Red Thread and Pink Patch Diseases of Turfgrasses

JONATHAN D. KAPLAN, Former Graduate Research Assistant, and NOEL JACKSON, Professor, Department of Plant Pathology/Entomology, University of Rhode Island, Kingston 02881

ABSTRACT

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Two groups of basidiomycetous fungi with pink mycelia were found to be associated with a turf ailment commonly referred to as red thread, pink patch, or Corticium disease. This disease complex is caused by a group of different fungi but was previously attributed exclusively to the pathogenic activities of *Corticium fuciforme*. The predominant group was composed of isolates of *Laetisaria fuciformis* forming red thread, needle, or antlerlike stromata on grass blades and had hyphae without clamp connections. A second heterogeneous group contained a number of fungi, including *Athelia fuciformis*, that had hyphae with clamp connections. Physiological and pathological studies on selected isolates from the two groups demonstrated differences in growth rate, temperature parameters, method of ingress, and response to certain fungicides. It is proposed that the name "red thread" disease be restricted to describe symptoms and signs of the turf disease caused by the fungus *L. fuciformis*. Turf exhibiting similar disease symptoms, but lacking needlelike red stromata should be referred to as "pink patch" caused by *A. fuciformis* and perhaps several other basidiomycetes with pink, clamped mycelium.

Red thread disease, also referred to as pink patch or Corticium disease, is documented as a common malady of turfgrasses in temperate regions throughout the world. Foliage of infected plants becomes water-soaked, then tan to bleached, shriveled and invested with pink to red, gelatinous, stranded hyphae. The hyphal aggregates may project as threadlike, needlelike, or antlerlike stromata from the tips of blighted leaves. Signs of the distinctive, colored mycelia have been used routinely to diagnose this turfgrass disease.

In 1873, Berkeley (3) described a hyphomycete collected from cereals in Australia by Baron Von Mueller in 1865. He named the fungus *Isaria fuciformis* Berk. and referred to a filiform stroma that burst through the leaf cuticle and bore minute conidia. Additional records of *I. fuciformis* as a pathogen of grass were reported from Ireland, England, and Australia late in the century, but it was not until 1906 that McAlpine (16) in Australia finally recognized this fungus as a basidiomycete and assigned it to the genus *Hypochnus* as *H. fuciformis* (Berk.) McAlp.

Present address of senior author: Department of Botany and Plant Pathology, University of New Hampshire, Durham.

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In 1917, Wakefield (22) reported a similar fungus on grasses in England. She described the causal organism as having vegetative hyphae that were pinkish, 2–4 μm in diameter, with clamp connections. The pinkish basidia were clavate and bore two to four stout sterigmata, 5.5–7 μm in length. After examining the fungus, and presuming it to be the same as McAlpine's fungus, she assigned both to the genus *Corticium*; hence, the causal fungus for the red thread/pink patch disease complex has been called *C. fuciforme* (Berk.) Wakef. for more than 60 yr.

Many other investigators have since worked on the red thread/pink patch disease complex; descriptions of the causal organism by some workers (6,7,9–12) conform with that of Wakefield (22). Others (2,8) have completed investigations into the physiology of *C. fuciforme* and the control of the disease, but these reports gave no description of the vegetative morphology of the fungus studied. Still others (14,19–21) have described the incitant of the disease as containing pink hyphae, 2–4 μm in diameter, but without clamp connections.

Burdsall (4) recently reexamined the type material of *I. fuciformis* from Australia and the Netherlands. He concluded that, because Berkeley's (3) naming of the fungus was based on the anamorph, any changes in nomenclature citing Berkeley as the original authority (including *C. fuciforme*) were invalid. Burdsall (4) credited McAlpine (16) with the correct disposition of the fungus to the genus *Hypochnus* but reassigned it to a new genus, *Laetisaria*, as *L. fuciformis* (McAlp.) Burds. Burdsall (4) also reexamined type material from which Wakefield (22) made her original description. He found this material not identical to *H. fuciformis* McAlp., as Wakefield (22) had believed, but closely resembling *Athelia singularis* Parm. He proposed a new name, *Athelia fuciformis* (Wakef.) Burds., for the fungus (4).

The implication of Burdsall's (4) findings is that more than one fungus may be involved in causing red thread/pink patch disease on grass plants. Specimens collected from England, and in the United States during 1979–1981, have indeed revealed that more than one fungus may be associated with "typical" red thread/pink patch symptoms. In light of these findings, it was deemed necessary to examine the causal fungi involved with this common turf disease more critically from a morphological, physiological, and pathological perspective to elucidate distinguishing features of these macroscopically similar groups of fungi.

MATERIALS AND METHODS

Isolation of fungi from infected material. During 1979, turf samples exhibiting "typical" red thread/pink patch symptoms and signs were obtained from England, and from Maryland, Missouri, Oregon, Rhode Island, and Washington in the United States. The associated fungi were isolated onto potato-dextrose agar (PDA) medium by plating out either stromal fragments or fragments of infected tissues that had been immersed in 10% sodium hypochlorite for 2 min before plating. The fungi were incubated at 20 C under intermittent subdued light. Morphological characteristics of the fungi thus isolated were in two categories: 1) rapidly growing fungi with pink mycelium bearing clamp connections, and 2) slower-growing fungi with pink mycelium lacking clamp connections.

Physiological tests. For physiological and pathological studies, five fungal isolates were chosen (see Table 1 for the origin of the isolates). Three isolates, STRI-3, RI4D, and RIBC, were chosen from category 1; and two, RIPRG and STRI-1, were chosen from category 2.

To test the effect of temperature on radial growth, 90-mm petri plates containing Difco cornmeal agar were seeded with 5-mm-diameter mycelial plugs, obtained from the edges of actively growing colonies of the isolates to be tested. These plates were then incubated at either 5, 12.5, 20, or 27.5 C. Growth in colony diameter was measured daily for 12 days. There were four replicates for each test, and the experiment was

repeated twice.

A basal salt medium (15), with glucose as a carbon source, was used to construct a growth curve for each isolate. Fifty-milliliter aliquots of this medium were placed in 125-ml Erlenmeyer flasks and sterilized at 15 psi for 17 min, after which the pH was adjusted to 6.0 using 1 M NaOH. For each isolate 40 flasks were seeded with two 5-mm-diameter mycelial plugs, and grown at 15 C on a bench shaker. The contents of four flasks were collected each day for 10 days on a dried, weighed filter paper by vacuum filtration. The mycelial pellets were dried at 40 C for 24 hr, and dry weights were determined.

Pathogenicity tests. The pathogenicity of the isolates was determined on two grasses; *Lolium perenne* L. 'Yorktown' and *Festuca rubra* L. 'Jamestown.' Tests were done on juvenile plants less than 3 mo old and on more mature plants that were at least 9 mo old. All plants were grown from seed in 16 × 7 × 9 cm pots containing autoclaved soil. The inoculum was prepared by growing each isolate on a medium containing wheat (*Triticum aestivum* L.) meal (100 g); ground, dry grass (200 g); sand (400 g); and tap water (750 ml) (18). When the medium was permeated with mycelium, it was broken into pea-sized fragments and three roughly equal fragments were evenly spaced in each pot. The plants were misted with distilled water until runoff and placed in 15 × 7.5 × 45 cm polyethylene bags, which were inflated and closed to keep atmospheric humidity near 100%. The plants were incubated at 15 C with 12 hr of artificial light and 12 hr of darkness. Control plants were inoculated with unseeded medium and grown in similar conditions. Daily observations were made, and symptom development was noted for each isolate.

To determine the mode of entry into the host plant by the pathogens, sections 15–25 μm thick were cut on a freezing microtome or a rotary microtome. Tissue sectioned on the freezing microtome was prepared immediately after removal from infected plants. For sections cut on the rotary microtome, tissue was fixed in Navashin's solution and then dehydrated and imbedded in paraffin (13). Sections were affixed to a clean microscope slide with Haupt's fixative, cured at 30 C for 7 days, and rehydrated. All sections were stained with 0.05% trypan blue in lactophenol and examined with a compound microscope.

Fungicide tests. Because physiological differences were established among the fungal isolates, it was decided to test four fungicides for any possible variation in response by pathogenic isolates. The fungicides tested were iprodione, benomyl, chlorothalonil, and triadimefon. A poisoned agar method was used to determine any differences as follows: under aseptic conditions, aliquots of the fungicides were added to sterile, molten PDA medium. Dilutions were made to give media with fungicide concentrations of 1,000, 500, 250, 125, 100, 50, 25, 12.5, and 0 (control) mg a.i./ml. The media were dispersed into 90-mm petri plates and allowed to harden. Each plate was seeded with a 5-mm plug of the isolate to be tested and incubated at 19 C. Growth was recorded by averaging the lengths of two perpendicular colony diameters every second day. The experiment was run until control plates for each isolate were filled with mycelium, and fungicide effectiveness was measured as a percentage of growth when compared with the control. At the conclusion of the experiments, mycelial plugs that had not grown were removed and placed on fresh PDA to determine whether the action of the fungicide had been fungistatic or fungicidal. There were three replicates for each treatment.

RESULTS

Description of isolates. All isolates cultured in this study were initially distinguished as lacking or possessing clamp connections in the mycelium (Table 1). All isolates whose hyphae lacked clamp connections were identified as *L. fuciformis*, the fungus most commonly associated with red thread/pink patch symptoms. These isolates came from diseased patches of turfgrass containing plants with water-soaked, later tan to bleached leaves that usually were yellowing and shriveling from the tips and matted together by strands of pink, gelatinous hyphae. The mycelia bridged diseased and healthy leaves and often formed prominent, cottony aggregates of hyphae that, upon drying, would fragment into arthroconidia.

Pink mycelium was frequently observed to invest the upper surfaces of healthy leaves. On colonized leaves, the fungus grew beyond the tips of the blades to form red threadlike, needlelike, or antlerlike stromata, 1–2 mm thick and 2–12 mm long, which contained hyphae 4–7 μm in diameter, lacking clamp connections.

In Rhode Island *L. fuciformis* fruited abundantly in the field, especially after prolonged periods of moisture. The effuse basidiocarps varied from hymenial fragments of less than 1 mm to those extending the length of an entire, invested, necrotic grass blade. Often the hymenia extended onto the red stromata. At ×20 magnification, the hymenium had a pink, mealy appearance. The basidia, which were slightly shorter and stouter than those previously described (4), were (11)-22-(30 μm) × 10 μm. Each produced four sterigmata, 6–7.5 μm long, bearing one hyaline, ellipsoid, apiculate spore (7)-10.4-(14 μm) × (4)-5.5-(8 μm).

In nature and in culture, vegetative hyphae were 4–7 μm wide. Branching and anastomosis occurred frequently, but the hyphae lacked clamp connections. Branch initials often gave the false appearance of clamp connections because they curved back toward the parent hyphae. The branch initials lost this clamplike appearance as growth proceeded and parallel hyphae were formed. In culture, *L. fuciformis* formed pink, tufted, slow-growing, irregularly shaped colonies. The mycelium consisted mostly of stranded hyphae, except on the extreme edge of the colony, where separate, branching hyphae could be detected.

Isolates whose hyphae contained clamp connections were collected much less frequently than *L. fuciformis* isolates, and they comprised a heterogenous group. Some isolates (RI4D and RIBC) were obtained from infected leaf tissue jointly colonized by *L. fuciformis*. Other isolates (STRI-3 and RIPJ) were taken from patches of diseased turf with which *L. fuciformis* was not associated. The turf in these patches appeared bleached and pink, although not as intense in coloration as turf infected with *L. fuciformis*. Pink, gelatinous mycelium was observed matting grass blades together and growing from blade to blade. Pink or red needlelike stromata were never present, although some

Table 1. Sources of selected basidiomycetous isolates associated with red thread/pink patch disease of turfgrasses in England and the United States

Isolate designation	Source	Host	Presence of clamp connections in the mycelium[a]
STRI-3	Bingley, U.K.	*Lolium perenne*	+
STRI-1	Bingley, U.K.	*L. perenne*	−
STRI-M	Bingley, U.K.	*L. perenne*	−
STRI-SW	Bingley, U.K.	*L. perenne*	−
STRI-954	Bingley, U.K.	*Festuca rubra*	−
RIF	Kingston, RI	*F. rubra*	+
RI4B	Wakefield, RI	*F. rubra*	−
RI4D	Kingston, RI	*L. perenne*	+
MDPRG	Beltsville, MD	*L. perenne*	−
WAPRG	Puyallup, WA	*L. perenne*	−
ORPRG	Corvalis, OR	*L. perenne*	−
RIPRG	Kingston, RI	*L. perenne*	−
RIBC	Kingston, RI	*Agrostis* sp.	+
RIPJ	Narrgansett, RI	*Poa pratensis*	+

[a] + = Clamps present (category 1), − = clamps absent (category 2) in the mycelium.

mycelium did grow out of cut leaf tips.

One clamped isolate, RIPJ, produced a pink, effuse hymenium in the laboratory on a piece of moistened paper towel that supported infected leaf fragments. The ephemeral basidia were similar in shape to those of *L. fuciformis*, but were slightly smaller (17–20 × 5–8 μm). Hyphae were 2–4 μm in diameter and contained clamp connections. From these observations, the fungus was identified as *A. fuciformis*. The circular colonies produced by this fungus, as well as by isolates RI4D, STRI-3, and RIF, contained pink-tinged surface mycelium with hyphae, 2–4 μm in diameter, that contained frequent clamp connections. Isolate RIBC produced a similar, although less pink, colony but hyphae of this fungus were slightly wider (3–5 μm in diam.).

Physiological tests. Physiological tests revealed several differences between the two groups of isolates (Table 2). In the temperature studies the isolates with clamp connections grew faster than the *L. fuciformis* isolates at all temperatures tested (Table 2). The clamped isolates, RI4D and STRI-3, accumulated dry matter at approximately six times the rate of the *L. fuciformis* isolates when grown at 15 C in liquid culture. The optimum temperature for growth was 20 C for all isolates. Most striking, the two *L. fuciformis* isolates, STRI-1 and RIPRG, did not grow at 27.5 C, a temperature at which growth of the clamped isolates was only marginally suboptimal (Table 2). Bennett (2) and Erwin (8) reported that growth of *C. fuciforme* was optimal at 18–20 C and that growth did not occur at 30 C, suggesting that both men had been working with *L. fuciformis*.

All isolates grew, although slowly, at 5 C, demonstrating the ability to tolerate colder temperatures. These results, coupled with the collection of several *L. fuciformis* isolates and one *A. fuciformis* isolate (RIPJ) from diseased patches in turfgrass during a February thaw in Rhode Island, indicated the potential for both groups of fungi to damage turf during the winter.

Reaction to fungicides. Some differences were noted in the reaction of the two groups of isolates to various fungicides in vitro. Iprodione and triadimefon, at all concentrations, completely prevented growth of the four isolates studied (*unpublished*). All fungicides tested reduced or prevented growth of the nonclamped isolates, RIPRG and STRI-1 (Table 2). Iprodione, benomyl, and triadimefon prevented any growth at all concentrations; the inhibitory effect of the former was fungistatic and the latter two were fungitoxic. Growth suppression elicited by chlorothalonil decreased as the concentration of fungicide in the media decreased.

With two exceptions, the responses of the clamped isolates (STRI-3 and RI4D) were similar to those of the *L. fuciformis* isolates. One exception was that triadimefon was only fungitoxic to these isolates at concentrations of 250 ppm or more, whereas the effect of this fungicide was fungitoxic at all concentrations against *L. fuciformis*. Second was the failure of benomyl to halt growth of the clamped isolates, except at 1,000 ppm, compared with complete control of *L. fuciformis*. These in vitro tests indicate that control of both fungal groups can be best attained by triadimefon or iprodione. Whether or not this tendency holds true in vivo awaits the result of field trials for control of both groups.

Pathogenicity tests. Pathogenicity tests under controlled environmental conditions revealed that one isolate, RIBC, was not pathogenic. The fungus grew out from the inoculum and spread up the surface of the leaves, presumably consuming surface carbohydrates and other exuded materials, but was not able to penetrate the plant tissue so no infection occurred.

The remaining four isolates caused infection on all inoculated plants in a similar manner and time frame, but with some differences. Within 48 hr, the fungi grew out from the inoculum and onto the leaves. The clamped isolates tended to grow up the leaf margins, whereas the *L. fuciformis* isolates grew up between the ribs of the grass blades. Pink, gelatinous mycelium grew over the tips of leaves and formed bridges, connecting colonized and healthy leaves, and spreading the fungus. Infected tissue became water-soaked and bleached, and leaves were soon matted together with mycelium. No pink stromata formed during the incubation period; however, stromata did form on leaf tips of plants inoculated with *L. fuciformis* (but not the clamped isolates) when previously incubated plants were placed out of doors and subjected to natural periods of wetting and drying.

Histology. Histological studies showed another difference between the two groups of isolates. Isolates RI4D and STRI-3 (clamped) were able to enter the host plant through the stomates, but more often did so by directly penetrating epidermal cell walls. The *L. fuciformis* isolates were observed entering host plants only through stomates. Erwin (8) reported that penetration by *C. fuciforme* was strictly via the stomates. Presumably, he also was working with *L. fuciformis*. Once inside the plant, both fungi were able to colonize the tissue extensively. Even under conditions of extensive colonization, no fungus isolate, clamped or unclamped, advanced to the crown of the plants, and all infected plants recovered completely when returned to the greenhouse and afforded optimum growing conditions.

DISCUSSION

This study has shown that more than one organism can induce the symptoms commonly described as those of the red thread/pink patch disease. Two general groups of pathogens have been distinguished morphologically, physiologically, and pathologically. One group consists of isolates of the fungus *L. fuciformis*, the predominant incitant of red thread disease in Rhode Island, and probably elsewhere. The second group contains fungi with pink mycelium containing clamp connections. Isolates in this category were collected much less frequently than were *L. fuciformis* isolates. Fruiting was not observed for all isolates with clamp connections, and the investigation indicated that these fungi comprise a heterogenous group.

Results of all experiments indicated that two of the isolates with clamp connections, STRI-3 and RI4D, are probably the same fungus species. Although not reported in this text (*unpublished*) several physiological tests identical to those described were done using the RIPJ isolate, which conforms closely to *Athelia fuciformis*. The results were similar to those obtained for the STRI-3 and RI4D isolates, and indicate that all of these are isolates of *A. fuciformis*.

In addition to *L. fuciformis* and *A. fuciformis*, other pink, resupinate basidiomycetes have been reported on senescing grass leaves or as superficial fungi on stems and leaf sheaths. Among them are *Exobasidiellum graminicola* (Bres.) Donk and *Galzinia culmigena* (Webster & Reid) Johri and Bandoni (1,17). Both fungi have pink mycelium, 2–4 μm in diameter, bearing clamp connections. Fungi closely resembling

Table 2. Growth rates of five fungal isolates associated with red thread (mycelium without clamp connections) and pink patch diseases (mycelium with clamp connections) and the effective doses of benomyl and chlorothalonil on four of them

Isolate		Growth rates[a]			Fungicide tests			
					Chlorothalonil		Benomyl	
Designation	Clamps	15 C (mg/day)	20 C (mm/day)	27.5 C (mm/day)	ED_{50}	ED_{100}	ED_{50}	ED_{100}
STRI-3	+	17	11	7	<12.5	>1,000	250	1,000
RI4D	+	19	12	7	<12.5	>1,000	250	1,000
RIPRG	−	3	8	0	<12.5	>1,000	<12.5	<12.5
STRI-1	−	3	10	0	<12.5	>1,000	<12.5	<12.5
RIBC[b]	+	2	14	12	...	...	...	...

[a] Values to nearest whole number.

[b] Not pathogenic on *Lolium perenne* 'Yorktown' or on *Festuca rubra* 'Jamestown.'

the latter have been found in Rhode Island, growing on senescing leaf sheaths of *L. perenne* and on culms of *Agrostis alba* L. and a *Glyceria* sp. Occasionally, they would encroach slightly onto healthy leaves, but they caused no damage to the plants. In our judgment, all the aforementioned fungi, with the exception of *L. fuciformis*, occur in the turf environment as antagonistic facultative symbionts (5). As such, they might attack plants that are severly stressed, perhaps by drought, cold, or an infection by some other fungus like *L. fuciformis*. On the other hand, *L. fuciformis* should be classified as an antagonistic obligate symbiont, indicating its need for a symbiotic association for normal, phenotypic development. Results of the physiological tests indicate that *L. fuciformis* is less suited for the saprophytic existence than the clamped isolates tested, but further, more complete studies must be done to substantiate this claim.

It is proposed that the name "red thread" disease be used to describe symptoms and signs, caused by *L. fuciformis*, that typically appear on turfgrass plants as water-soaked, then tan to bleached, shriveled leaves, matted together by pink mycelium and bearing numerous needlelike or antlerlike red stromata. Diseased turf exhibiting similar symptoms, but caused by basidiomycetes with pink clamped mycelium that lack needlelike red stromata, should be referred to as "pink patch" disease. This differentiation will provide a means of distinguishing between the two similar diseases. In addition, it is recommended that a reexamination of red thread disease be undertaken in other laboratories. Only by surveying the fungi involved from diverse locations will the incitants of the red thread/pink patch disease complex be identified and their relative importance be determined.

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