Techniques for Inducing Summer Patch Symptoms on *Poa pratensis*

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ABSTRACT

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Two techniques were evaluated for use in studies on the ecology and control of turfgrass patch diseases caused by soilborne pathogens. Sod of mature Kentucky bluegrass (*Poa pratensis*) produced in field plots was used to determine whether controlled temperatures in growth chambers could be used to manipulate the expression of summer patch symptoms caused by *Phialophora graminicola*. The pathogen did not induce disease symptoms on sod at 14 C but did so very slowly at 21 C and rapidly at 29 C. Preliminary evidence indicated that the pathogen grew through sods at 21 C but did not kill plants quickly unless the temperature was increased to 29 C. A sod of Kentucky bluegrass was also produced on a mobile cart in controlled-environment chambers, then inoculated with isolates of *P. graminicola* and *Leptosphaeria korrae*. Both pathogens formed circular zones of restricted root growth before foliar symptoms were expressed. After an incubation period of 21 wk at 21–24 C, a patch of grass affected by *P. graminicola* became visible. Symptoms of the summer patch disease, which became more clear after the temperature was increased to 30 C, included the ring (frogeye) pattern of well-developed or older patches, a sunken-pocket effect, and heat-stress banding of leaves on tillers marginally affected by root rot. This is the first report of a patch disease with characteristic field symptoms being produced under semicontrolled conditions on this host. These techniques may be used to increase the efficiency of ecological, etiological, and control studies on patch diseases of turfgrasses. Observations of circular patterns of restricted root growth during the harvest of commercially produced sod could also be used as a quality control measure to reject areas of fields that are otherwise presymptomatic of these diseases.

Additional key words: Fusarium blight syndrome

Distinct circular to arc-shaped patch diseases of turfgrasses are caused by soilborne pathogenic fungi that produce darkly pigmented, ectotrophic runner hyphae that spread along roots, rhizomes, and stolons of Gramineae and move from one plant to another (3,9,10). Representative diseases and their agents include take-all patch of *Agrostis* spp. (*Gaeumannomyces graminis* (Sacc.) von Arx & Oliv. var. *avenae* (E. M. Turner) Dennis) and spring dead spot of *Cynodon* spp. (*Leptosphaeria korrae* Walker & Smith, *L. narmari* Walker & Smith).

Fusarium blight (1), also named Fusarium blight syndrome (6), is another important disease characterized by distinct patches. Although *Fusarium* spp. are often the dominant fungi isolated from symptomatic plants, their role as primary incitants of this patch disease syndrome has been questioned (4,5). *Phialophora graminicola* (Deacon) Walker and *L. korrae*, either alone or together, were recently found to be consistently associated with Fusarium blight syndrome (8). Both of these ectotrophic fungi were shown capable of causing typical first-season patches or small rings (frogeye patches) in inoculated field trials, and both were highly pathogenic to seedlings in the greenhouse. These newly described components of the Fusarium blight syndrome were named summer patch, caused by *P. graminicola*, and necrotic ring spot, caused by *L. korrae* (7). These fungi, however, had not been used on large sods in the greenhouse or controlled-environment chambers to form the distinct circular to ring-shaped patches (1,4–6) exhibited on Kentucky bluegrass (*Poa pratensis* L.) in the field (Fig. 1A). These steps were necessary to provide investigators with the full capability to study the biology of these patch diseases and to improve disease control strategies. Smith (9) demonstrated that *L. narmari* and *L. korrae* could be used to produce characteristic patches of spring dead spot on greenhouse-produced sods of bermudagrass (*Cynodon dactylon* (L.) Pers.).

We report on a technique for evaluating the growth and pathogenicity of *P. graminicola* in field-produced sod incubated under controlled environmental conditions. We also report typical field symptoms of summer patch (Fusarium blight syndrome) that developed on an inoculated bench of Kentucky bluegrass sod grown from seed in controlled-environment chambers and the greenhouse. This is the first report of an experimentally induced patch disease on Kentucky bluegrass.

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MATERIALS AND METHODS

Temperature effects on symptom development. Seven-year-old Kentucky bluegrass (cultivar Merion) was cut at a depth of 2 cm from a plot at the Cornell University Turfgrass Field Research Nursery. The sod was then cut into segments (37 × 7 × 2 cm), which were placed over a 1-cm-deep layer of sterilized sandy loam in plastic trays (37 × 7 × 4.5 cm). Fifteen holes (4 mm in diameter) had been drilled in the bottoms of the trays to allow water drainage and air exchange. Six trays were incubated at 29 C, and 21 trays each were incubated at 14 and 21 C. The turf was mowed to a height of 1.5 cm each week and watered as needed to maintain continuous wetness. After 2 wk of incubation at the three temperatures, all except three trays in each chamber were inoculated with *P. graminicola*. Inoculation consisted of placing five to seven colonized perennial ryegrass seeds in 1-cm-deep slits centered along the long axis of each tray and positioned between 1 and 2 cm from each end of the tray. Three replicate trays were transferred from the chambers (14 and 21 C) immediately after inoculation and at the end of 2, 4, and 6 wk of incubation. Three inoculated and three uninoculated trays of turf were maintained at each temperature throughout the experiment.

Weekly observations of turfgrass quality were made for 13 wk after inoculation. Disease symptoms did not occur at 14 C. Grass incubated at 21 C was killed within a 6-cm zone at the end of each tray. When sods were incubated at 29 C, the advancing zone of killed tillers had advanced 16 cm to the centers of the trays at 10 wk after inoculation. Sods transferred from 14 or 21 to 29 C had patches that were intermediate in size between those at the original and final incubation temperatures (Fig. 2). *P. graminicola* grew through sods at 21 C without causing immediate death of the colonized tillers. An increase in temperature did, however, cause a sudden and complete zone of death in patch areas colonized by the pathogen at lower temperatures.

Patch symptom development. Sod of Kentucky bluegrass (cultivar Merion) was produced on a stainless steel mobile cart (61 × 92 cm) used in controlled-environment chambers at the Dimock

Environmental Control Laboratory, Cornell University, Ithaca, NY. The steel mesh surface of the cart was covered with two layers of cheesecloth to retain a 3-cm layer of steam-pasteurized soil mix (2:1:1:1 mixture of sandy loam-sand-vermiculite-peat). Seed (24 g/m^2) was shallowly incorporated into the soil surface on 11 November 1983, and the flat was incubated at 20 C with a 12-hr photoperiod for 9 wk, during which a uniform stand of seedlings became established. Waterings, mowings (at 2 cm), and applications of a dilute fertilizer solution (1:1:1, NPK, at 200 μg N/ml) were performed at daily, weekly, and monthly intervals, respectively.

Inocula of *P. graminicola* and *L. korrae* were placed in three locations in the 9-wk-old sod. Two isolates of the latter pathogen were used: a virulent isolate collected from the field (ATCC 56289) and a single ascosporic isolate that was later shown to be nearly avirulent. Inoculum consisted of air-dried perennial ryegrass (*Lolium perenne* L.) grains that had been autoclaved and then colonized by a pathogen (1.2 g). Inoculum was placed at the soil surface, and each inoculation site was marked with a plastic garden stake.

One week after inoculation, the sod was moved to a chamber at 24 C to increase its rate of growth. The sod developed a 2-cm-deep mat of tightly knit roots and rhizomes under these conditions, and turfgrass quality gradually declined. The sod was sprayed twice (3 and 6 wk after inoculation) with mancozeb to control Drechslera leaf spot (*D. poae* (Baudys.) Shoem.), and once (6 wk after inoculation) with dienochlor to control spider mites (*Tetranychus* spp.) These applications controlled the targeted pests but did not noticeably improve turfgrass quality. The sod was therefore moved (9 wk after inoculation) to a greenhouse at 21 C and placed over a 2-cm layer of the soil mix on a greenhouse bench. Sod quality improved rapidly and achieved a high standard within 2 wk.

A distinct 25-cm-diameter patch of chlorotic green tillers became apparent around the locus of *P. graminicola* inoculum 21 wk after inoculation, and it became progressively more distinct during the following 2 wk. Leaves in the affected zone became chlorotic, ceased to elongate, and then died back from the tips. A sunken pocket (4,5) developed as entire tillers died, but some living tillers remained in the affected area. These tillers had rotted roots, but their foliar growth and appearance seemed equal to that of healthy tillers outside of the patches. By the end of 23 wk, the patch was about 36 cm in diameter and had a sharply defined interface with areas of apparently healthy grass. A zone (17 × 20 cm) of partially thinned grass was centered over the inoculum locus, thus producing the ring (frogeye) pattern that

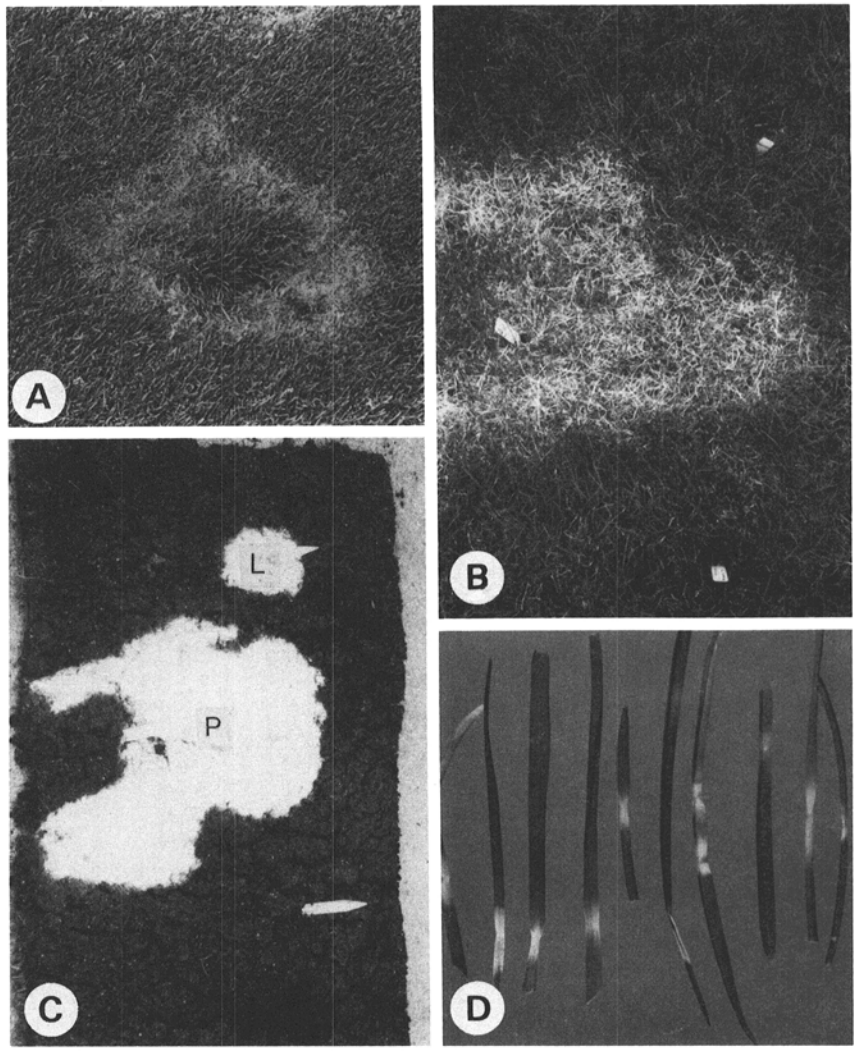

Fig. 1. Summer patch of Merion Kentucky bluegrass. **(A)** Ring pattern characterizing advanced stages of the disease in the field. **(B)** Ring pattern resulting from growth chamber-produced sod inoculated with *Phialophora graminicola* at the center left marker. **(C)** Patches of necrotic roots around inoculation loci for *P. graminicola* (P) or *Leptosphaeria korrae* (L) 25 wk after inoculation. (Areas devoid of roots failed to retain soil below the thatch layer and were covered with white sand for photographic contrast.) **(D)** Heat-stress bands on leaves of plants affected by sublethal root rot and incubated outdoors on hot, bright days with little irrigation.

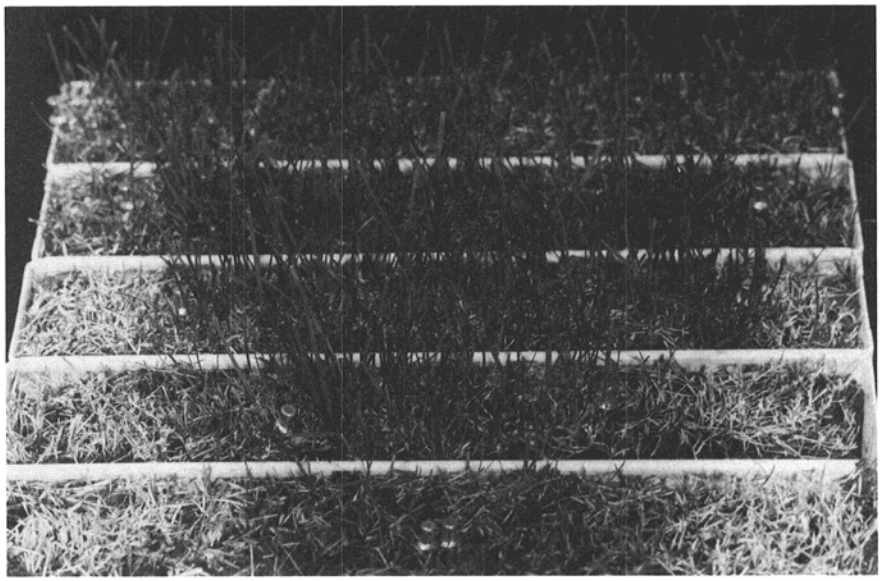

Fig. 2. Regulation of incubation temperatures to modify the size of summer patch symptoms on field-produced Kentucky bluegrass sod: incubation for (top to bottom) 10 wk at 21 C; 3 wk at 29 C after 2, 4, or 6 wk at 21 C; and 10 wk at 29 C.

characterizes well-developed patches in the field. By inverting the sod, we also noted that root development into the soil layer that had been added 9 wk after inoculation was excellent under zones of healthy grass and absent under tillers killed by *P. graminicola*. An exception was that rooting was also absent under the surface 2 cm of root/rhizome mat in a 12-cm-diameter zone centered under the inoculum locus for the field isolate of *L. korrae*. The foliage above that zone remained asymptomatic, thus failing to reveal the severe nature of root rot caused by *L. korrae*. Soil from the basal layer did not adhere to the sod in the two areas devoid of roots. In an attempt to induce visible symptoms on the turf infested by *L. korrae*, the sod was transferred (23 wk after inoculation) to a greenhouse at 30 C and very high humidity. After 2 wk at 30 C, the sod infested with *L. korrae* had still failed to develop visible symptoms. At this temperature, nearly all tillers in the patch of turf infested by *P. graminicola* died except for many of those near the original inoculation site. The foliar symptoms and lack of rooting in these patches were photographed (Fig. 1B,C) 25 wk after inoculation. To enhance the photographic clarity in zones devoid of roots beneath the surface root/rhizome mat, photographs of the inverted sod were taken with or without a thin layer of white sand that was placed over the poorly rooted areas.

The sod was then reinstalled on the mobile growth-chamber cart and incubated outdoors for 3 days during hot (daily highs of 26–32 C and daily lows of 13–20 C), dry weather. Watering was kept to a minimum to induce severe plant stress, as indicated by wilting of healthy foliage each day and by development of heat-stress banding (Fig. 1D) on green tillers immediately in front of patch margins. The banding occurred on plants that were subsequently determined to have sublethal root dysfunction caused by the pathogenic fungi. The bands were often (55%) devoid of fungal colonists, but isolation attempts onto 2% water agar or potato-dextrose agar yielded species of *Fusarium* (14% of sections), *Curvularia* (14%), *Myrothecium* (10%), and *Alternaria* (6%). Leaves incubated in a moist chamber yielded only *Myrothecium* sp. A 3-cm-diameter patch of dead tillers with a sunken pocket developed over the site of *L. korrae* inoculation during the second and third days of exposure to the outdoor incubation conditions.

RESULTS AND DISCUSSION

We have demonstrated that patch diseases of Kentucky bluegrass can be induced to occur on one- and two-dimensional sod surfaces in controlled or semicontrolled environments. This finding presents an improved means to study the etiology of patch diseases and the ecology of soilborne turfgrass pathogens that have a prolonged ectotrophic colonization stage. Unfortunately, the two-dimensional system used for circular patch symptom development involves large spatial and time requirements in controlled-environment chambers or greenhouses. If temperatures in these facilities are not capable of being modified through the experimental period, multiple units are necessary. These limitations will restrict the utility of studies in which full patch symptom expression is necessary.

The environmental conditions that favor root colonization by *P. graminicola* and *L. korrae* are mostly unknown, but it is clear that these pathogens are able to move through the root zone and colonize roots without causing visual symptoms of disease. In our demonstrations, *P. graminicola* grew through field- and greenhouse-produced sods at temperatures of about 21–24 C. Disease symptoms developed slowly in both studies at these temperatures. Patch disease severity was amplified by increasing the temperature to 29 or 30 C. Although the precise conditions necessary for root colonization and expression of disease remain unknown, it may be inferred from this work and from field observations (4,5) that one or more of many plant stresses are involved in the final stage of symptom development. We modified the extent of patch development by varying the length of *P. graminicola* colonization at 21 C and then caused colonized tillers to die within 7–10 days by increasing the temperature to 29 C.

Summer patch and necrotic ring spot usually appear when seeded turfs are 3 yr or older or when sodded turfs are 2 yr or older (4,5). We observed that root development was severely restricted in the zones colonized by virulent isolates of both root pathogens, and this appears to occur well before foliar symptoms are expressed. When the sod was inverted, these poorly rooted zones were characterized by their inability to hold soil below the surface mat.

Although foliar symptoms of these diseases seldom appear on commercially produced sod (which is marketed 6–15 mo after seeding), there are occasions when circular zones of restricted root growth have been observed on some sods when they are harvested. Our observation of restricted zones of root growth before the appearance of visible symptoms provides a means whereby marketing decisions can be made during final stages of sod production. The obvious benefits of incorporating this parameter into market quality procedures would be to decrease the potential for patch development on recently sodded sites and therefore to increase consumer satisfaction and reduce the replacement and litigation costs associated with sod that performs unsatisfactorily. It is unlikely, however, that zones of restricted root development can be detected routinely on seeded turfs.

Alternating bands of green and bleached tissues on turfgrass leaves have been associated with plants in the vicinity of those that have become affected by patch diseases (5). Such symptoms have been described as heat-stress banding on cereal grains (2) and turfgrasses (6). The bands are presumed (5) to reflect leaf growth rates during periods in which chlorophyll formation is inhibited when a rapid rise in leaf temperature is inadequately compensated by transpirational cooling. *Fusarium* spp. and other facultative parasites are generally not recoverable from the youngest bleached bands but are readily isolated from older bands. Transpirational efficiency is known to be reduced when the vascular system of plant roots becomes restricted by pathogens or any other cause. The abiotic bands that developed on plants affected by root rot in this experiment, and not those without colonization by the soilborne pathogens, appears to be the first experimental demonstration of this phenomenon and to support the association of heat-stress banding with conditions leading to development of summer patch symptoms in the field.

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LITERATURE CITED

1. Couch, H. B., and Bedford, E. R. 1966. Fusarium blight of turfgrasses. Phytopathology 56:781-786.
2. Harris, M. R. 1964. Diseases of cereal crops in Washington. Wash. Agric. Exp. Stn. Ext. Bull. 559. 16 pp.
3. Nilsson, H. E., and Smith, J. D. 1981. Take-all of grasses. Pages 433-448 in: Biology and Control of Take-all. M. J. C. Asher and P. H. Shipton, eds. Academic Press, London. 538 pp.
4. Sanders, P. L., and Cole, H., Jr. 1981. The Fusarium diseases of turfgrass. Pages 195-209 in: Fusarium: Diseases, Biology and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park. 457 pp.
5. Smiley, R. W. 1980. Fusarium blight of Kentucky bluegrass: New perspectives. Pages 155-178 in: Advances in Turfgrass Pathology. P. O. Larsen and B. G. Joyner, eds. Harcourt, Brace, Jovanovitch & Co., Duluth, MN. 197 pp.
6. Smiley, R. W. 1983. Compendium of Turfgrass Diseases. American Phytopathological Society, St. Paul, MN. 136 pp.
7. Smiley, R. W. 1984. "Fusarium blight" of *Poa* spp. re-described as patch diseases caused by *Phialophora graminicola, Leptosphaeria korrae,* or related species. (Abstr.) Phytopathology 74:811.
8. Smiley, R. W., and Fowler, M. C. 1984. *Leptosphaeria korrae* and *Phialophora graminicola* associated with Fusarium blight syndrome of *Poa pratensis* in New York. Plant Dis. 68:440-442.
9. Smith, A. M. 1967. Spring dead spot of couch grass turf in New South Wales. M.Sc. thesis. University of Sydney, Australia. 114 pp.
10. Walker, J., and Smith, A. M. 1972. *Leptosphaeria narmari* and *L. korrae* spp. nov., two long-spored pathogens of grasses in Australia. Trans. Br. Mycol. Soc. 58:459-466.