Yellow Ring on *Poa pratensis* Caused by *Trechispora alnicola*

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ABSTRACT

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Trechispora alnicola was identified as the causal agent of yellow ring disease of *Poa pratensis*. The fungus was isolated from surface-sterilized roots and crown tissues of naturally infected sod. Mycelium and conidia were effective forms of inoculum when placed adjacent to the grass crowns or roots or drenched into sod. Kentucky bluegrass seedlings developed yellowed leaves, typical of yellow ring disease, 6–8 wk after inoculation. Inoculated sod required 16 wk to develop yellowed leaves. Disease severity was greatest at 20 and 25 C, and no disease symptoms developed at 30 C. Bluegrass susceptibility was not affected by maturation; both 3-wk-old and 2-yr-old sod were susceptible. The fungus was capable of saprophytic colonization of naturally produced thatch. The fungus was readily recovered from inoculated grass roots after surface sterilization but not from crown tissue. In culture, the fungus grew most rapidly on thatch agar medium at 20–25 C and was capable of growth at 15 and 32 C.

Yellow ring on *Poa pratensis* L. is a fairy ring disease dissimilar from those described by Filer (2), Redhead and Smith (5), and Smith (7). Yellow ring disease does not result in necrosis of grass plants, and disease symptoms are not always visible in the sward (9). The symptoms of this disease are rings of yellowed grass leaves that may appear each year from May through October. Wilkinson (9) identified *Trechispora alnicola* (Bourd. & Galz.) Liberta associated with grass plants displaying yellow ring symptoms. This fungus was previously described as a wood-rotting microorganism but not as a pathogen (4). Jackson (3) described a related fairy ring disease occurring on *Agrostis tenuis* Sibth., *Festuca rubra* L., and meadow-grass turfs and identified *T. confinis* (Bourd. & Galz.) Liberta as the probable causal agent. The symptoms and progression of this fairy ring are unlike those of yellow ring. *T. confinis* does not cause yellow symptoms, and the disease appears in a grass sward as a patch, not a ring (3). Yellow ring on *P. pratensis* has been observed in Illinois, Iowa, Indiana, New York, New Jersey, Pennsylvania, and Ohio (9). The etiology of this disease is unknown. The objective of this research was to determine if *T. alnicola* is the causal agent of yellow ring. A brief report of this work has been presented (9).

MATERIALS AND METHODS

Isolation. *P. pratensis* sod with yellow ring symptoms was collected from near St. Anne, IL. *T. alnicola* was isolated by transferring mycelia or conidia from diseased turf to petri dishes containing acidified thatch agar medium, pH 4.5, or acidified malt agar medium, pH 4.2. Isolations were also made from surface-sterilized (45 sec, 0.65% sodium hypochlorite), naturally infected crowns and roots collected in the field. Thatch agar medium was prepared by removing soil and leaves from field-grown sod, air-drying and fragmenting (0.5 mm diameter) the thatch, and boiling (15 min) 200 g in 500 ml of deionized water. The thatch was separated from the extract by filtering in through eight layers of cheesecloth. The extract was combined with 17 g of Difco-Bacto agar, 20 g of sucrose, and sufficient water to make 1 L. The thatch medium was then sterilized (6.8 kg pressure/160 C, 20 min) and dispensed into petri dishes (9-mm-diameter). The medium was about pH 6.8. Cultures were routinely incubated at 21–22 C on a laboratory bench.

Growth in culture. *T. alnicola* grown on thatch agar was transferred to malt agar, potato-dextrose agar (PDA), one-fifth-strength PDA, and V-8 juice agar and incubated at 22 C for 3 wk to "condition" the fungus to each medium. A 5-mm-diameter plug of each conditioned culture was inverted and placed on fresh medium in a petri dish. The radial growth of the fungus was measured daily and recorded. Five replicates on each medium were incubated in the dark at 15, 20, 25, 27, 30, 32, and 35 C. The experiment was repeated three times.

Thatch used to assay the saprophytic capability of *T. alnicola* was prepared by cutting plugs (4 cm diameter) with a thatch layer 3 cm thick from 2-yr-old sod (an equal blend of the bluegrass cultivars Baron, Majestic, and Touchdown). The thatch plugs were air-dried for 7 days, and all soil and green plant materials were removed. The thatch plugs were placed in plastic tubes (5 × 25 cm) (Conetainer Co., Canby, OR) partially filled with 100 ml of vermiculite. *T. alnicola* was placed in the thatch plugs as a spore suspension or buried beneath them as a culture plug (1 cm diameter). The thatch plugs were watered every other day to keep them moist and incubated at 20 C.

Pathogenicity tests. Spore suspensions, plugs from vigorously growing cultures, and naturally colonized bluegrass plant tissue were used as sources of inoculum in pathogenicity tests. Spore suspensions were prepared by flooding 5 ml of deionized water containing Tween 80 (0.01%) onto a fresh culture of *T. alnicola* grown on thatch agar medium and dislodging the spores by gentle scraping. The spore suspension was filtered through four layers of cheesecloth to remove mycelial fragments. Spore density was determined with a hemacytometer and adjusted to 1×10^4 spores per milliliter. Culture plugs (1.5 cm diameter) were cut from a culture of *T. alnicola* grown on thatch agar medium for 3 wk. *T. alnicola*, in air-dried field sod, was used as inoculum after removal of green leaves and soil. The dried sod was fragmented to 1 mm diameter using a Wiley mill. The fragmented material was air-dried and weighed.

Pathogenicity studies were carried out in tapered plastic tubes or 20-cm-diameter clay pots. The tubes were partially filled with 100 ml of vermiculite or fumigated Drummer clay-loam (0.34 kg of methyl bromide per 90 kg of soil). Kentucky bluegrass cultivar Baron seed or sod were then placed in contact with the vermiculite or soil. Sod plugs (4 cm diameter) used for these tests were grown for 6 wk in tubes starting from seed (i.e., young sod) or 2-yr-old sod collected from the field (i.e., field sod). Just before sod inoculation, sod roots further than 1.25 cm from the crown tissue were excised and discarded. The prepared sod plugs were placed directly on the vermiculite or soil surface. For pathogenicity experiments requiring longer than 6–8 wk of incubation, sod plugs were initially transplanted to 20-cm clay pots filled

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with fumigated Drummer soil.

Grass seedlings were inoculated by drenching with a spore suspension, placing culture plugs beneath or beside the grass, or by adding infested debris to the grass seedlings. Five milliliters of a spore suspension (1×10^4/ml) were used to inoculate seedlings grown in the plastic tubes. Suspensions were topically applied to the 3-wk-old grass with a pipette. Inoculum consisting of culture plugs of *T. alnicola* grown on thatch agar medium was placed in the tubes before seeding or adding sod. If the grass was seeded, the culture plug was covered with vermiculite or soil. Sod was placed directly on top of the culture plug. Colonized debris (0.3 or 5 g per tube) was used to inoculate both seedlings and sod. Seed was placed directly on the debris at planting or the inoculum was placed around the bases of 3-wk-old seedlings and covered with a thin layer (0.5 cm thick) of vermiculite. Sod was inoculated by placing of debris in the plastic tube (0.3 g) or clay pot (5.0 g) and planting the sod directly on top of the inoculum. All treatments were watered every third day, fertilized to maintain vigorous growth, and incubated in a greenhouse (natural light, 20–23 C) for up to 30 wk.

A disease severity rating scale (1–5) was used to describe grass susceptibility: 1 = darkened green leaves, 2 = faint yellow leaves, 3 = light yellow leaves, 4 = canary yellow leaves, and 5 = dark yellow leaves. In addition, soil and plants were removed from each tube or clay pot and inspected for signs of fungal colonization or aberrant growth of crowns and roots. Fungi colonizing the turf were microscopically observed, isolated, and identified. Discolored areas in the roots and/or crowns were dissected with a scalpel, surface-sterilized (45 sec) in sodium hypochlorite (0.065%), rinsed in sterile, deionized water, and seeded onto acidified thatch agar medium. Fungal isolates growing from the plant tissues were identified and compared with *T. alnicola* in culture.

RESULTS

T. alnicola was isolated from diseased bluegrass sod showing yellow ring symptoms in the field. After surface sterilization of the crowns and roots of diseased plants, the fungus was recovered from 20 of 200 crowns and 49 of 311 roots collected. The crowns and roots from these plants were slightly discolored and stunted compared with grass plants not showing yellow ring symptoms in the field. Isolating *T. alnicola* by direct transfer of mycelial fragments colonizing turf to an agar medium proved difficult because of contamination by other fungi and bacteria. Direct transfer of conidia (adhering to a needle) to an acidified agar medium was an effective means of isolation.

The growth of *T. alnicola* in culture was affected by the source of nutrients and the temperature of incubation. Thatch agar medium supported the fastest growth compared with malt agar, one-fifth-strength PDA, PDA, or V-8 juice agar media, which supported slower growth rates. On thatch agar medium, the fungus grew about 1 mm/24 hr at 17 and 25 C and 0.8 mm/24 hr at 15 and 20 C. On malt agar medium, the fungus grew 0.7, 0.6, and 0.5 mm/24 hr at 27, 30, and 32 C, respectively. The fungus failed to grow on malt agar medium at 35 C. The fungus produced conidia on thatch agar, malt agar, and one-fifth-strength PDA media but not on PDA or V-8 juice agar media.

Kentucky bluegrass inoculated 3 wk after seeding was infected by *T. alnicola* and developed root symptoms and yellow leaves similar to diseased plants associated with this fungus in the field (Fig. 1). Three-week-old seedlings inoculated with a suspension of conidia, a culture plug, or infected debris developed infections and discolored roots after 6 wk. Infected roots were slightly stunted and tan to light brown compared with roots of uninoculated plants. Distinct lesions were not observed on infected roots. *T. alnicola* was isolated on acidified thatch agar medium from 89 of 113 discolored roots, representing 59 inoculated plants. The fungus was not isolated from the crowns of inoculated plants. Infected roots had thin hyphae (2 μm diameter) ramifying through the epidermal and upper palisade layers. Colonization appeared to be intracellular, but detailed histological observations were not made. Inoculation of Kentucky bluegrass at planting did not result in disease symptoms.

Young sod incubated at 20 C was infected by *T. alnicola* after inoculation with 5 ml of a spore suspension applied as a drench (Fig. 1). Yellowed leaves developed 18–20 wk after inoculation. At 25 and 30 C, disease was not observed on seedlings or young sod. Inoculated field sod did not develop yellow ring symptoms. When inoculated with culture plugs or colonized debris, *T. alnicola* saprophytically colonized the thatch material of field sod, but attempts to isolate the fungus from living roots 18 wk after inoculation were unsuccessful. The fungus also saprophytically colonized thatch plugs devoid of living bluegrass. Mycelia and conidia (saprophytic colonization) were observed in thatch at 15, 20, and 25 C but not at 30 C.

Severity of yellow ring was affected by temperature and turf age. Disease severity ratings of yellow ring in 3-wk-old bluegrass seedlings and young sod were 2.1 (C.V. = 10.5%) and 1.2 (C.V. = 1.8%), respectively, at 25 C. At 20 C, disease severity ratings were 4.0 (C.V. = 5.9%) and 3.6 (C.V. = 8.9%) for seedlings and young sod, respectively. At 25 and 30 C, inoculated seedlings developed no symptoms. At 17 C, only 3-wk-old seedlings responded to inoculation (C.V. = 8.9%). Only 10% of all inoculated treatments resulted in disease severity ratings greater than 2.0, and symptoms remained visible for no longer than 6 wk after their initial appearance.

DISCUSSION

T. alnicola isolated from *P. pratensis* root and crown tissues was demonstrated to be a parasitic pathogen of bluegrass. Generally, the response of *P. pratensis* to infection by *T. alnicola* was plesionecrotic. Slight discoloration and stunting of the roots and leaf yellowing, which appear to be limited and temporary, were the typical symptoms observed, and these symptoms were similar to those observed in the field (9). The name yellow ring is

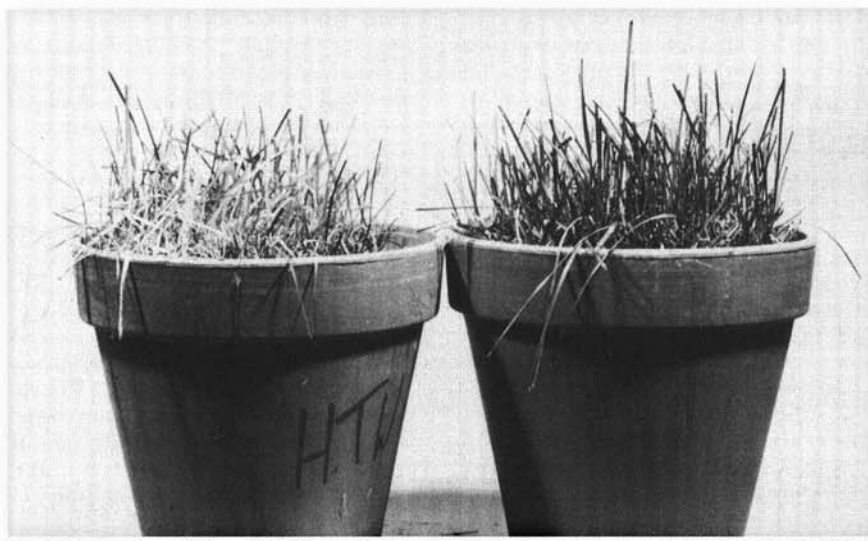

Fig. 1. *Poa pratensis* 'Baron' (left) inoculated with *Trechispora alnicola* and (right) uninoculated. A conidial suspension (1×10^4/ml) was used to inoculate the sod 6 wk after seeding. All treatments were incubated at 20–23 C under natural light. Symptoms were first observed about 18 wk after inoculation.

proposed for this disease of bluegrass.

This is the first report of *T. alnicola* as a pathogen on any plant species. *T. alnicola*, though parasitic on *P. pratensis*, is a weak pathogen. Based on the degradation of thatch to form a mat layer in sod (9), this fungus appears to be well suited as a saprophyte. Others have also noted this characteristic (4). The fungus saprophytically colonized thatch material at 15, 20, and 25 C, representing a greater temperature range than for pathogenesis. Liberta (4) reported that *T. alnicola* and other *Trechispora* species are commonly found colonizing dead plant material. Because conducive conditions for saprophytism and parasitism overlap, it might be expected that both processes occur simultaneously in a bluegrass sward. This is supported by the observation that dense mycelial colonization of thatch in field sod occurred when yellow leaves were present or absent (9). *Marasmius oreades* (Bolt. ex Fr.) Fr., a pathogen of *P. pratensis* causing a fairy-ring disease, also colonized thatch in addition to infecting grass (7).

The long incubation time between inoculation and symptom development and the sublethal nature of pathogenesis would suggest that *T. alnicola* is a slow-growing microorganism. This is supported by the observation that the fungus grew only 1 mm/24 hr or slower in culture. The slow growth rate and weak pathogenesis suggest that yellow ring symptoms may not develop until after numerous infections have occurred on a plant. The lack of distinct necrotic lesions or root destruction indicates that individual infections do not result in extensive cellular death.

T. alnicola is capable of infecting grass when conidia or mycelia are associated with debris or removed from it. The conidia germinate in water, which could serve as a dissemination medium. The survival potential of conidia in turf is not known. Survival of mycelium, which is hyaline and thin (8), infecting living grass roots and crowns or in turf debris, could be potentially great because of the protection afforded by these sources. The conditions conducive for infection also support vigorous bluegrass growth, but the pathogen did not produce disease symptoms when inoculum was buried under field sod. The pathogen did produce symptoms when topically drenched into young sod but not field sod. These observations suggest that the parasitism by *T. alnicola* could be inhibited by microorganisms inhabiting the turf. This idea is supported by the low frequency of plants inoculated with *T. alnicola* that developed yellow ring. In addition, the remission of symptoms 6 wk after their initial appearance indicates that pathogenesis is a sensitive process that is easily inhibited.

T. alnicola infected 3-wk-old seedlings and young sod, but not 2-yr-old sod, indicating that under field conditions, the entire population of mature plants may not be susceptible. Bluegrass shoots generally survive 6–18 mo (1), however, which would result in a continuously available supply of susceptible plants in a sod. *T. alnicola* apparently does not have the potential to kill a bluegrass plant. Based on the intermittent display of symptoms, it is suggested that in response to conducive edaphic conditions, *T. alnicola* increases its parasitic activities, which partially destroy root tissue and leaf chlorophyll. This type of interaction is supported by the observation that disease severity was greatest when *T. alnicola* extensively colonized the thatch (6).

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