

# Control of Yellow Ring in Kentucky Bluegrass Swards

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## ABSTRACT

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*Trechispora alnicola* is the causal agent of yellow ring of *Poa pratensis*. In culture, isolates collected from the same diseased pieces of sod or from different swards did not grow or produce conidia after the two thalli intersected. Transplantation of diseased sod to cultivated soil or inoculation of symptomless field sod with plugs of diseased sod failed to develop yellow ring after 3 yr. Twenty-one cultivars of *P. pratensis* were susceptible to infection by *T. alnicola*, but 10 of these cultivars showed low disease ratings. In addition, 50 of 89 bluegrass selections and crosses showed less disease. Bluegrass mixtures consisting of two to five cultivars developed severe yellow ring in the field. *Agrostis palustris*, *Festuca rubra*, and *Lolium perenne* were not susceptible to infection by *T. alnicola*. The chemical PCNB (6.1 kg/ha) prevented the effective dissemination of *T. alnicola* and limited the severity of yellow ring in sod but did not eliminate disease symptoms. The fungicide propiconazole (6.1 kg/ha) reduced both the occurrence of new infections and disease severity but was less effective than PCNB.

*Trechispora alnicola* (Bourd. & Galz.) Liberta is the causal agent of yellow ring in bluegrass swards (7). Yellow ring is a fairy ring but differs from the fairy ring caused by *Marasmius oreades* (Bolt. ex Fr.) Fr. (1,4,6) in that pathogenesis does not result in necrosis and disease symptoms are not always obvious in a sward (9). Jackson (2) reported that *T. confinis* (Bourd. & Galz.) Liberta was the probable cause of a fairy ring in *Agrostis tenuis* Sibth., *Festuca rubra* L., and meadowgrass, but the symptoms in these swards were not rings of yellowed leaves that characterize the yellow ring disease. It also is unknown if the disease observed by Jackson resulted from pathogenesis by *T. confinis*. It has not been reported that *T. alnicola* will infect turfgrass species other than *P. pratensis* L. Yellow ring has been observed in bluegrass swards in Illinois, Iowa, Indiana, New York, New Jersey, Pennsylvania, and Ohio (9), but the susceptibility of individual bluegrass cultivars is unclear. Similarly, it is unknown if mixing bluegrass cultivars will reduce the development of yellow ring. The objectives of this research were to determine the susceptibility of turfgrasses to *T. alnicola* and to investigate potential chemical and biological management of this disease. A brief report of this work has been presented (7).

## MATERIALS AND METHODS

**Isolation of *T. alnicola*.** *P. pratensis* sod infested with *T. alnicola* was collected from sod farms near St. Anne

and Harvard, IL, Adelphi, NJ, and South Bend, IN. Isolations of *T. alnicola* were made by direct transfer of mycelium or conidia from diseased turf into petri dishes containing acidified thatch agar medium (pH 4.5) (9). Cultures were transferred to and maintained on thatch agar medium (pH 6.8) at 21–22 C on a laboratory bench.

The compatibilities of isolates of *T. alnicola* from St. Anne (group 1), Harvard (group 2), Adelphi (group 3), and South Bend (group 4) were assayed on thatch agar medium. Group 1 consisted of four isolates (1a–1d), each recovered from different rings of yellowed grass within 1 ha. Groups 2–4 represented isolates from single rings of diseased grass from different fields. Using two isolates, pieces (2 mm<sup>2</sup>) of actively growing culture were placed 3 cm apart on a microscope slide (1.9 × 7.5 cm) coated with a 2-mm-thick layer of thatch agar medium. The slides were placed on glass supports in a glass petri dish containing three layers of filter paper saturated with water. Observations were made every 48 hr after the mycelia intersected on the medium. General mycelial appearance, growth, and conidial production were recorded. All combinations of the seven isolates were tested in triplicate and repeated twice.

**Pathogenicity tests.** The susceptibility of *A. palustris*, *F. rubra*, and *Lolium perenne* L. to infection by *T. alnicola* was tested using a tube assay previously described by Wilkinson (8). Plastic Conetainers (Conetainer Co., Canby, OR) were partially filled with 100 ml of vermiculite and seeded with about 100 grass seeds. The seeds were covered lightly with vermiculite, watered every other day, and maintained in a greenhouse

at 20–22 C. About 3 wk after seeding, the grass in each cone was inoculated with 5 ml of a spore suspension ( $1 \times 10^4$  ml in 0.01% Tween 80). The inoculum was pipetted directly onto the crowns. Inoculated grass was watered every other day and incubated in a growth chamber (16 hr of light, 20 C). Disease severity (DS) was rated (1–5) on leaf symptoms (1 = dark green, 2 = faint yellow, 3 = light yellow, 4 = canary yellow, and 5 = dark yellow) 8 wk after inoculation. Fourteen replicates of each treatment were used, and the experiment was repeated three times.

Five hundred square meters of 3-yr-old sod from near Peoria, IL, was transferred to Urbana, IL, in May 1983 and placed on a Drummer silt loam. The sod was an equal blend of the bluegrass cultivars Baron, Majestic, and Touchdown. It was free of yellow ring disease and had a thatch layer 3 cm thick. In June 1983, the sod was exposed to plugs of bluegrass infested with *T. alnicola* collected from near St. Anne, IL. Plugs of sod 5 cm thick and 10 cm in diameter were taken from turf showing yellow ring symptoms, and 3 hr later, they were transplanted into the sod at Urbana. A total of 200 diseased turf plugs and 47 disease-free plugs were transplanted into the disease-free sod. Each plug was placed 60 cm from the next nearest plug. All turf plugs were randomly distributed, and permanent corner markers were used to identify the location of each plug. The sod was watered to maintain vigorous grass growth and fertilized (193 kg NPK/93 m<sup>2</sup>/yr), and broadleaf herbicides were applied as needed. The infested sod was rated for severity of yellow ring and colonization by *T. alnicola* from June 1983 through November 1985.

Samples of two severely diseased sods (DS = 5) were collected from near St. Anne and Harvard, IL, in 1982, cut into pieces 0.45 × 1.8 m, and transplanted on Drummer silt loam at Urbana. The two sods were equal blends of the bluegrass cultivars Baron, Bristol, Majestic, Parade, Touchdown, and Victa and of Adelphi, Glade, Nassau, and Ram I. Sod pieces showing no symptoms of yellow ring also were collected from both areas and transplanted. Two pieces of diseased and disease-free sod were arranged as areas 0.9 × 1.8 m, and each sod area was replicated four times. The sod was maintained as described earlier. The grass was mowed weekly to a height of about 5 cm, and leaf symptoms were

rated (1–5) monthly and recorded from June 1982 through November 1985.

Twenty-one commercially available cultivars of *P. pratensis* were evaluated for susceptibility to *T. alnicola* under field conditions at the Soils and Crops Research Center, Rutgers University, Adelphi, NJ. This research was conducted in cooperation with R. E. Funk, Department of Crops and Soils, Rutgers University. The sources of the seeds were: A-20 (Warren's Turf Nursery), Adelphi and America (International Seeds, Inc.), Banff and Barbie (Baren Brug Co.), Baron (Lofts, Inc.), Bayside, Birka, and Bristol (O. M. Scott & Sons), Eclipse, Enmundi, and Enoble (North American Plant Breeders), Midnight (Turfseed, Inc.), Touchdown and Trenton (Northrup King), Vanessa (Mommersteeg, Int.), and Victa, Wabash, and Welcome (Tib Szego Assoc.). The cultivars were tested as pure stands. Each bluegrass sward was replicated three to five times in a completely randomized design and seeded at a rate of 17 kg/ha in September 1979. All sod received two applications (May and September) of 24.0, 48.9, 34.4, 28.9, and 44.0 kg of nitrogen per hectare per year in 1979, 1980, 1981, 1982, and 1983, respectively. The herbicides 2,4-D-dicamba, bensulide (Betasan), and chlorthal dimethyl (Dacthal) were used

as needed. The sod was mowed to a height of about 5 cm. Yellow ring was first observed in the sod in June 1982. In August 1983, the severity of yellow ring was rated for each plot. The rating scale of 1–5 was used. All plots rated  $\geq 1.0$  were examined for *T. alnicola* in the thatch.

**Chemical control of yellow ring in the field.** Chemicals to control yellow ring were tested on sod farms near Peoria, Harvard, and St. Anne, IL, and South Bend, IN. The chemicals used were triadimefon (0.38, 0.76, and 1.52 kg/ha), propiconazole (0.21, 0.42, and 0.84 kg/ha), PCNB (3.05 and 6.11 kg/ha), and flurmeicyclox (3.05 and 6.11 kg/ha). Treatments were applied in 1983 during May and 6 wk later during mid-July. Each treated area (0.2 ha) was replicated three times and arranged as a completely randomized block. Disease symptoms were rated at least once a month starting on the first day of May using two systems. Twenty-five yellowed rings of grass were randomly selected in each replicated area and the leaf symptoms rated (1–5). The area of each replicate bordered by rings of yellow grass was also estimated and recorded as a percentage of a treated area.

## RESULTS

**Compatibility of *T. alnicola* isolates.** Isolates of *T. alnicola* from different geographic areas and from different areas within a common sward showed incompatibility in culture, and each isolate was also self-incompatible. Isolates grew at similar rates (1 mm/24 hr) until 48 hr after the mycelia had intersected on the medium, at which time growth of both isolates ceased. In the area of intersection (4–6 mm wide), mycelia were hyaline and measured about 2  $\mu$ m in diameter; conidia were not observed. Conidia were produced on mycelia from the distal ends of the slide to within 3–5 mm of the area of intersection. All mycelia were about 2  $\mu$ m in diameter and hyaline.

**Susceptibility of grass species in controlled environment.** *A. palustris*, *F. rubra*, and *L. perenne* failed to show symptoms, and *P. pratensis* showed extensive yellowing after inoculation with *T. alnicola*. Mean disease severity ratings were 0.6, 0.8, 0.3, and 4.2, respectively, and only the rating for *P. pratensis* was significantly different from uninoculated treatments according to a Duncan's multiple range test ( $P = 0.05$ ). The roots of each nonsusceptible species showed no discoloration or stunting, and attempts to isolate *T. alnicola* after surface sterilization were unsuccessful.

**Susceptibility of field-grown sod.** Bluegrass sod exposed to plugs of diseased sod in the field failed to develop yellow ring 3 yr after infestation. Using permanent markers to locate the plugs, we attempted to isolate *T. alnicola* from

50 randomly selected inoculum plugs in 1984 (1 yr after transplantation) but were unsuccessful. Mycelium originally visible in the thatch material in the inoculum plugs in 1983 was not visible in June 1985.

Severely diseased pieces of sod (DS = 5) established at Urbana showed yellow ring symptoms for about 8 wk after transplantation. With the disappearance of the original symptoms in 1982, yellow ring was not observed in the sod through 1985.

**Susceptibility of grasses to *T. alnicola*.** Cultivars of *P. pratensis* that showed typical yellow ring symptoms had a dense layer of mycelia and conidia in the thatch. All sods examined were 3 yr old and had a thatch layer  $\geq 2$  cm thick. All disease in this experiment resulted from indigenous sources of inoculum; therefore, the evaluation provides information on susceptible cultivars, and it cannot be used to distinguish cultivars that are resistant from those that escaped infection. All cultivars evaluated were susceptible to *T. alnicola* (Table 1). The mean disease severity rating ranged from 1.0 to 4.5. Ten cultivars received a mean DS  $\leq 2.0$  and six at  $2.0 < DS \leq 3.0$ . Only three cultivars received a mean DS  $\geq 4.0$ . In addition, 89 other field selections and experimental hybrids were susceptible to *T. alnicola*. Fifty of these sods were scored means at DS  $\leq 2.0$ , 13 at  $2.0 < DS \leq 3.0$ , 10 at  $3.0 < DS \leq 4.0$ , and 16 at  $4.0 < DS \leq 5.0$ .

Thirteen of 21 sods consisting of two to five *P. pratensis* cultivars mixed before seeding showed severe (DS = 4.0–5.0) yellow ring symptoms. These data are from bluegrass in Illinois, Indiana, and Wisconsin, and infections resulted from natural sources of inoculum. The remaining eight swards examined had fairy ring diseases caused by fungi other than *T. alnicola*. In no case did yellow ring appear on sod less than 21 mo old or on sod with a layer of thatch  $< 2$  cm thick. Mean DS values for the swards ranged from 3.8 to 4.9.

**Control of yellow ring disease with fungicides.** The fungicides PCNB and propiconazole reduced the severity of yellow ring in the field (Fig. 1A). PCNB was equally effective at 3.05 and 6.11 kg/ha in limiting the severity of yellow ring. Four weeks after treatment, PCNB reduced the disease severity but did not provide complete control. PCNB was also effective in preventing an increase in the total area of diseased turfgrass (Fig. 1B). During May through July 1983, the area of diseased turfgrass increased from 20 to 33% in swards receiving only water. Propiconazole at 0.84/ha had a significant but temporary effect on the severity of yellow ring by delaying the development of severe yellow ring until August (Fig. 1A). It limited the total area of diseased turfgrass compared with turf receiving only water (Fig. 1B). In turfgrass receiving propiconazole at 0.84 kg/ha,

**Table 1.** *Poa pratensis* cultivars susceptible to *Trechispora alnicola*<sup>1</sup>

Cultivar	DS <sup>2</sup>
Banff	1.0 a
Bristol	1.0 a
Enmundi	1.0 a
Bayside	1.5 a
Birka	1.5 a
Enoble	1.5 a
Adelphi	1.9 a
Touchdown	2.0 a
Victa	2.0 a
Welcome	2.0 a
America	2.5 ab
Sydsport	2.5 ab
Eclipse	2.8 b
Midnight	2.8 b
Barbie	3.0 b
Trenton	3.0 b
Vanessa	3.2 b
Blacksburg	3.5 bc
Baron	4.0 c
Wabash	4.0 c
A-20	4.5 c

<sup>1</sup>Each cultivar was seeded as five replicates at 16 kg of seed per hectare in September 1979 at the Soils and Crops Research Center, Rutgers University, Adelphi, NJ. In August 1983, all cultivars listed showed typical symptoms of yellow ring, and *T. alnicola* was identified in each.

<sup>2</sup>Disease severity (DS) was rated in 1983, using leaf symptoms, on a scale of 1–5, where 1 = dark green, 2 = faint yellow, 3 = light yellow, 4 = canary yellow, and 5 = dark yellow. All cultivars were replicated five times. Values with the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

about 27% of the treated area showed yellow ring symptoms compared with 33% of the area for turfgrass receiving no fungicides. Propiconazole at 0.21 or 0.42 kg/ha did not reduce disease severity. Triadimefon applied at 1.5 kg/ha, but not at lower rates, retarded the development of severe disease until mid-June but was ineffective after that date (Fig. 1A).

## DISCUSSION

Each isolate of *T. alnicola*, representing a single ring of diseased grass, was incompatible with other isolates when grown together on the same culture medium. Mycelial growth and conidiation were halted when isolates intersected. In field sod, one isolate of *T. alnicola* did not invade grass previously colonized by another isolate (H. T. Wilkinson, unpublished). This incompatibility suggests that only conidia that are dispersed to uncolonized turf will infect a grass plant or colonize thatch. Isolates of *M. oreades* that cause fairy rings are also incompatible, but the fungus does not produce conidia (5). *T. alnicola* and *M. oreades* do not appear incompatible, that is, disease development in sod is not interrupted when these organisms intersect (H. T. Wilkinson, unpublished).

The competitive ability of *T. alnicola* compared with other soil microorganisms was not determined, but the observed lack of pathogenism or saprophytism upon transplantation of diseased sod would support the idea that *T. alnicola* is not an aggressive soil colonist. Furthermore, the absence of disease development in sod with <2 cm of thatch and the lack of fungal colonization at the thatch-soil interface suggest that growth of *T. alnicola* is limited to thatch.

Susceptibility to *T. alnicola* appeared to be limited to *P. pratensis* in this study. Greenhouse pathogenicity tests demonstrated that *A. palustris*, *L. perenne*, and *F. rubra* were not susceptible to infection. This was supported by a lack of observations or reports of yellow ring developing in sod lacking *P. pratensis* or sod consisting of a bluegrass-ryegrass or bluegrass-fescue mixture.

Twenty-one cultivars of *P. pratensis* were susceptible to infection by *T. alnicola* in the field. Differences in disease severity among infected bluegrass cultivars indicated that resistance may exist among the cultivars tested, but data supporting this are tentative because of

limited field evaluation. The use of natural inoculum for field evaluation of resistance may have compensated for nonuniform distribution of inocula and permitted grass plants to escape infection. If genetic resistance does exist, it is possible that the use of resistant cultivars or the blending of bluegrass cultivar(s) with other turfgrass species could impede the development of yellow ring disease in sod. The inability of *T. alnicola* to infect creeping bentgrass, perennial ryegrass, and red fescue would support this, but in bluegrass swards that included Adelphi, Touchdown, or Victa, disease severity was great even though they appeared to have some resistance. Careful selection and blending of highly resistant cultivars may provide significant disease resistance. Disease severity values for 50 of the 89 field evaluated selections and commercial cultivars were  $\leq 2$ , a mild disease symptom. It is possible that this level of resistance, combined with a fungicide application, could reduce disease severity to an acceptable level.

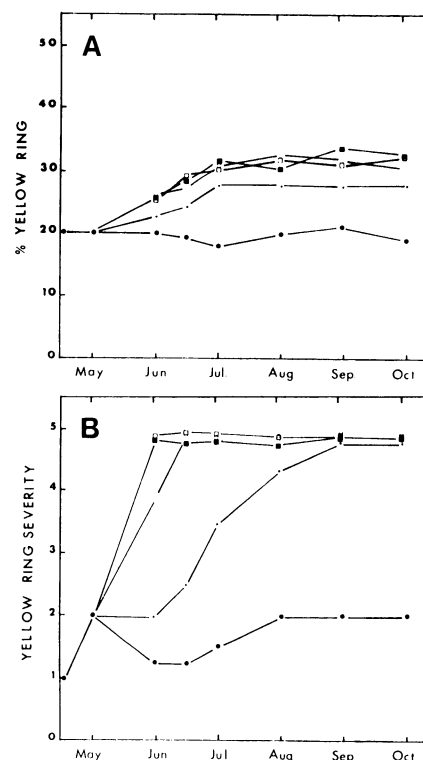
Several fungicides limited the area of diseased grass and reduced disease severity, but none of the four fungicides tested reduced DS values to  $\leq 1$ . The fungicides were applied to swards that were heavily infested with *T. alnicola*; thus effective disease control required therapeutic and preventative activity. PCNB (30.5 and 6.11 kg/ha) was effective in preventing additional infection foci from developing, which indicates that, if used preventatively, it may prevent effective dissemination of the pathogen. PCNB was also markedly effective in limiting the severity of yellow ring. Had the compound been used before the establishment of yellow ring, it may have prevented disease symptoms. A combination of this fungicide and the use of resistance may effectively control this disease.

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**Fig. 1.** Control of yellow ring of *Poa pratensis* with fungicides. Treatments were applied on 11 May and repeated on 16 July 1983 and consisted of (●) PCNB, 6.11 kg/ha; (+) propiconazole, 0.84 kg/ha; (○) furmecycloz, 6.11 kg/ha; (□) triadimefon, 1.52 kg/ha; and (■) water, 2.0 kl/ha. (A) Percentage of treated area showing yellow rings; (B) Mean disease severity (1-5) of 25 randomly selected yellow rings. All data are mean values for three replicates.

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