamber and field inoculations have demonstrated the pathogenicity of the isolated Rhizoctonia sp. on a Poa pratensis host. The production of symptoms similar to those of natural infections supports an association of these fungi with the cool season blighting of turf in Michigan in 1981 and 1982. Based on the demonstrated pathogenicity of the Rhizoctonia sp. isolated from cool seson blighted turfgrass it is hypothesized that these fungi are isolates of "cool weather Rhizoctonia" or R. cerealis, the causal agent of the turfgrass disease yellow patch.

The success of the inoculation technique is of paramount importance due to the difficulty researchers have had conducting field studies. Although there was considerable variation between the replicates of each isolate this may have been due to some variation in the inoculum such as unequal amounts of mycelial growth on the seeds. If that was the problem, it could be easily rectified and this inoculation method used in fungicide and fertility studies. At the present time there are no

Table 2. Results after 30 days of field inoculations using five *Rhizoctonia*-like fungi isolated from cool season blighted *Poa pratensis* L.

Block No./Isolate No.	Diameter of Affected Area (cm)	Symptom Severity Within Measured Area (as described below)
1 / 5	7	1
	-	-
	9	2
	12	2
2 / 4	6	1
	6	1
	8	3
	12	1
3 / Control	-	-
	-	-
	-	-
	-	-
4 / 2	6	2
	5	1
	14	5
	8	3
5 / 1	-	-
	11	1
	-	-
	5	1
6 / 3	-	-
	-	-
	-	-
	-	-

Symptom Severity Scale:

1 = symptoms just visible; foliar lesions or reddening.
2 = up to 25% of the plants within measured area blighted.
3 = 25-50% of the plants within measured area blighted.
4 = 50-75% of the plants within measured area blighted.
5 = 75-100% of the plants within measured area blighted.

evaluations regarding the susceptibility of various Kentucky bluegrass cultivars to disease symptoms caused by these Rhizoctonia sp. Field inoculations on different cultivars of seeded and sodded turf may also be useful in establishing the role of genetic factors in disease development.

Environmental Conditions Associated with Natural Symptom Development. The rainfall and air temperature conditions which preceded the appearance of the Rhizoctonia sp. incited foliar blight symptoms on a seeded block of 'Fylking' Kentucky bluegrass in Michigan were recorded (Figure 8). This area did not receive any irrigation. The natural symptoms (Figure 5) appeared over the weekend of May 31, 1982.

The average daily air temperature was between 19 and 23°C for approximately five days before the time of symptom appearance. A temperature pattern very similar was noted between the 12th and 16th, however, no symptoms developed. Although air temperatures were similar during these two time periods the symptoms appear to have developed only after an increase in the amount of rainfall. This is preliminary data, however, it supports the hypothesis that these Rhizoctonia fungi are "cool weather Rhizoctonia" as described by Sanders et al. (31).

Figure 8. The average daily air temperature and rainfall which preceded the development of naturally occurring foliar blight symptoms on or close to May 31, 1982.

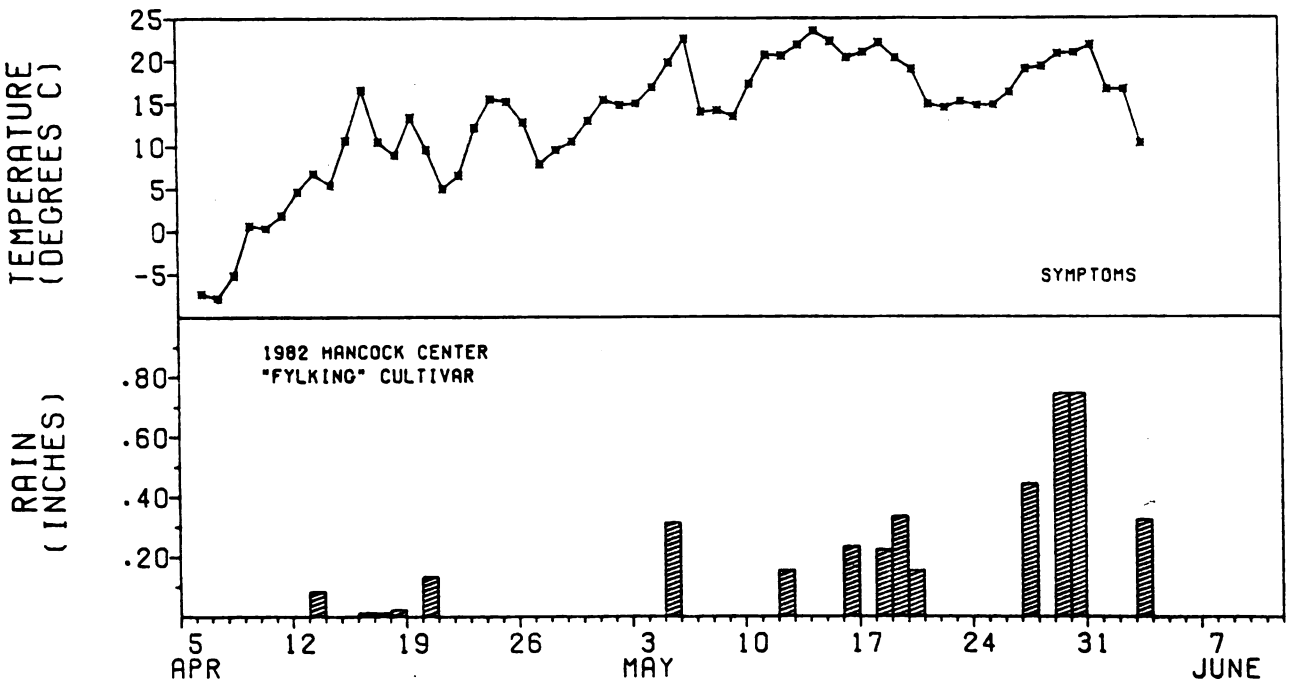
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Figure 8.

SECTION II

CHARACTERIZATION/IDENTIFICATION OF THE *RHIZOCTONIA* SP. ISOLATED FROM COOL SEASON BLIGHTED *POA PRATENSIS* L.

In the previous findings a *Rhizoctonia*-like fungus was consistently associated with cool season blighted Kentucky bluegrass in Michigan. The objectives of these investigations are to characterize and identify the isolated *Rhizoctonia* fungi. Culture on agar, temperature-growth relations, nuclear state and hyphal anastomosis are characteristics that are commonly used in identifying *Rhizoctonia* spp.

Materials and Methods

Cultural Characteristics. Agar disks 5 mm in diameter were cut from the margins of actively growing colonies and transferred to 15 mm X 100 mm plastic petri plates containing 20 ml of PDA. The plates were incubated in darkness at 21±1°C. After 40 days the cultural and sclerotial characteristics of each of the Michigan isolates, the *Ceratobasidium* anastamosis group testers (CAG1-CAG7) and an isolate of *R. solani* were compared. The CAG testers were obtained from Patricia Sanders, Pennsylvania State University, University Park, Pennsylvania and Brian Olsen, Cornell University, Geneva, New York.

Temperature-Growth Relations. To compare the *in vitro* growth rates of each isolate at various temperatures agar disks 5 mm in diameter were cut with a cork borer from the margins of actively growing colonies and

transferred to 15 mm X 100 mm plastic petri plates containing 20 ml of PDA. Each isolate was incubated at temperatures of 10, 16, 23 and 28°C in the dark. Colony diameter was measured at 24 hour intervals for up to 6 days. Each treatment was replicated three times.

Nuclear Staining. Each isolate was stained using an HCl-Giemsa nuclear staining technique developed by Herr (13). This technique allows the determination of the number of nuclei in vegetative cells. An isolate known to be R. solani was stained as a control.

Hyphal Anastamosis. To observe hyphal anastamosis a technique described by Herr and Roberts (14) was employed. The method involves the pairing of unknown isolates with known tester isolates followed by examination of the point of contact between the hyphal growth of the two isolates. Autoclaved microscope slides were dipped in molten autoclaved 2% water agar and placed in petri plates containing solidified water agar for the maintenance of moisture. 3 mm X 15 mm sections of the fungal isolates to be paired were removed from the margins of colonies actively growing on PDA and placed approximately 2.5 cm apart on the agar-coated slide (Figure 9). The petri plates were covered, placed in plastic bags and incubated at room temperature. When the advancing hyphae from the opposing colonies had made contact and were slightly overlapped the slides were removed from the petri plate. A drop of 0.05% cotton blue was placed on the area of hyphal contact, covered with a cover slip and examination for hyphal anastamosis was made. The Michigan isolates were opposed in all possible combinations and with known testers from CAG1 (Ceratobasidium anastomosis group) through CAG7.

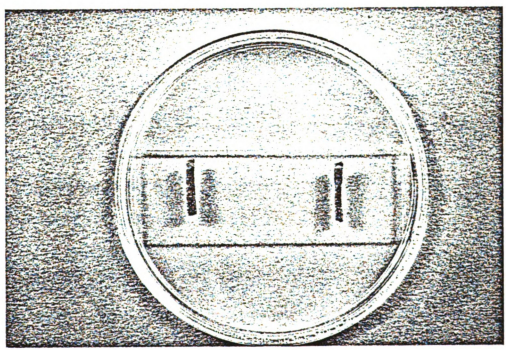


Figure 9. Mycelial fragments of the isolates were placed parallel on the water agar coated slides. The area of hyphal contact was stained and observations made for the occurrence of hyphal anastamosis.

Results and Discussion

Cultural Characteristics. Variation existed in the mycelial and sclerotial characteristics of the Rhizoctonia isolates examined, but some trends were apparent (Table 3). With the exception of isolate 4, all of the Michigan Rhizoctonia fungi isolated from the cool season blighted turfgrass produced mycelium which was buff in color. The pigmentation of isolates increased with time to darker shades of brown (Figure 6). Yellow to brown pigmented sclerotia formed on the culture surface of Michigan isolates 1, 2, 3, 5 and 6. Michigan isolates 1, 3 and 6 showed obvious zonation. Michigan isolate 4 produced mycelium white in color and no surface sclerotia. The CAG 1 and 3 testers produced a buff colored mycelium and brown sclerotia. Testers from CAG 2, 4, 6 and 7 all produced mycelium closer to white in color. The CAG 5 tester produced mycelium that was darker in color, more similar in pigmentation to that of the R. solani isolate. A similarity was apparent between the mycelial pigmentation of the CAG 1 and 3 testers and Michigan isolates 1, 2, 3, 5 and 6. Burpee (6) reported that the pigmentation of R. cerealis isolates from turfgrasses was buff to light brown in color after eight weeks of growth at 23°C in the absence of light.

Temperature-Growth Relations. The influence of temperature on the in vitro mycelial growth rate of the Michigan isolates and the CAG testers is shown in Figure 10. The data are presented in Appendices 1 and 2. There was limited variation in the growth rates of Michigan isolates 1, 2, 3, 5 and 6 at all temperatures. Compared to those isolates, Michigan isolate 4 exhibited significantly different growth rates at all temperatures tested. The CAG 1 tester and Michigan isolates 1, 2, 3, 5 and 6 exhibited similar growth relations at all of

Table 3. Mycelial pigmentation of several Rhizoctonia isolates on PDA after 40 days at 23°C in the absence of light.

Dirty White	Buff	Light Brown
CAG 2, 4, 6 and 7	Michigan 1, 2, 3, 5 and 6	CAG 5 tester
Michigan 4	CAG 1 and 3 testers	R. solani

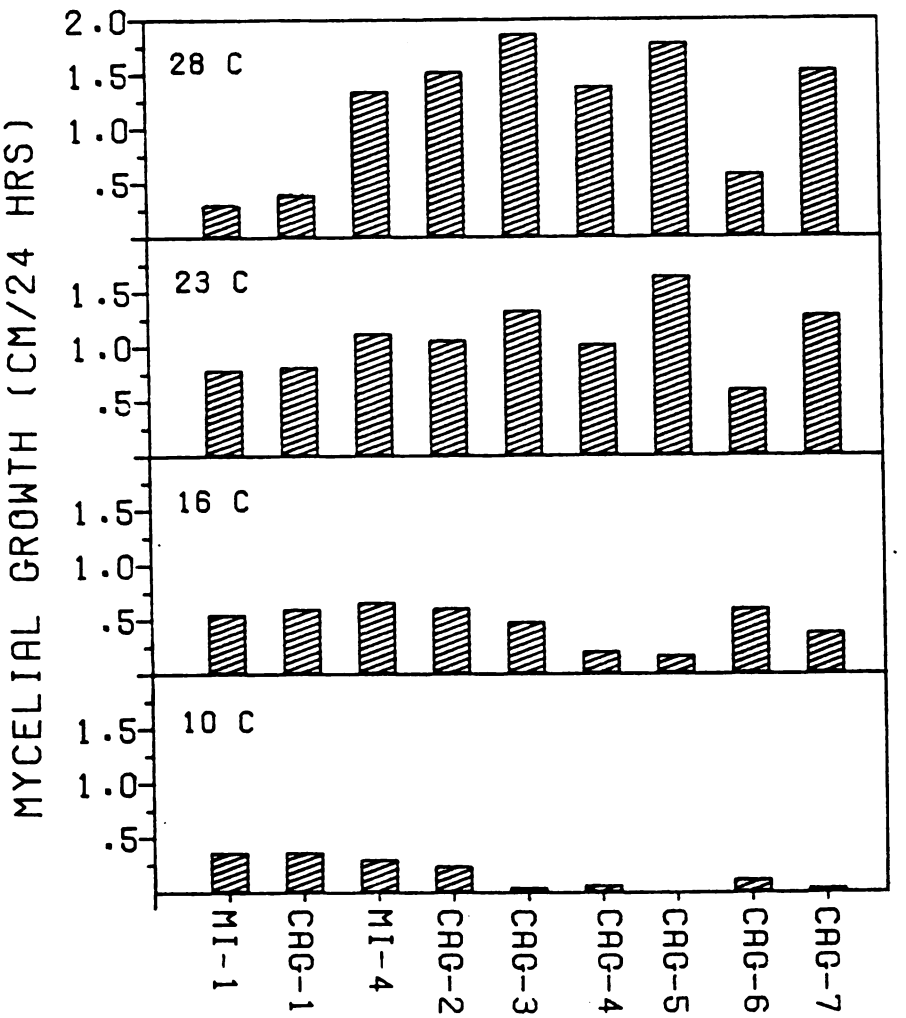


Figure 10. The mycelial growth rates of several binucleate Rhizoctonia isolates incubated at different temperatures in the absence of light. MI-1 is an average of the growth rates of Michigan isolates 1, 2, 3, 5 and 6.

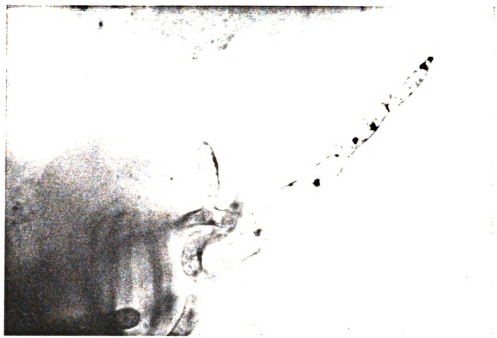
the temperatures tested. Compared to the other Rhizoctonia isolates, Michigan isolates 1, 2, 3, 5 and 6 and the CAG 1 tester grew more rapidly at 10°C and more slowly at 28°C than any of the other isolates with the exception of CAG 6. The average optimal growth rate of Michigan isolates 1, 2, 3, 5 and 6 was 0.78 cm/24 hours at 23°C. This is close to the growth rate of the CAG 1 tester, 0.80 cm/24 hours at 23°C. These results compare favorably with those of other researchers. Burpee (6) reported the optimal in vitro growth rate of several R. cerealis isolates from turfgrasses occurs at 23°C. Lipps and Herr (21) reported the optimal growth rate of an R. cerealis type culture to be 0.72 cm/24 hours at 23°C.

Nuclear Staining. Based on the nuclear staining, Michigan isolates 1 through 6 were all found to have binucleate vegetative hyphal cells (Figure 11a) and by comparison, the R. solani isolate appeared to be multinucleate (Figure 11b). Of the Rhizoctonia spp. recognized as pathogens of turfgrasses, R. cerealis is the only one with predominantly binucleate hyphal cells (32).

Hyphal Anastamosis. The vegetative hyphae of five Michigan isolates (isolates 1, 2, 3, 5 and 6) anastamosed with the CAG 1 tester isolate and failed to anastamose with the CAG 2 through CAG 7 testers (Table 4). Michigan isolates 1, 2, 3, 5 and 6 anastamosed with each other in all possible combinations. Cell wall and cytoplasmic fusion as described by Parmeter (25) indicate the occurrence of hyphal anastamosis (Figure 12). Michigan isolate 4 failed to anastamose with the CAG 1 tester or any of the other Michigan isolates.

The CAG 1 is known to be made up of R. cerealis and is distinct from R. solani and other binucleate Rhizoctonia spp. (6). Based on the

a.



b.

Figure 11. Nuclear staining of several Rhizoctonia isolates employing the HCl-Giemsa technique. The cool season turfgrass pathogens were all found to be binucleate (a) and the R. solani isolate appeared multinucleate (b).

Table 4. Hyphal pairing among the Rhizoctonia sp. isolated from cool season blighted Poa pratensis L. and the Ceratobasidium anastamosis group (CAG) testers to observe the occurrence of hyphal anastamosis.

	Michigan Isolate						Ceratobasidium Anastamosis Group Testers						
Michigan Isolate	1	2	3	4	5	6	CAG1	CAG2	CAG3	CAG4	CAG5	CAG6	CAG7
1		+	+	-	+	+	+	-	-	-	-	-	-
2	+		+	-	+	+	+	-	-	-	-	-	-
3	+	+		-	+	+	+	-	-	-	-	-	-
4	-	-	-		-	-	-						
5	+	+	+	-		+	+	-	-	-	-	-	-
6	+	+	+	-	+		+	-	-	-	-	-	-

+ Denotes the observation of hyphal anastamosis (cell wall and cytoplasmic fusion).

- No apparent hyphal anastamosis.

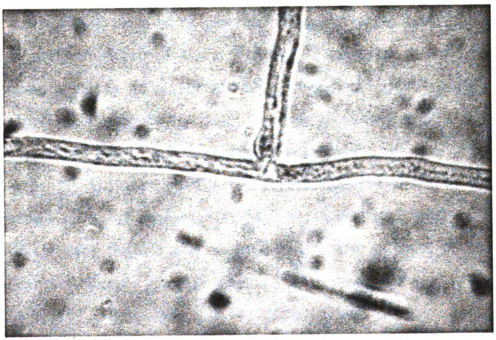


Figure 12. The cell wall and cytoplasmic fusion which indicate the occurrence of hyphal anastamosis.

observation of hyphal anastamosis, Michigan isolates 1, 2, 3, 5 and 6 were identified as R. cerealis.

The Identification of R. cerealis. In the proceeding experiments several Rhizoctonia isolates were characterized. The isolates examined included six unidentified Rhizoctonia sp. found to be cool season pathogens of turfgrass in Michigan, seven CAG testers and an isolate of R. solani. A comparison was made of the cultural characteristics and the influence of temperature on the growth rate of each isolate. The nuclear condition of each isolate was determined and where possible the isolates were placed into a hyphal anastamosis group. Five of the six cool season turfgrass pathogens were identified as isolates of R. cerealis, the causal agent of yellow patch disease of turfgrasses. The basis for this identification was the placement of Michigan isolates 1, 2, 3, 5 and 6 into CAG 1, the group comprised of R. cerealis.

Hyphal anastamosis testing is a method which has been shown to be useful in fungal identification (12). Burpee (6) suggested hyphal anastamosis testing to be of potential value in separating R. cerealis from other binucleate Rhizoctonia spp. The data presented above illustrate that along with a positive hyphal anastamosis reaction, the Michigan R. cerealis isolates and the CAG 1 test have shown similarities in nuclear state, cultural characteristics and temperature-growth relations. These data suggest that examination of two or three of these other characteristics could provide adequate information to accurately identify R. cerealis. This may be particularly useful for the diagnostician who may not have access to CAG tester isolates.

There are currently four Rhizoctonia spp. that are recognized as turfgrass pathogens and of these R. cerealis is the only one which forms

vegetative hyphae with predominantly binucleate hyphal cells (32). The nuclear staining technique employed in these experiments provided for excellent results and photography, however, it is an involved procedure. Burpee (5) has suggested some rapid staining techniques which may be more practical. Unfortunately, these alternative methods do not stain nuclei with the same clarity as the HCl-Giemsa method. Determination of the nuclear conditions of a Rhizoctonia-like fungus should be done as an initial step in species identification.

The mycelial pigmentation of R. cerealis isolates from turfgrasses appears to be a consistent feature. The observation of buff to light brown colored mycelium produced by all of the Michigan R. cerealis isolates from turfgrasses is in agreement with the findings of Burpee (6). In comparison with the other CAG testers examined, the Michigan R. cerealis isolates were the most similar culturally to the CAG 1 and 3 testers. The production of yellow to brown sclerotia was consistent in all of the Michigan R. cerealis isolates, however, this feature appears to be of limited taxonomic value due to reports that some R. cerealis isolates have failed to form sclerotia on PDA (6). One must also consider the type of medium and environmental conditions under which the isolates will be incubated.

In combination with the above described cultural characteristics determination of the growth rates of Rhizoctonia-like fungi isolated from turfgrasses should prove to be useful in identifying R. cerealis isolates. The results described here illustrate the close positive correlation between the temperature-growth relations of the Michigan R. cerealis isolates and the CAG 1 tester (Figure 10). The greatest difference between the growth rates of the R. cerealis isolates and CAG

testers 2 through 7 occurred at 28°C. The temperature-growth relations exhibited by the CAG 6 tester were similar to those of the R. cerealis isolates at 16, 23 and 28°C, however, the CAG 6 tester appeared dissimilar culturally. The CAG 1 tester and Michigan R. cerealis isolates were culturally similar to the CAG 3 tester, however, they exhibit very dissimilar temperature-growth relations. Our results were also very similar to those reported by a number of other researchers. Of the Rhizoctonia spp. recognized as pathogens on turfgrasses, damage from R. solani generally occurs when temperatures are above 28°C (32). R. zeae grows best at 32°C and R. oryzae has been associated only with tropical and subtropical regions. In contrast, damage from R. cerealis occurs between 15-25°C (32).

The identity of Michigan isolate 4 was not established in these investigations. It is a binucleate pathogen of Poa pratensis, however, it does not appear to be R. cerealis. It appeared different in culture than any of the R. cerealis isolates and exhibited significantly different temperature-growth relations. This isolate may be from another CAG. Burpee et al. (8) reported that the majority of the binucleate Rhizoctonia spp. isolated from turf were placed into CAG 1, however, one binucleate Rhizoctonia sp. isolated from a Poa sp. crown in New York was placed into CAG 2 and another binucleate Rhizoctonia sp. isolated from turf in Germany was placed into CAG 6.

SECTION III

FUNGICIDE SCREENING FOR THE CHEMICAL MANAGEMENT OF R. CEREALIS ON A TURFGRASS HOST

Because yellow patch is a relatively new disease there are no fungicide recommendations available for the control of R. cerealis on a turfgrass (17). The objective of these investigations was to screen several antifungal compounds for use in a yellow patch management program.

Materials and Methods

In Vitro Fungicide Bioassay. An agar plate bioassay method was employed to determine the effect of several antifungal compounds on the mycelial growth rate of five R. cerealis isolates from Kentucky bluegrass. The fungicides were suspended in sterile deionized water at 10,000 µg active ingredient (a.i.)/mL and aliquots were pipetted directly into autoclaved partially cooled PDA. Chemicals were serially diluted to obtain fungicide concentrations of 1, 100 and 1000 µg a.i./ml PDA (1 ppm, 100 ppm and 1000 ppm, respectively). Approximately 20 ml of the amended PDA was poured into 15 x 100 mm plastic petri dishes. Agar plugs of mycelium 5 mm in diameter were taken from the margin of actively growing colonies of the test isolates and transferred to the center of the fungicide amended PDA plates. Each isolate was placed on three replicate plates for each fungicide treatment and on three non-fungicide amended

PDA controls. The fungicides to be tested were anilazine (Dyrene, Mobay Chemical Corporation, Kansas City, Missouri), benomyl (Tersan 1991, DuPont DeNemours, Wilmington, Delaware), chlorothalonil (Daconil 2787, SDS Biotech International, Cleveland, Ohio), cyclohexamide + PCNB (Actidione RZ, Upjohn, Kalamazoo, Michigan), iprodione (Chipco 26019, Rhone-Poulenc, Monmouth Junction, New Jersey), propiconazol (CGA-64250, CIBA-Geigy, Greensboro, North Carolina), triadimefon (Bayleton, Mobay Chemical Corporation, Kansas City, Missouri) and vinclozolin (Vorlan, Mallinckrodt, St. Louis, Missouri).

The petri plates were incubated under a 12 hour light/dark cycle at 21±1°C and the fungal colony growth on each plate was measured along the same diameter 2, 5 and 10 days after seeding the plates. The data from the 5th day measurements were subjected to Analysis of Variance (ANOVA) and a multiple comparison using Duncan's Multiple Range Test (DMR). This set of data was chosen for statistical analysis because good differentiation of the inhibitory compounds was apparent and beyond this day the mycelium of some isolates had grown the radius of the petri dish.

In Vivo Fungicide Bioassay. Six week old 'Fylking' Kentucky bluegrass plants were treated with six of the fungicides screened in the agar plate bioassay. These included the five most effective fungicides and the least effective fungicide in vitro. Each fungicide was applied to the foliage of seven different pots of 'Fylking' using a CO_2 small plot sprayer at a water volume of 40 gals/acre. The rate of application for each fungicide was considered separately. Each rate was selected based on the rate used in the field for the management of a turfgrass pathogen which exhibited a similar reaction to the particular fungicide

in vitro. Following the fungicide application the pots remained in the greenhouse and care was exercised not to wet the foliage.

Inoculations were made 24 hours after fungicide treatment by placing three kernels of *R. cerealis* infested wheat seed (prepared as in Section I) at the base of the grass foliage in each pot. Inoculum of isolates 1 and 2 were placed each in three replicate pots for each fungicide treatment and three non-fungicide treated pots. The plants were maintained at 100% relative humidity and 20±2°C in plastic bags in a temperature controlled growth chamber. The chamber had a 12 hour photoperiod. Disease severity evaluations were made four days and eight days after the inoclations. A rating scale from 0-100% was used and readings were made by approximating the percent of the total number of leaf blades in each pot which exhibited foliar necrosis. The data were subjected to ANOVA and DMR.

Results and Discussion

In Vitro Fungicide Bioassay. Considerable variation existed in the degree of growth inhibition elicited by the fungicides at the three tested rates. The data are presented in Table 5.

At the 1 ppm a.i. concentration the *R. cerealis* isolates are the most sensitive to iprodione. At this rate iprodione is extremely effective at inhibiting mycelial growth. Propiconazol, chlorothalonil and triadimefon also induced growth suppression, but not to the degree of iprodione. Cyclohexamide + PCNB, anilazine, benomyl and vinclozolin were less effective than the other fungicides at inhibiting fungal growth at this rate.

Table 5. *In vitro* fungicide bioassay. Mycelial growth (cm) after 5 days of *R. cerealis* isolates on PDA amended with three rates of various fungicides.

Fungicide	Isolate 1			Isolate 2			Isolate 3			Isolate 5			Isolate 6		
	1 ppm	100 ppm	1000 ppm	1 ppm	100 ppm	1000 ppm	1 ppm	100 ppm	1000 ppm	1 ppm	100 ppm	1000 ppm	1 ppm	100 ppm	1000 ppm
Chlorothalonil	2.6 c	0.0 a	0.0 a	1.4 b	0.5 b	0.3 b	1.0 b	0.4 c	0.3 a	0.7 a	0.3 c	0.1 ab	1.5 b	0.5 bc	0.3 cd
Triadimefon	2.4 c	0.0 a	0.0 a	3.8 d	0.6 b	0.4 b	2.6 d	0.2 b	0.1 a	2.8 c	0.3 c	0.1 ab	3.0 c	0.4 b	0.2 bc
Benomyl	4.4 d	0.0 a	0.0 a	6.4 f	0.0 a	0.0 a	3.2 f	0.0 a	0.0 a	4.5 d	0.0 a	0.0 a	4.2 d	0.0 a	0.0 a
Anilazine	4.1 d	0.6 b	0.2 a	6.7 f	1.4 d	0.3 b	5.2 f	1.9 e	0.3 a	5.6 3	0.9 d	0.2 b	5.2 f	2.2 d	0.3 d
Propiconazol	1.3 b	0.0 a	0.0 a	2.3 c	0.0 a	0.0 a	1.8 c	0.1 a	0.0 a	1.7 b	0.2 b	0.0 a	1.7 b	0.0 a	0.0 a
Iprodione	0.5 a	0.0 a	0.0 a	0.6 a	0.0 a	0.0 a	0.4 a	0.0 a	0.0 a	0.5 a	0.0 a	0.0 a	0.5 a	0.0 a	0.0 a
Vincozalin	4.8 e	2.1 d	1.8 b	7.0 f	3.3 e	2.7 c	5.9 g	2.7 f	2.3 b	5.4 d	3.2 f	2.2 c	5.8 g	2.4 d	1.9 e
Cyclohexamide + PCNB	4.2 d	1.0 c	0.0 a	5.3 e	1.2 c	0.0 a	3.9 e	1.5 d	0.1 a	4.7 d	1.1 e	0.2 b	4.4 e	0.6 bc	0.1 b
Control	5.0 e	5.0 e	5.0 c	6.9 f	6.9 f	6.9 d	5.8 g	5.8 g	5.8 c	5.9 f	5.9 g	5.9 d	5.9 g	5.9 e	5.9 f

Values within a vertical column with the same letter are not significantly different according to Duncan's Multiple Range Test, P = 0.05.

At the 100 ppm a.i. concentration iprodione, propiconazol and benomyl produced the most significant and consistent growth inhibition of all isolates. Benomyl had produced little growth inhibition at the 1 ppm a.i. concentration, however, at this rate it prevented any fungal growth. Triadimefon and chlorothalonil provided significant growth inhibition, however, statistically they were not as efficacious as iprodione, propiconazol and benomyl (Figure 13). The degree of growth inhibition elicited at this rate by cyclohexamide + PCNB and anilazine was significantly less than the other fungicides. The least effective compound was vinclozolin.

With the exception of vinclozolin all of the fungicides virtually prevented fungal growth at the 1000 ppm a.i. concentration. For some of the compounds, i.e., chlorothalonil and triadimefon, the increase in the a.i. concentration from 100 ppm to 1000 ppm incited litle additional growth suppression.

Figure 13 illustrates the similarity in the reactions of isolates 2 and 3 to each of the fungicides at the 100 ppm a.i. concentration. Isolates 1, 5 and 6 exhibited similar reactions. The repeat of this experiment produced the same results.

In Vivo Fungicide Bioassay. All of the fungicides provided the inoculated plants with excellent protection from disease even after incubation for eight days in conditions which have been shown to be favorable for disease development (Section I). The fungicide treatments had a significant effect on the development of foliar disease symptoms (Figure 14).

In all cases where no disease symptoms developed there was little or no fungal mycelial growth away from the infested wheat seeds. There was

Figure 13. The effect of fungicides on the *in vitro* growth of two *R. cerealis* isolates. Mycelial growth was measured after five days on PDA amended with 100 µg fungicide active ingredient/ml pDA. Within each block, bars topped with the same letter are not significantly different according to Duncan's Multiple Range Test, P = 0.05.

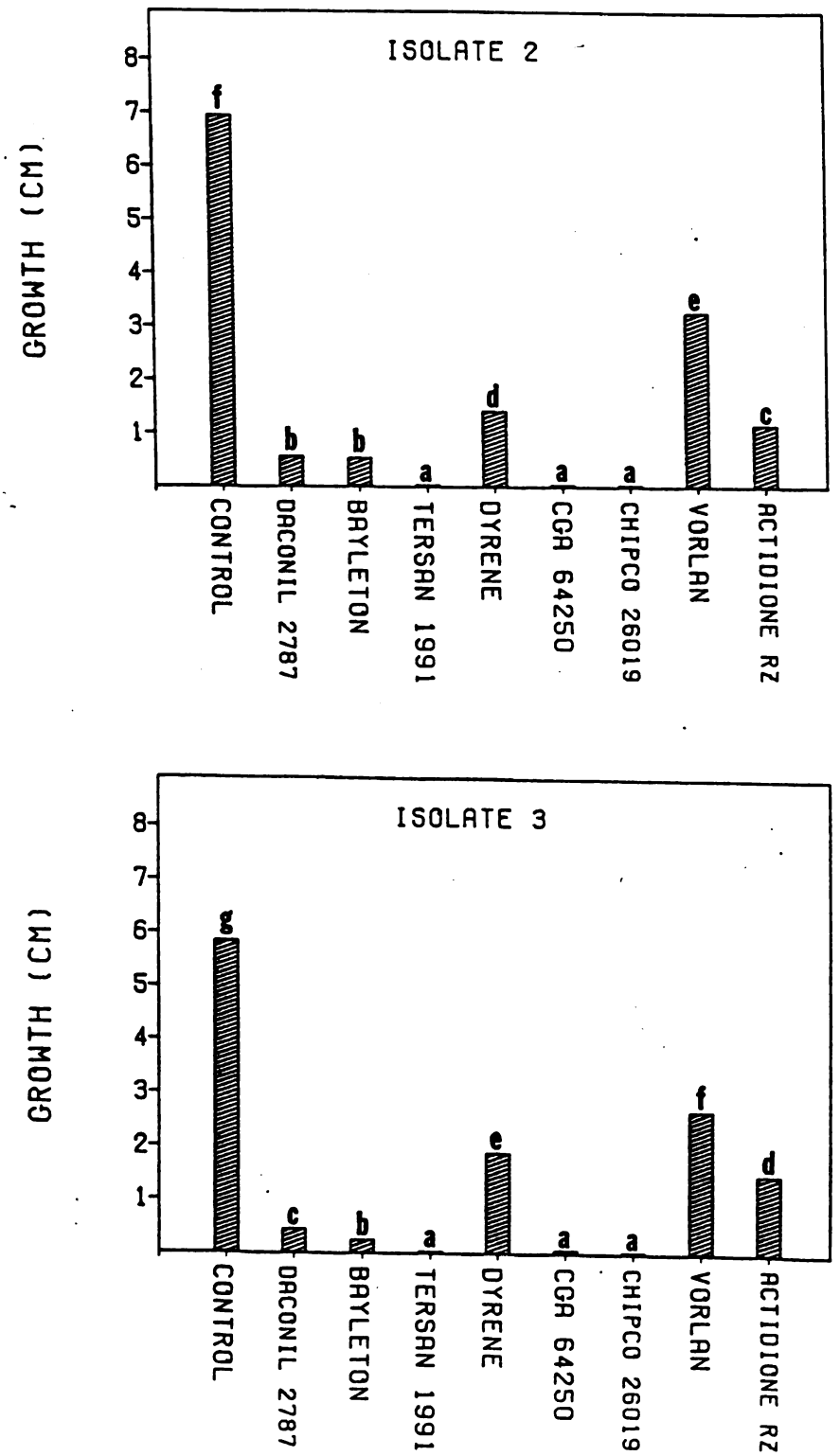

ISOLATE 2
GROWTH (CM)
8
7
6
5
4
3
2
1
f
b
b
a
d
a
a
e
c
CONTROL
DACONIL 2787
BAYLETON
TERSAN 1991
DYRENE
CGA 64250
CHIPCO 26019
VORLAN
ACTIDIONE RZ
ISOLATE 3
GROWTH (CM)
8
7
6
5
4
3
2
1
a
c
b
a
e
a
a
f
d
CONTROL
DACONIL 2787
BAYLETON
TERSAN 1991
DYRENE
CGA 64250
CHIPCO 26019
VORLAN
ACTIDIONE RZ

Figure 13.

Figure 14. The effect of fungicide applications on the development of foliar blight symptoms. The rate of each of the fungicides is as follows:

Daconil 2787 (Chlorothalonil)	4 oz/1000 sq. ft.
Bayleton (Triadimefon)	.5 oz/1000 sq. ft.
Tersan 1991 (Benomyl)	1 oz/1000 sq. ft.
CGA-64250 (Propiconazol)	.5 oz/1000 sq. ft.
Chipco 26019 (Iprodione)	2 oz/1000 sq. ft.
Vorlan (Vinclozolin)	2 oz/1000 sq. ft.

Within each block, bars topped with the same letter are not significantly different according to Duncan's Multiple Range Test, P = 0.05.

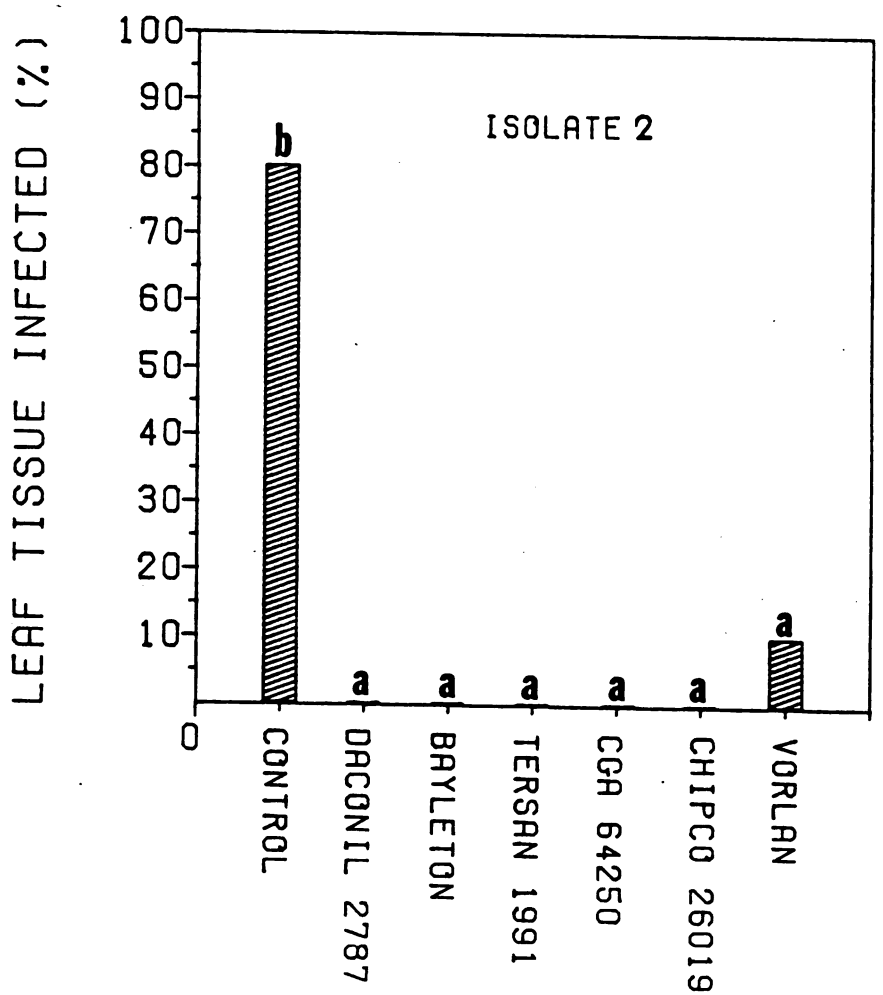

LEAF TISSUE INFECTED (%)
100
90
80
70
60
50
40
30
20
10
0
ISOLATE 2
b
a
a
a
a
a
a
CONTROL
DACONIL 2787
BAYLETON
TERSAN 1991
CGA 64250
CHIPCO 26019
VORLAN

Figure 14.

no significant difference in the efficacy among any of the fungicides based on the DMR test. All of the treatments gave significant control compared to the untreated inoculated plants. The vindozolin plants did, however, show more infected foliage than the other fungicide treated plants. On the fourth day following the inoculations the fungus in the vincozalin treated plants had grown an average of 2.0 cm away from the wheat seed, but no symptoms of necrosis were apparent. By the eighth day foliar symptoms were apparent, however, little additional mycelial growth had occurred. The data for the inoculations of isolates 1 and 2 are presented in Table 6. The same results were obtained in the repeat of this experiment.

Fungicide Screening for the Chemical Management of R. cerealis. The agar plate method is one of several methods designed to detect the antifungal activity of a compound. This method was employed in the preceding studies because it is efficient, economical, requires only limited resources and is an ideal initial step in screening a number of fungicides for activity against a microorganism. A few researchers (18,20) have reported poor correlation between agar plate and field and greenhouse studies when evaluating fungicides against soil borne pathogens, however, this method is commonly used by plant pathologists and can provide very useful information.

Klomparens and Vaughn (19) reported good correlation between the laboratory in vitro performance and the field control of several turfgrass pathogens. Pelletier (29) cited their work and suggested that a reason for the good correlation between the results of such tests against soil-borne turfgrass pathogens was that "in order to be effective turfgrass fungicides need not enter and be subjected to the complexities

Table 6. *In vivo* fungicide bioassay

Fungicide/Rate (a.i./1000 sq. ft.)	Percent of total leaf blades exhibiting necrotic lesions	
	Isolate 1	Isolate 2
Chlorothalonil/4 oz.	0,0,0 a	0,0,0 a
Triadimefon/.5 oz	0,0,0 a	0,0,0 a
Benomyl/1 oz	0,0,0 a	0,0,0 a
Propiconazol/.5 oz	0,0,0 a	0,0,0 a
Iprodione/2 oz	0,0,0 a	0,0,0 a
Vinclozolin/2 oz	10,10,10 a	10,10,10 a
Control	80,80,70 b	80,80,80 b

Values within a vertical column with the same letter are not significantly different according to Duncan's Multiple Range Test, P = 0.05.

of the soil environment." Such a statement may be valid in the use of some fungicides, however, it appears to be a gross oversimplification of the situation. There is no discussion of whether the statement considers both systemic and contact fungicides. Systemic fungicides are used to manage turfgrass diseases and in some cases must be drenched into the root zone to be effective (36). No mention is made of the effect the thatch layer may have on fungicide activity. The thatch layer is also a complex component of the turfgrass environment and is known to reduce the effectiveness of some pesticide applications (2). Pelletier does not consider whether one is testing for the growth suppression of a foliar pathogen or a crown and root pathogen. Treatment for pathogens which attack different plant organs may require different application strategies.

All of the fungicides tested in the preceding in vitro bioassay elicited significant growth inhibition of the R. cerealis isolates and these results are useful to suggest compounds which may warrant further testing. A number of factors may, however, alter the effectiveness of these fungicides in actual field use and these must be kept in mind when evaluating such bioassay results.

The in vivo bioassay included the host plant in the testing system. Following the application of fungicides symptom development was greatly reduced or prevented on the fungus inoculated plants. A comparison of the results of the in vivo and in vitro bioassay tests is difficult because of the different rates used, however, there does appear to be a correlation. The in vitro test demonstrated that iprodione, propiconazol, benomyl, triadimefon and chlorothalonil elicited excellent fungal growth inhibition. These fungicides all prevented symptom

development *in vivo*. Vindozolin was the least effective growth inhibitor *in vitro*. The vindozolin treatments were the only fungicide treated plants to develop foliar symptoms. The amount of infection was surprisingly low, however, considering the comparatively poor performance of this product *in vitro*.

With the exception of vindozolin and propiconazol all of the fungicides used in the preceding tests are labelled for some degree of control of the disease brown patch caused by *R. solani*. Triadimefon, iprodione and vindozolin are all broad spectrum systemics and have recently been labelled for use on turfgrasses (23). Benomyl and propiconazol are also broad spectrum systemics, however, propiconazol has not yet been released for commercial use. The three contact fungicides used were chlorothalonil, cyclohexamide + PCNB and anilazine.

The inoculation technique *in vivo* induced a foliar blight symptom. The results of these inoculations provide useful information, however, a crown and root inoculation technique would be desirable. The crown and root rot symptom is more common and causes damage considered more severe than the foliar blight.

The preceding experiments have suggested several fungicides to be tested further. The success of iprodione at the lowest rate is of particular interest as that rate may be more near the actual rates found in the field. These experiments were conducted under artificial conditions. What is needed currently is field control on naturally infected turf. It should be established which fungicides are most effective in the field and at what rates and timing schedules. The use of fungicides in a yellow patch management program may require the use of specific application procedures such as those recommended for *Fusarium*

blight. *Fusarium* blight which also affects crown and root tissues is chemically managed by using high concentrations of a benzimidazole fungicide applied to the turf and drenched into the root zone (36). The use of higher fungicide rates and a drench application method may be necessary to inhibit *R. cerealis* which is a soil-borne fungus and likely to be a thatch inhabitor. Systemic fungicides such as iprodione and triadimefon may be particularly useful because they are transported basipetally and acropetally in the plant.

SUMMARY AND CONCLUSIONS

The unresolved cool season blighting of Kentucky bluegrass in Michigan has been identified as the disease yellow patch caused by Rhizoctonia cerealis. The diagnosis was based on the appearance of the macroscopic symptoms and the identification of the associated fungal pathogen. Yellow patch has been reported in several midwestern states, the transition zone and Bermuda (6,17). This is the first confirmed report of yellow patch in Michigan.

The yellow patch symptoms observed in Michigan occurred during the spring and fall and appeared as "frog-eyes" or patches of dead grass or patches of foliar blighted turf. These observations coincided with those of other turfgrass researchers (6,11,15,17,31). The fungi isolated from diseased tissues resembled Rhizoctonia solani in hyphal and cultural appearance. Characterization of the isolated Rhizoctonia solani-like fungi included the determination of the nuclear state, cultural characteristics, temperature-growth relations and hyphal anastamosis grouping of each isolate, leading to the identification of five Michigan R. cerealis isolates. The pathogenicity of the R. cerealis isolates on Kentucky bluegrass was demonstrated in growth chamber and field inoculation studies. The inoculation incited a foliar and possibly a crown and root rot symptom.

Variability in the natural symptoms of yellow patch and their resemblance to those of other turfgrass diseases has contributed to the

confusion in diagnosing this disease. Upon attempting the diagnosis of yellow patch, as with any other turfgrass problem, one must first consider the time of symptom appearance. Yellow patch and Fusarium blight both cause "frog-eye" symptoms on Kentucky bluegrass turf, however, the symptoms of each disease have been associated with temperatures of 50-75°C and 75-90°C, respectively (10,36). Fairy rings also produce symptoms in a ring pattern, however, fairy rings are usually wider in diameter than yellow patch rings and induce a different type of turf discoloration. The plants associated with a fairy ring become either chlorotic or darker green in color (36).

Symptomatic plants should be closely examined for signs of the pathogen. Sclerotia (also referred to as bulbils by Smiley (32) are found in association with dark fungal hyphae on the crown, sheath and rhizomes of infected plants. Observation of sclerotia is less likely to be confused if plants are viewed through some type of magnification aid. The sclerotia serve as overwintering structures and may be more likely found at the later stages of disease development as the food source (the host plant) depletes in supply. Researchers (16) have reported that occasionally turfgrass crowns are found that are covered with dark hyphae which resemble Rhizoctonia, however, no Rhizoctonia sp. can be isolated. For this reason diagnosis should not be based only on the observation of dark hyphae and attempts should be made to isolate the pathogen.

If a suspected Rhizoctonia fungus is isolated from necrotic tissues several fungal characteristics have been discussed which may be used in identifying the organism. Nuclear staining is critical and should be followed by an examination of the cultural characteristics and/or growth rates of the isolate. Lipps and Herr (21) reported some variation in the

mycelial pigmentation of R. cerealis isolates from wheat and it was noted that the Michigan R. cerealis isolates from turfgrass became differing shades of brown pigmentation after extended periods of time. Researchers (5,26) have also reported some overlap in the optimal in vitro growth rates of some R. cerealis and R. solani isolates. For these reasons it is important to not base an identification on one fungal characteristic. For the most conclusive species identification hyphal anastamosis can be performed. The perfect state is used to identify R. solani isolates (31), however, at the present time it is not used to identify the binucleate Rhizoctonia turfgrass pathogens. In 1967, Parmeter et al. (26) reported the induction of fruiting of a binucleate Rhizoctonia sp. isolated from bermudagrass in California. More recent attempts by researchers to do the same with other binucleate Rhizoctonia turfgrass pathogens have not bee as successful. Basidiospore production has not been induced in vitro or observed in vivo for R. cerealis isolates from turfgrass or wheat (5,31).

In vitro and in vivo fungicide screening was performed to evaluate the efficacy of several antifungal compounds for the management of R. cerealis on turf. All of the fungicides tested exhibited some degree of efficacy in mycelial growth inhibition and foliar blight symptom suppression. The fungicides tested should be further screened and included in field studies. Lucas (22) has emphasized the need to identify Rhizoctonia sp. isolated from turfgrasses because of species variation in fungicide sensitivity.

Still unresolved are the factors which have led to the emergence of yellow patch as a turfgrass disease. Genetic resistance plays an important role in the control of turfgrass diseases, however, the last

two decades have shown that the release of a cultivar with resistance to a major disease can be followed by the emergence of "new" diseases. Prior to the release of the cultivar 'Merion' in 1947 (2) the primary disadvantage of Kentucky bluegrass was its inherent susceptibility to Dreschlera melting-out (formerly Helminthosporium) (36). Following the release and widespread planting of 'Merion', Fusarium blight, stripe smut and powdery mildew which were previously unknown or of minor importance on Kentucky bluegrass developed on the new cultivars. These diseases replaced melting-out as the most troublesome Kentucky bluegrass diseases (36). During the 1970s several new "improved" cultivars were developed that showed varying degrees of resistance to Fusarium blight and stripe smut (2). The introduction of the new "improved" cultivars resistant to those major diseases may lead to the emergence of a disease previously unknown on Kentucky bluegrass, the disease yellow patch.

Genetic factors are likely to influence the severity and occurrence of yellow patch, however, the resistance of a plant to a disease is also conditioned by external factors (1). Rhizoctonia spp. are classified as unspecialized facultative parasites and by definition their parasitism is usually restricted by general host resistance (32). The Rhizoctonia spp. usually infect seedling host plants, immature or senescent tissues of older plants or tissues of plants that are growing under particularly adverse conditions (32). Adverse conditions of turf establishment such as those associated with poor rooting and thatch accumulation may be adequate to predispose inherently susceptible turf to disease.

This study has resolved the etiology of the unusual cool season blighting of home lawn turf in Michigan. This study has also provided a discussion of the symptoms and pathogen characteristics which may be used

to diagnose this disease. A new inoculation technique was developed for the implementation of R. cerealis field research. Many questions still remain unanswered.

Epidemiological data would be useful to clarify what environmental conditions are associated with disease development. This type of information might promote the most efficient use of fungicides in the management of yellow patch. Investigations should be conducted to determine the cause of the apparent sod susceptibility to this disease. The sod susceptibility may be a result of the genetic makeup of the plant species currently used in sod or it may be a combination of genetic and external factors. Perhaps yellow patch would occur more frequently on seeded turfs if they were established as stands of the new "improved" cultivars.

Other areas that need to be resolved are the role cultural practices such as fertility and irrigation may play in disease development. Any procedures one might attempt to avoid a yellow patch outbreak would be useful along with information on the renovation and reestablishment of yellow patch damaged turf.

LIST OF REFERENCES

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1. Agrios, G.N. 1978. Plant Pathology. Academic Press, New York. 703 pp.

2. Beard, J.B. 1973. Turfgrass: Science and Culture. Prentice-Hall, Incorporated, Englewood Cliffs, New Jersey. 658 pp.

3. Beard, J.B., P.E. Rieke, K.T. Payne, R.C. Shearman and J.F. Wilkinson. 1973. Lawn Establishment. Michigan State University Extension Bulletin E-673.

4. Boerma, G.H. and A.A. Verhoeven. 1977. Check-list for scientific names of common parasitic fungi. Netherlands Journal of Plant Pathology 83:165-204.

5. Burpee, L.L. 1980. Identification of Rhizoctonia species associated with turfgrass. pp 25-28 in B.G. Joyner and P.O. Larsen, eds. Advances in Turfgrass Pathology, Harcourt Brace Jovanich, Inc., Duluth, Minnesota.

6. Burpee, L.L. 1980. Rhizoctonia cerealis causes yellow patch of turfgrasses. Plant Disease 64:1114-1116.

7. Burpee, L.L., P.L. Sanders, H. Cole, Jr. and R.T. Sherwood. 1980. Pathogenicity of Ceratobasidium cornigerum and related fungi representing five anastamosis groups. Phytopathology 70:843-846.

8. Burpee, L.L., P.L. Sanders, H. Cole and R.T. Sherwood. 1980. Anastamosis groups among isolates of Ceratobasidium cornigerum and related fungi. Mycologia 72:689-701.

9. Converse, J. 1982. Scotts Guide to the Identification of Turfgrass Diseases and Insects. O.M. Scott and Sons Company, Marysville, Ohio. 105 pp.

10. Couch, H.B. 1973. Diseases of Turfgrasses. Robert E. Kreiger Publishing Company, Huntington, New York. 348 pp.

11. Dale, J.L. 1978. Atypical symptoms of Rhizoctonia infection on zoysia. Plant Disease Reporter 62:645-647.

12. Flentje, N.T., H.M. Stretton and A.R. McKenzie. 1970. Mechanisms of Variation in Rhizoctonia solani. p 11 in J.R. Parmeter, Jr. ed. Rhizoctonia solani, Biology and Pathology. University of California Press, Berkeley.

13. Herr, L.J. 1979. Practical nuclear staining procedures for Rhizoctonia-like fungi. Phytopathology 69:958-961.

14. Herr, L.J. and D.L. Roberts. 1980. Characterization of Rhizoctonia populations obtained from sugarbeet fields with differing soil textures. Phytopathology 70:476-480.

15. Hirrel, M.C. and M.C. Shurtleff. 1981. Recognizing new diseases of turfgrass. American Lawn Applicator Vol II, No. 3.

16. Joyner, B.G., L. Burpee and R.E. Partyka. 1980. Discussion: Rhizoctonia Diseases. pp 29-32 in B.G. Joyner and P.O. Larsen, eds. Advances in Turfgrass Pathology, Harcourt Brace Jovanich, Inc., Duluth, Minnesota.

17. Joyner, B.G. 1982. Yellow Patch: New turf disease problem with no known controls. Lawn Care Industry Vol 6, No. 4.

18. Kendrick, J.B. and J.T. Middleton. 1954. The efficacy of certain chemicals as fungicides for a variety of fruit, root and vascular pathogens. Plant Disease Reporter 30:350-353.

19. Klomparens, W. and J.R. Vaughn. 1952. The correlation of laboratory screening of turf fungicides with field results. Michigan Quarterly Bulletin, Vol 34:425-435.

20. Latham, A.J. and M.B. Linn. 1968. A comparison of soil column and petri dish techniques for the evaluation of soil fungitoxicants. Phytopathology 58:460-463.

21. Lipps, P.E. and L.J. Herr. 1982. Etiology of Rhizoctonia cerealis in sharp eyespot of wheat. Phytopathology 72:1574-1577.

22. Lucas, L.T. 1982. Brown patch in the transition zone. American Lawn Applicator Voll III, No. 4.

23. Lucas, L.T. 1983. Fungicides and Nematicide Update: Recently labelled fungicides for use on turfgrasses. Plant Disease 67:120.

24. Martin, S.B., Campbell, C.L. and L.T. Lucas. 1981. Reactions of R. solani and Rhizoctonia-like fungi to selected fungicides. Phytopathology (Abstract) 71:893.

25. Parmeter, J.R., R.T. Sherwood and W.D. Platt. 1969. Anastamosis grouping among isolates of Thanatephorus cucumeris. Phytopathology 59:1270-1278.

26. Parmeter, J.R., H.S. Whitney and W.D. Platt. 1967. Affinities of some Rhizoctonia species that resemble mycelium of Thanatephorus cucumeris. Phytopathology 57:218-223.

27. Parmeter, J.R. and H.S. Whitney. 1970. Taxonomy and nomenclature of the imperfect state. pp 6-19 in J.R. Parmeter, Jr., ed. Rhizoctonia solani, Biology and Pathology. University of California Press, Berkeley.

28. Patchan, G., T.M. Smith, P.E. Rieke and K.T. Payne. 1981. Turf Tips: Sodding a Lawn. Michigan State University Extension Bulletin E-1490.

29. Pelletier, E.N. 1977. Detecting potential protective and systemic antifungal compounds. pp 51-68 in M.R. Siegel and H.D. Sisler, eds. Antifungal Compounds Volume 1. Marcel Dekker, Inc., New York, New York.

30. Sanders, P.L., L.L. Burpee, R.T. Sherwood and H. Cole, Jr. 1976. Ceratobasidium: A pathogen of turfgrass. Proceedings of American Phytopathological Society (Abstract) Vol 3:310.

31. Sanders, P.L., L.L. Burpee and H. Cole, Jr. 1978. Preliminary studies on binucleate turfgrass pathogens that resemble Rhizoctonia solani. Phytopathology 68:145-148.

32. Smiley, R.W. 1983. Compendium of Turfgrass Diseases. American Phytopathological Society, St. Paul, Minnesota. 102 pp.

33. Talbot, P.H.B. 1965. Studies of 'Pellicularia' and associated genera of Hymenomycetes. Persoonia 3:372-406.

34. Tu, C.C. and J.W. Kimbrough. 1978. Systematics and phylogeny of fungi in the Rhizoctonia complex. Botanical Gazette 139(4):454-466.

35. Van der Hoeven, E.P. and G.J. Bollen. 1980. Effect of benomyl on soil associated with rye. 1. Effect on the incidence of sharp eyespot caused by R. cerealis. Netherlands Journal of Plant Pathology 86:163-180.

36. Vargas, J.M. 1981. Management of Turfgrass Diseases. Burgess Publishing Company, Minneapolis, Minnesota. 204 pp.

APPENDICES

APPENDIX I

IN VITRO MYCELIAL GROWTH RATES (CM/24 HRS) OF RHIZOCTONIA-LIKE FUNGI ISOLATED FROM COOL SEASON BLIGHTED POA PRATENSIS L. IN MICHIGAN

	Temperature °C			
Isolate	10°	16°	23°	28°
1	0.35	0.51	0.81	0.34
2	0.35	0.57	0.83	0.34
3	0.37	0.52	0.74	0.27
4	0.28	0.64	1.10	1.32
5	0.36	0.52	0.78	0.29
6	0.33	0.53	0.73	0.28

APPENDIX II

IN VITRO MYCELIAL GROWTH RATES (CM/24 HRS) OF SEVERAL RHIZOCTONIA ISOLATES AT VARIOUS TEMPERATURES

	Temperature °C			
Isolate	10°	16°	23°	28°
M-1	0.35	0.53	0.78	0.30
M-4	0.28	0.64	1.10	1.32
CAG-1	0.36	0.58	0.80	0.39
CAG-2	0.22	0.59	1.04	1.50
CAG-3	0.03	0.46	1.31	1.85
CAG-4	0.05	0.19	1.00	1.36
CAG-5	0.00	0.15	1.62	1.76
CAG-6	0.10	0.58	0.58	0.57
CAG-7	0.02	0.36	1.26	1.51

M-1 = Average of Michigan isolates 1, 2, 3, 5 and 6.

M-4 = Michigan isolate 4.

CAG = Ceratobasidium anastamosis group testers.