Temperature and Osmotic Potential Effects on *Phialophora graminicola* and Other Fungi Associated with Patch Diseases of *Poa pratensis*

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ABSTRACT

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Phialophora graminicola and *Leptosphaeria korrae* cause patch diseases in Kentucky bluegrass turfs. *Fusarium crookwellense* is also commonly associated with these diseases. Temperature and osmotic potential effects on growth and pathogenicity of isolates of these fungi from the United States were unknown. Maximum growth rates for *P. graminicola, L. korrae*, and *F. crookwellense* occurred in vitro at 28–31, 14–28, and 21–32 C, respectively, and at osmotic potentials >−0.3, >−1.5, and from −0.2 to −4.0 MPa, respectively. *P. graminicola* grew along rhizomes and through field-produced sod at rates up to 2 cm/wk at 21–29 C, but grew very slowly through sod and on rhizomes at 14 C. Rapid necrosis of colonized plants occurred only at 29 C and, through a system of elevation in the incubation temperatures, this property was used to assay the extent of the pathogen's growth through sods at lower temperatures. Susceptibility of plants to *P. graminicola* was pronounced when plants were mowed at 2-cm height compared to being unmowed. *L. korrae* colonized roots but did not affect plant health under the conditions examined in this study. *F. crookwellense* thoroughly colonized and halted further growth by cultures of the other fungi on agar medium, but did not contribute to progress of disease caused by *P. graminicola*, or to symptom expression on plants colonized by *L. korrae*. Field conditions in which growth of the ectotrophic pathogens are most likely to occur are discussed with respect to conditions associated with the first appearance of disease symptoms.

Additional key words: Fusarium blight, necrotic ring spot, summer patch, turfgrass, wheat.

Fusarium blight syndrome of Kentucky bluegrass (*Poa pratensis* L.) (20,24) was recently recognized to be a mixture of two or more patch diseases caused by ectotrophic root-infecting fungi related to *Gaeumannomyces graminis* (Sacc.) Arx & Olivier (25,27). Two of these ectotrophs are *Phialophora graminicola* (Deacon) Walker, which causes summer patch, and *Leptosphaeria korrae* Walker and Smith, which causes necrotic ring spot (25). *Fusarium acuminatum* Ell. & Ev., *F. crookwellense* Burgess, Nelson & Toussoun., *F. culmorum* (W. G. Smith) Sacc., *F. graminearum* Schwabe, and other species are typically the most prevalent fungi isolated from foliage, crowns, and roots of plants expressing symptoms of patch diseases (5,10,23,26).

Repeated attempts to reproduce the full spectrum of Fusarium blight syndrome symptoms with *Fusarium* spp. have failed (20,23). We recently reported that inocula of *P. graminicola* and *L. korrae* induced typical patches of Fusarium blight syndrome in the field (27) and in controlled-environment chambers (28). It is now unclear which primary pathogen(s) was associated with Fusarium blight syndrome investigations reported from about 15 states, and whether secondary infections by *Fusarium* species accelerate the rate of patch development. It is also unclear why our observations conflict with those in Europe and Australia, where *P. graminicola* is considered to be an avirulent root parasite (1,8,9,13,14,34) that has value as a biocontrol agent against take-all patch of turfgrasses (7,39) and take-all of small grains (1,9). Although the virulence of isolates of *P. graminicola* could differ in these countries, the temperatures at which the studies were conducted had important effects. European and Australian studies with *P. graminicola* were conducted at 22 C or lower temperatures. Disease symptoms on closely mowed grasses in our field (35) and controlled environment (28) studies were associated with temperatures above 25 C.

Contradictory reports on relationships of temperature and moisture to the incidence of Fusarium blight syndrome (20,23) were possibly caused by differences in primary incitants for the diseases studied. The effects of these parameters on North American isolates of *P. graminicola* and *L. korrae* in controlled experiments are unknown, but it is clear that summer patch and necrotic ring spot are influenced differently by environmental conditions in the field. Summer patch symptoms usually occur first on closely mowed turfs in July and August, during hot, sunny days directly after a period of excessive rainfall or irrigation (23). In contrast, necrotic ring spot occurs from late spring through early winter in New York. It is important to determine the sensitivities of these newly recognized pathogens to environmental parameters that could influence their growth and, therefore, the timing of disease control strategies. Effects of environmental parameters on isolates of *P. graminicola* (1,7,9,38) and *L. korrae* (32) from other countries have been determined, but this information is lacking for North American isolates.

This paper reports the effects of temperature and osmotic potential on the in vitro growth of *P. graminicola, L. korrae*, and *F. crookwellense*. The influence of temperature on summer patch and necrotic ring spot development, of temperature on the growth of rhizomes of *P. graminicola*, and of mowing on summer patch, also were evaluated on Kentucky bluegrass in controlled-environment chambers. The role of *F. crookwellense* in patch disease development was studied by conducting challenge tests of this fungus against *P. graminicola* or *L. korrae* in vitro, and by dual inoculations of host plants with these fungi in pot tests.

MATERIALS AND METHODS

The fungi. Isolates of *P. graminicola* and *L. korrae* were collected by the trap-crop technique (21,27). Root and crown samples from patch-affected turfgrasses were buried in pots of fine gravel in the greenhouse, in which wheat (*Triticum aestivum* L.) was grown. Isolations were made from surface-sterilized segments of seedling wheat roots on which ectotrophic, darkly pigmented hyphae were

observed through a dissecting microscope. Single-hyphal-tip cultures were used for each of the ectotrophs. Isolates of *P. graminicola* were trapped from plants taken from the margins of patch-affected areas of Kentucky bluegrass at Farmingdale, NY, during August 1983. Isolates of *L. korrae* were from similar-appearing patches at Skaneateles, NY, in November 1981. Single-ascospore cultures of *L. korrae* were made from pseudothecia developed in the greenhouse on rotting, naturally infected plants (27) from diseased patches at Farmingdale, NY, during July 1983. Isolates of *F. crookwellense* pathogenic to bluegrasses (15) were obtained from single conidial cultures from crowns of Kentucky bluegrass exhibiting symptoms of a patch disease at Oyster Bay, NY, in August 1982. After isolation, all fungi were maintained at 5 C on half-strength, potato-dextrose agar (PDA) medium, or on sterile wheat leaf agar. Two isolates of each fungus were used in each of the in vitro tests reported. Inocula for the in vitro studies consisted of 4-mm-diameter disks of colonized agar taken from the advancing margin of young cultures on PDA. Inocula for the in vivo tests consisted of perennial ryegrass (*Lolium perenne* L.) grains that had been autoclaved, infested with a single isolate of *P. graminicola* or *L. korrae*, and then dried.

Osmotic potentials. Osmotic potentials of a minimal salts medium (33) were adjusted with potassium chloride (37). This medium was selected because of its high unadjusted osmotic potential (−0.1 MPa, as compared to −0.3 MPa for half-strength PDA; 1 MPa = 10 bars) and because it is commonly used in studies with similar fungi (4,38). Ten concentrations of the medium, with potentials of −0.1 to −7.0 MPa, were prepared and, 1 day after pouring, the osmotic potentials were measured with a Wescor C-52 Sample Chamber and a Wescor PCT-55 Soil Hygrometer (Wescor, Inc., Logan, UT). The intended and measured potentials had a correlation coefficient of 0.992.

Two isolates of each fungus were transferred onto four replicate plates of media at each osmotic concentration 3 days after the media had been prepared. Plates were incubated in darkness at 20 C, and the colony diameters were marked at two locations on each plate at 48-hr intervals beginning 5 days after transfers were made. Growth rates (millimeters per day) at a time when colony growth remained stable during consecutive rating intervals are reported. The coefficient of variation (CV) for mean growth rate of each fungus at each salt concentration was determined by grouping the isolates, replicate plates, and duplicate measures per plate ($n = 16$).

In vitro temperature study. Replicate plates of each isolate on half-strength PDA were incubated in darkness in incubators maintained at 3–5 C intervals from 6 to 33 C. Colony diameters were marked at 48-hr intervals after the plates had become about 30% colonized at the higher temperatures, and over longer time periods at the lowest temperatures. Growth rates and CV were determined as described for the osmotic potential study.

In vivo temperature study in pots. Kentucky bluegrass cultivar Merion was grown from seed in 8-cm-diameter clay pots containing sterile gravel (percentages in size ranges >2, 1-2, 0.5-1, 0.25-0.5, and <0.25 mm were 28, 38, 25, 7, and 2, respectively). Incubation of seedlings was at 14, 21, or 29 C in controlled-environment chambers (all with 12-hr photoperiod). Watering was performed twice daily during the first 2 wk and once daily thereafter. Plants were fertilized with a complete nutrient source about once every other week. Seven weeks after seedling emergence, all pots except three in each chamber were infested with inoculum consisting of dry perennial ryegrass grains that had been autoclaved and then colonized by *P. graminicola* or *L. korrae* (an ascospore-derived culture). The numbers of infested pots at 14, 21, and 29 C were 12, 12, and 6, respectively. Inoculum (1.2 g) was placed at the soil surface in the center of each pot. Three weeks later, three pots infested with each fungus were transferred from 14 or 21 C to 29 C. Eleven weeks after inoculation, all pots remaining at 14 and 21 C were moved to 29 C. Weekly records of turfgrass foliar quality (scale 1–9: 1 = plants dead, 9 = vigorous foliage growth and appearance) and rate of leaf elongation were made throughout the study period. CVs were determined for the means of each treatment. Relative leaf elongation rates were used as nondestructive estimates of vascular dysfunction caused by

pathogenesis (17). Periodic microscopic observations of randomly selected roots in some treatments were also made to determine the extent of colonization by the pathogens, but quantitative assessments were not made.

Disease progress study in trays. The rate of patch disease progress in Kentucky bluegrass sod inoculated with *P. graminicola* and incubated at 14, 21, or 29 C was evaluated by using a technique described earlier (28). Seven-year-old Kentucky bluegrass cultivar Merion was cut at 2 cm depth from a plot at the Cornell University Turfgrass Field Research Nursery. The soil under the sod was a deep Hudson silty clay loam of pH 6.0. Sod segments of $37 \times 7 \times 2$ cm were placed over a 1-cm-deep layer of sterilized Arkport sandy loam in $37 \times 7 \times 4.5$-cm plastic trays. Six sod segments were placed in a controlled-environment chamber for 2 wk of equilibration at 29 C, and 21 sod segments were conditioned to growth at 14 or 21 C. The turf was mowed to 1.5 cm height each week, and was watered as needed to maintain continuous wetness. All except three sods in each of the three chambers were then inoculated with *P. graminicola*. Inoculation consisted of placing five to seven colonized seeds of perennial ryegrass into 1-cm-deep slits centered along the sod's long axis, and positioned between 1 and 2 cm from each end. Three inoculated and three uninoculated sod segments were maintained at each temperature throughout the experiment. Other sod segments were transferred at 2-wk intervals from the lower temperatures to 29 C to determine the extent of pathogen movement through the turf at the lower temperatures. We had previously determined that asymptomatic sod colonized at low to intermediate temperatures was killed quickly when the temperature became elevated to 29 to 30 C (28), and that this procedure is a reliable indicator that the pathogen is present on susceptible turfgrass species (*unpublished*). Three replicate sod segments were therefore transferred to 29 C from the 14 and 21 C chambers immediately after inoculation (week 0) and at the end of 2, 4, and 6 wk of incubation.

Weekly records of turfgrass foliar quality and mean rate of leaf elongation were made throughout the study period. The ratings and measurements were made at 2-cm intervals across the long axis of each sod, beginning at 2 cm from the inoculation point. Visual and microscopic observations of roots and the bases of tillers were made periodically (qualitatively) to determine the relationship between colonization of tissue by the pathogen and progress of disease symptoms. Mowing was performed immediately after the records were collected. Data from both ends of each sod were combined. Treatment means and CVs were, therefore, based upon six measurements (two ends of each sod and three sods per treatment).

Growth of *P. graminicola* on rhizomes. The influence of temperature on growth of *P. graminicola* on rhizomes of Kentucky bluegrass was studied using a technique similar to that described by Deacon (8). Rhizome segments 3–8 cm long were collected in early spring from a peat moss mulch in a rose garden adjacent to a residential lawn. The rhizomes were very white, except for brown nodes and roots, and microscopic examination yielded no evidence for the presence of ectotrophic, darkly pigmented fungi. Leaf sheaths and roots were pulled from all nodes except the youngest, nearest the emerging tiller. Four rhizomes were buried at 1-cm depth in each of 12 nonsterile gravel-filled trays ($37 \times 7 \times 4.5$ cm). The rhizomes were parallel to one another, and were separated by about 1 cm. Tillers at the end of each rhizome were placed so that the shoots (three- to five-leaf stage) were above the gravel surface and the roots were buried. Four trays were placed in each of three controlled-environment chambers operated at 14, 24, and 29 C. Two rhizomes in each tray were inoculated with *P. graminicola* immediately after burial by placing a 1 cm^3 block of pathogen-colonized PDA medium directly on the cut end of the rhizome (e.g., the end farthest from the tiller). After 2 or 4 wk of incubation, the rhizomes in two trays were removed from the gravel and the extent of colonization by *P. graminicola* and of rhizome and root discoloration was determined by microscopic observation.

The preceding study evaluated the growth potential of *P. graminicola* in a single direction (from the mother clone toward the daughter clone) on severed rhizomes. A study with intact rhizomes

was established to determine if the pathogen's growth rate toward the mother clone differed from that toward the daughter clone. Plants with mother and daughter clones connected by a white rhizome that was over 10 cm long were arranged in flats containing nonsterile gravel. The experimental procedure described previously was repeated, except that the inoculum block was placed midway between the mother and daughter clones. Three plants were incubated for 2 wk at a temperature of 14, 24, or 29 C.

In vitro study of dual cultures of ectotrophic fungi and *F. crookwellense*. Opposing culture tests were conducted on PDA to study the interaction of an ectotroph with *F. crookwellense*. Fungi were transferred from parent cultures onto fresh plates of the same medium. Inoculum of *F. crookwellense* was introduced at either 0, 4, or 8 days following that of *P. graminicola* and *L. korrae*. Incubation was on a laboratory bench at 24 C. Plates seeded with individual fungi were used as controls. Observations of growth rates and habits of each fungus were made daily following challenge.

In vivo study with dual inoculations. Dual inoculation tests were performed in controlled-environment chambers to determine if *F. crookwellense* increases the death rate of plants affected by root rot caused by *P. graminicola* or *L. korrae*. Thirty-six pots of Merion Kentucky bluegrass were prepared and infested as described for in vivo temperature tests. Treatments consisted of 12 pots treated with *P. graminicola*, *L. korrae*, or no primary root pathogen. Incubation was at 14 C prior to inoculation and for an additional 4 wk. The pots were then transferred to an 18/24 C alternating temperature (12 hr each) chamber, incubated for 1 wk, and then the seedlings in six pots of each treatment were infested with two isolates of *F. crookwellense*. The inoculation was accomplished by spraying a turbid conidial suspension of macroconidia from both isolates to the point of runoff on freshly mowed foliage (6). Control pots were sprayed with water. Each pot was then enclosed in a plastic bag and maintained at 100% relative humidity for 3 days, whereupon they were removed from the bags and transferred to a 29 C chamber. The overall foliar quality of plants in each pot was noted each week, as described for the in vivo temperature study. A final rating was made 5 wk after *Fusarium* had been added, and CVs were determined for the treatment means. The data also were evaluated by analysis of variance and Fisher's least significant difference test.

Mowing versus not mowing. Merion Kentucky bluegrass was grown for 4 mo in 8-cm-diameter clay pots containing a soil mixture containing sandy loam, fine sand, vermiculite, sand peat moss (2:1:1:1, v/v). Plants were grown in a chamber (18 C nights, 25 C days, 12 hr of light), mowed at 2-cm height, and watered and fertilized as necessary. They were then moved to a greenhouse (typically ranging from 17 to 24 C, but rising to a high of 41 C on several hot, clear days), and 2 wk later 20 pots with uniform stands were selected. The root mat that had formed along the inside walls of the pots was removed (day 0), and the plants were repotted into

sterile sandy loam. At repotting, turf in 10 pots was inoculated with *P. graminicola* by placing four infested ryegrass seeds deep into the turf sward. The inoculated and uninoculated turfs were then subdivided so that five pots of each were mowed at 2-cm height once each week, and five pots were not mowed. Air temperatures at plant height were monitored continuously. Each week, just prior to mowing the appropriate treatments, assessments were made of leaf elongation rates and turfgrass foliar quality. CVs for treatment means were determined for each weekly assessment.

RESULTS

Osmotic potentials. Sensitivities of the fungi to gradients in osmotic potentials differed significantly among species (Fig. 1A) but not among isolates within species. Data are therefore presented as means of all isolates and replications within species. Growth rates for *P. graminicola* and *L. korrae* were greatest at the highest potential evaluated (-0.1 MPa) and were reduced to 50% of maximum by potentials of about -0.6 and -2.5 MPa, respectively. Growth was completely inhibited at -2.2 and -7.0 MPa for *P. graminicola* and *L. korrae*, respectively. Growth rates for the ascosporic cultures of *L. korrae* were greater than those for the hyphal tip cultures at potentials of -0.1 to -1 MPa. *F. crookwellense* produced maximum growth at about -1.0 MPa. Growth was 50% of maximum at about -0.2 and -4.0 MPa, but growth was not inhibited completely even at the lowest potential studied (-7 MPa).

In vitro temperature study. Maximum growth rates for *P. graminicola*, *L. korrae*, and *F. crookwellense* on PDA occurred at 27 to 31, 20 to 25, and 27 C, respectively (Fig. 1B). The range of temperatures at which growth of these fungi were more than 50% of their maximum rates was 16–33 C, 14–29 C, and 22–31 C, respectively. The two tested isolates of each fungus differed little in response to temperatures, except that the growth rate of one isolate of *P. graminicola* continued to increase from 27 to 31 C, whereas the other exhibited a maximum at 27 C and a very slight reduction at 31 C. Growth rates for the two ascosporic cultures of *L. korrae* from 15 to 28 C were greater than those for the hyphal tip cultures, but the temperature response curves for these four isolates were otherwise equal.

In vivo temperature study in pots. Uninoculated control plants in controlled-environment chambers remained symptomless (Fig. 2) at 14 and 29 C. A decline in foliar quality at 21 C was caused by Helminthosporium leaf spot (caused by *Bipolaris sorokiniana* (Sacc. ex Sorok.) Shoem). Leaf growth rates, when plotted against time, exhibited trends nearly identical to the foliar quality ratings in Fig. 2 and are, therefore, not presented.

Plants in the centers of all pots infested with *L. korrae* became colonized by the fungus at all temperatures. Root cortices became blackened, especially at 21 C, but we did not perform histological examinations to determine if vascular tissues also were invaded by this fungus. All plants infected by *L. korrae* had foliar quality ratings (Fig. 2) and leaf growth rates (data not presented) nearly equal to those of the uninoculated controls. At 21 C, however, the growth and foliar quality of the colonized plants were generally superior to those of the controls. Specific observations of possible interactions between necrotic ring spot and Helminthosporium leaf spot were not made.

P. graminicola colonized all plants in each pot at 21 and 29 C, and only the plants near the centers of pots were colonized at 14 C. Visible symptoms of summer patch did not appear at 14 or 21 C, but all plants in each pot were killed within 3–4 wk at 29 C. When plants colonized at the low and intermediate temperatures were transferred to 29 C, they were killed within 2 wk. At 14 C, the plants colonized by *P. graminicola* generally had foliar quality ratings and leaf growth rates that exceeded those of the uninoculated controls.

Disease progress study in trays. Although foliar quality ratings (1 to 9 scale) and leaf growth rates at 2-cm intervals across the sod segments were made at weekly intervals, only selected data are presented here to illustrate the principal findings. The data are presented (Fig. 3) as the number of weeks after inoculation when the foliar quality of turfgrass at a given distance from the inoculum
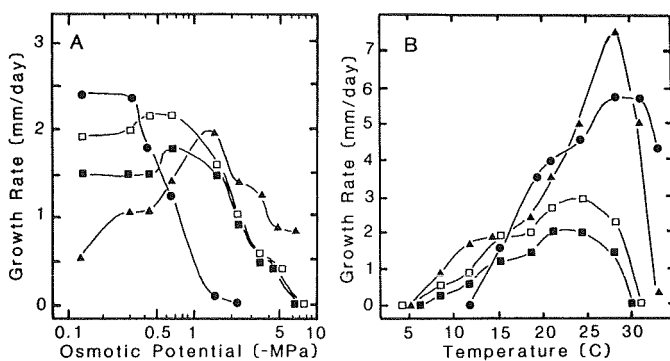

Fig. 1. Growth rates for *Phialophora graminicola* (●), *Leptosphaeria korrae* (□ = field isolates, ■ = ascospore isolates), and *Fusarium crookwellense* (▲) on **A**, an osmotically adjusted minimal salts medium (1 MPa = 10 bars) or **B**, potato-dextrose agar medium incubated over a range of temperatures. Coefficients of variation for each datum point varied from 0 to 0.83 (mean = 0.21) in **A** and from 0.02 to 0.42 (mean = 0.14) in **B**.

locus declined to a rating of 5 (Fig. 3A; acceptable quality), 3 (Fig. 3C; unacceptable), or 1 (Fig. 3E; dead turf). Comparable data on leaf growth rates are presented in Fig. 3B, D, and F. Fig. 3 also includes data for uninoculated controls.

Sod segments incubated at 14 C always sustained uniform declines in foliar quality and leaf growth rates in inoculated and uninoculated turfs. The foliar quality at 14 C declined to a rating of 5 (Fig. 3A) 3 wk after leaf growth rates declined to 4 cm/wk or less (Fig. 3B). The foliar quality in sod segments at 14 C never declined to unacceptable levels (Fig. 3C), and the low temperature influence on leaf growth rates (Fig. 3D) was uniform in the inoculated and uninoculated turfs 10 wk after inoculation.

P. graminicola influenced the quality and growth of turf at 21 C, but did not cause severe symptoms of summer patch at this temperature. As compared to the uninoculated controls, the fungus caused a measurable reduction in foliar quality 4 cm from the inoculum point 6 wk after inoculation (Fig. 3A), and in the leaf growth rate 1 wk earlier (Fig. 3B). Although the pathogen's affect on the rate of leaf growth became more pronounced (Fig. 3D) with prolonged incubation, the development of unacceptable turf quality did not expand beyond 6 cm from the inoculation point (Fig. 3C) during the 12-wk experimental period. *P. graminicola* never caused the grass to die (Fig. 3E) or cease growing (Fig. 3F) at 21 C even at the inoculum locus. The rate at which leaf growth rates became 3 or less at 21 C (Fig. 3D) suggests that the pathogen grew through sod at a rate at least 1.5 cm/wk at this temperature.

Incubation of sod segments at 29 C adversely influenced growth and foliar quality of the turf. This temperature is well above the maximum for root initiation and growth (2,16). The leaf growth rates in uninoculated sods declined uniformly to 4 cm/wk 3 wk following inoculation (Fig. 3A) and to 3 cm/wk after another 3 wk of incubation (Fig. 3D). All plants had died by 11 wk after inoculation (Fig. 3E and F). It was readily apparent, however, that
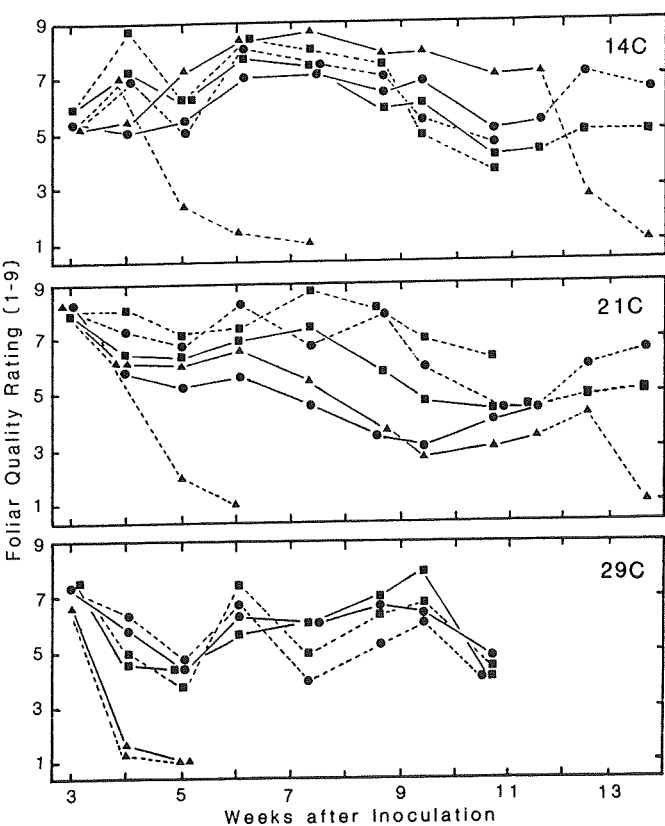
inoculation with *P. graminicola* accelerated the rate of death, and that the rate of increase in summer patch dimensions at this temperature was 3 cm/wk (Fig. 3E). Leaves of plants in inoculated sods at 29 C stopped growing (Fig. 3F) about 1 wk before they died (Fig. 3E).

The growth of *P. graminicola* through sod was further evaluated by inducing symptom development on plants that were colonized by the fungus at low to intermediate temperatures. Sods incubated for 0, 2, 4, or 6 wk at 14 or 21 C were moved to a 29 C chamber to achieve this objective (Fig. 4). Very little movement of the pathogen was detected at 14 C (Fig. 4A and B). Incubations for from 0 to 6 wk at 14 C did not alter the progress of patch development once the temperature had been raised to 29 C (Fig. 4A). There was some evidence, however, that the leaf growth rate was reduced 1 wk sooner at 29 C if the sods had been incubated for 6 wk at 14 C (Fig. 4B). The regression lines for the 6 wk and 0-4 wk data sets differed significantly at $P = 0.05$.

Growth of *P. graminicola* through sod at 21 C was clearly detected (Fig. 4C and D). The disease progressed about 3 cm/wk at this temperature. Each week of incubation at 21 C caused the disease to occur about 1-2 wk earlier when the temperature became elevated. For each distance from the inoculum locus, incubation at 21 C for 4 wk caused disease to appear about 2 wk earlier than when the sod was incubated at 14 C. The rate of disease progress through the sods at 29 C was reduced as the incubation period at 21 C was increased.

Growth of *P. graminicola* on rhizomes. The pathogen did not produce ectotrophic growth on rhizomes after 2 wk at 14 C (Table 1). Dematiaceous hyphae of the fungus grew at 2 cm/wk along the
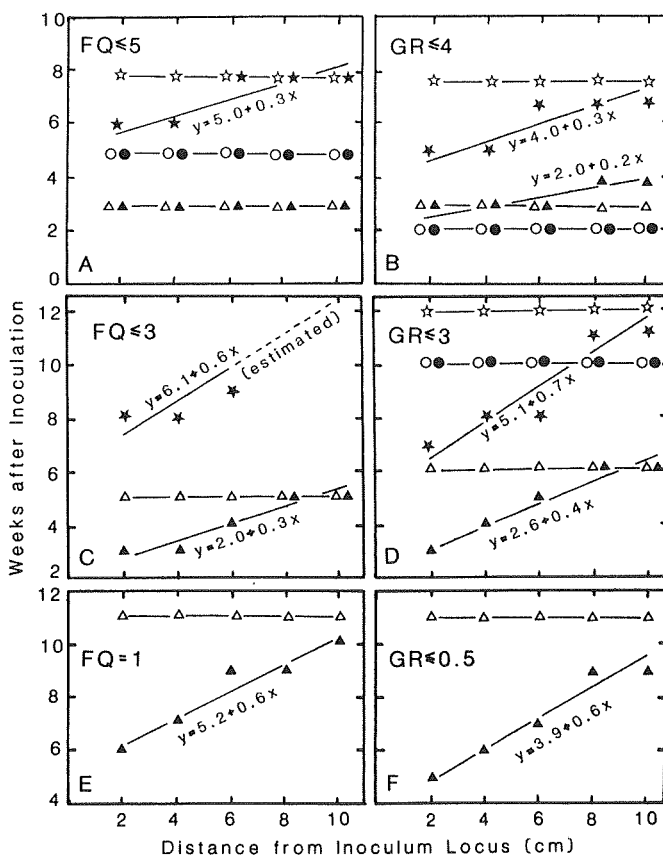

Fig. 2. Turfgrass quality (1 = dead, 4 = acceptable quality, and 9 = healthy) in pots of Merion bluegrass infested with *Phialophora graminicola* (▲), *Leptosphaeria korrae* (■), or uninoculated control (●), and incubated at the indicated temperature (continuous line) or transferred to 29 C (broken line) at 3 or 11 wk after inoculation. Coefficients of variation for each datum point varied from 0 to 0.54 (mean 0.16)

Fig. 3. Influence of temperature (● = 14 C, ★ = 21 C, ▲ = 29 C) on the progress of summer patch (solid symbols = inoculated with *Phialophora graminicola*, open symbols = uninoculated) through mature sod segments of field-grown Merion Kentucky bluegrass, as quantified by the number of weeks required for turfs to decline to the designated foliar quality ratings ([FQ] scale of 1-9; 1 = dead, 4 = acceptable quality, and 9 = healthy) and the designated leaf growth rates ([GR] centimeters per week) at 2-cm intervals from the inoculum locus. Coefficients of variation for each datum point varied from 0 to 0.62 (mean = 0.22).

rhizomes surfaces equally well at 24 and 29 C. The fungus moved past as many as three nodes during the 2-wk incubation period. When fungus encountered a node with roots, it continued to colonize the rhizome and also began to move down the root axis. Growth from a specific reference node was 50–83% slower along the rhizome than down the root axes.

After 4 wk of incubation at 29 C, all inoculated rhizomes became dark brown to black over their entire length (up to 8 cm), and the cortical and stelar tissues of roots were also thoroughly invaded by the pathogen. At 24 C, the discoloration of rhizomes also crossed nodes but extended only up to 47 mm from the inoculation point. Fungal hyphae were present over the entire rhizome segment at 24 C. Incubation of rhizomes at 14 C for 4 wk led to a brown to black discoloration of the internode inoculated with the pathogen. Discoloration occurred up to 2 cm from the inoculation point, but it was never observed to move past the first node. Ectotrophic hyphae were not observed on rhizomes incubated at 14 C, but histological studies were not conducted to determine if *P. graminicola* was able to move past the nodes through the vascular tissue. Dematiaceous ectotrophic growth or tissue discoloration did not occur on uninoculated rhizomes during this experiment, indicating that the fungus did not grow through the gravel to colonize uninoculated rhizomes as close as 1 cm from the inoculated series. The ability of the fungus to grow through gravel was not directly evaluated.

Growth of *P. graminicola* on intact rhizomes connecting mother and daughter clones showed a directional preference at 24 C but not at 29 C. Since the growth rates toward the daughter rhizomes were equal to those measured on severed rhizomes at each temperature,
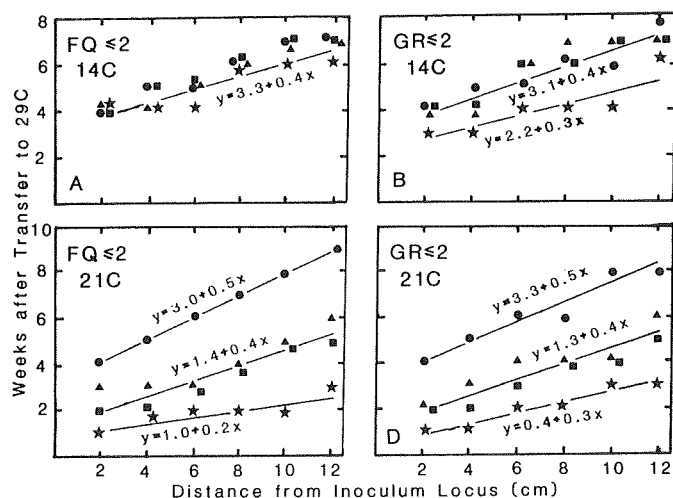
data for this experiment are not presented. Growth was 5 to 6 times faster toward the daughter clone than toward the mother clone at 24 C, but the growth was equal in both directions on rhizomes incubated at 29 C. Ectotrophic hyphae were not observed on rhizomes of plants incubated at 14 C.

In vitro study of dual cultures of ectotrophic fungi and *F. crookwellense*. *Fusarium* always grew over and into the slower-growing cultures of *P. graminicola* and *L. korrae* (*unpublished*). Microscopic observations and growth measurements indicated that the ectotrophs did not slow or alter growth of *Fusarium*, but mycelia of the ectotrophs did not advance through the agar or aerial environments after contact had been made by *Fusarium*.

In vivo study with dual inoculations. Although the isolates of *F. crookwellense* we used were known to be pathogenic to Kentucky bluegrasses (15), they did not cause significant leaf necrosis or crown and root rot under the conditions examined in this study (Table 2). This fungus also failed to amplify the severity of disease incited by *P. graminicola* or to accelerate the rate at which the summer patch disease appeared. This facultative parasite also did not incite disease on asymptomatic plants whose roots were observed to be colonized by *L. korrae*.

Mowing versus not mowing. Grass mowed at 2-cm height was much less tolerant of *P. graminicola* than was the unmowed grass (Fig. 5). The mowed, inoculated grass died (foliar quality rating = 1) within 5 wk. The quality of unmowed, inoculated grass also declined to an unacceptable level (rating = 3) but then began a swift recovery after 7 wk. The period of decline in turfgrass quality in inoculated grass coincided with a period in which greenhouse temperatures became elevated to as high as 41 C on several days. Improvement in quality of the inoculated, unmowed plants occurred when temperatures did not exceed 32 C. An initial period of stress from the root pruning was expressed in the mowed grasses by an early decline in quality and then a period of partial (inoculated treatment) or complete (uninoculated) recovery. Leaf growth rate curves for these treatments were more variable than the foliar quality rating curves but, as could be expected, they were reciprocals of the latter, and are therefore not presented.

DISCUSSION

Results of this study suggest that isolates of *P. graminicola* and *L. korrae* from different continents may have quite different adaptations to edaphic conditions. Although direct comparisions of isolates were not made, New York isolates of these fungi appear to have temperature optima 3 to 6 C higher than those reported from comparable studies in Northern Europe (9) and Australia (32). Osmotic potential tolerances for isolates of *P. graminicola* from New York are much lower than those reported from Australia (38). Isolates of *P. graminicola* from New York and Australia grow at near-maximum rates at the highest potentials studied (−0.1 MPa), but are reduced to 50% of their maximum rates at −0.6 and from −2 to −3 MPa, respectively. Similarly, the potential at which growth is prevented is −2.2 MPa for the New York isolates and

Fig. 4. Growth of *Phialophora graminicola* through mature, field-grown Merion Kentucky bluegrass at 14 or 21 C, as assessed by symptom development on colonized plants when inoculated sods were transferred to 29 C after 0 (●), 2 (▲), 4 (■), or 6 (★) wk of incubation at 14 or 21 C. The number of weeks after transfer at which foliar quality ratings ([FQ] scale of 1–9; 1 = dead, 4 = acceptable quality, and 9 = healthy) and leaf growth rates ([GR] centimeters per week) became 2 or less were measured at 2-cm intervals from the inoculum locus. Coefficients of variation for each datum point varied from 0 to 0.60 (mean = 0.19).

TABLE 1. Influence of temperature on growth of *Phialophora graminicola* on the surfaces of unsterile, field-produced rhizomes of Merion Kentucky bluegrass

Temperature (C)	Mean growth rate (cm/week)
14	0 (0)[a]
24	2.0 (0.2)
29	2.1 (0.3)
FLSD (*P* = 0.05)	0.2

[a] Coefficients of variation are in parenthesis.

TABLE 2. Influence of *Fusarium crookwellense* on expression of foliar symptoms on Merion Kentucky bluegrass plants alone or in combination with *Phialophora graminicola* or *Leptosphaeria korrae*[a]

Treatment	Mean foliar quality rating[b]
None	8.5 (0.1)[c]
+ *F. crookwellense*	8.3 (0.1)
P. graminicola	3.8 (0.4)
+ *F. crookwellense*	4.7 (0.3)
L. korrae	8.3 (0.1)
+ *F. crookwellense*	8.3 (0.1)
FLSD (*P* = 0.05)	1.3

[a] Refer to the text for inoculation procedures and incubation temperatures.
[b] Ratings 1–9; 1 = dead, 4 = acceptable quality, 9 = healthy.
[c] Coefficients of variation are in parentheses.

from −6 to −7 for those from Australia. The ecological attributes of *P. graminicola* and *L. korrae* from North America must therefore be evaluated separately from their counterparts in other regions.

Our isolates of *P. graminicola* grow very well on nonsterile rhizomes of Kentucky bluegrass (Table 1), and are therefore in an excellent position to colonize roots of new tillers, provided that environmental conditions are not limiting to such growth and pathogenesis. Although Kentucky bluegrass is a perennial plant, it maintains individual roots and tillers for only 6–18 mo (11). The greatest proportion of senescing tillers and roots occurs in early summer and regeneration of tillers and roots from surfacing rhizomes occurs mostly in late summer to early autumn (2,16). The ability of ectotrophic pathogens to incite disease will be determined by the relative balance among the rates of pathogen growth, rates of root regeneration, and environmental stresses. Cook et al (4) reported that osmotic and soil matric potentials had comparable effects on growth rates of *Gaeumannomyces* and *Fusarium* spp. Therefore, it is likely that effects of osmotic potentials on growth of the taxonomically and ecologically similar fungi that we studied are nearly equivalent to effects that could be expected from variations in soil matric potentials in the field. Although this was not examined directly, we wished to compare results of our in vitro studies on the ectotrophs with estimates of temperatures and osmotic potentials for optimal and maintenance growth rates by Kentucky bluegrass roots (Fig. 6) (2,16).

Summer patch, caused by *P. graminicola*, appears during midsummer when temperatures are high. Regrowth of turf into affected areas does not begin until the air and soil temperatures begin to decline during late summer and early autumn. In New York, the disease is recognized most frequently on high-quality Kentucky bluegrass turfs that are mowed closely and irrigated (23). Frequently, "Fusarium blight" first becomes evident on very hot days that follow major rains or irrigation. These observations were made at locations where *P. graminicola* is now known to be the cause of the patch disease (31) and where temperatures in the root zone reach at least 36 C during the summer (35). Disease caused by *P. graminicola* was most favored by warm to hot, wet conditions in our study. Comparisons in Fig. 6 indicate that the pathogen could gain an advantage over the host at about 20 C, and become increasingly important up to about 33 C, provided moisture is not limiting. In our pathogenicity studies with field-produced sod, plants colonized by *P. graminicola* at 21 C did not develop severe symptoms in the foliage until the plants were transferred to 29 C, or the incubation period was lengthy. This result would be predicted from the comparisons made in Fig. 6. Summer patch is, however, also recognized for its occurrence on irrigated turfs which are suddenly subjected to drought during periods of moderate temperatures (23), as may occur when an irrigation head fails to activate or to distribute water uniformly. Although the ability of related fungi to grow at low osmotic potentials increases as the temperature is increased (4,38), we feel that it is unlikely that pathogen growth and pathogenesis could occur so rapidly as to cause large and distinct patches at the onset of sudden droughts. In this instance, plants previously colonized by the pathogen presumably suffer from levels of vascular dysfunction that are sublethal at higher soil water potentials and which contribute to a lethal condition when additional stresses are also imposed. We have confirmed that close mowing during conditions of high temperature increases the susceptibility of turfgrass to this disease. The chronology of infection and symptom development in the field require further investigation. Other stresses that also have been implicated with expression of patch disease symptoms include high nitrogen nutrition (36), arsenical herbicides (31), phytotoxins in turfgrass thatch (30), and others (20,23).

We determined that the maximum growth rates of *P. graminicola* on unsterile rhizomes, in field-grown turf, and on an agar medium were remarkably similar. This result does not agree with that of Grose et al (12) who studied the effects of temperature and water potential on growth of *G. graminis* var. *tritici* in soil. In her study, the pathogen grew most rapidly at high temperatures on agar medium and at lower temperatures in unsterile soil. The apparent shift in optimal temperature for growth of the pathogen

was attributed to increasing levels of microbial competition in soil as temperature increases. This observation is in harmony with the fact that take-all of wheat is most severe in cool, wet soils (3,18). With summer patch of turf, however, the disease occurs during warm to hot weather, when microbial activity in the root zone is near maximum. Since *P. graminicola* is capable of growing at comparable rates in sterile media and in unsterile turf, it is important to determine whether this pathogen is less sensitive than *G. graminis* var. *tritici* to microbial competition.

Symptoms of necrotic ring spot, caused by *L. korrae*, have first been observed during all months from April to November in New York, on nonirrigated as well as irrigated turfs. This disease, therefore, occurs over a wider range of environmental conditions than does summer patch. *L. korrae* began to grow at near-maximum rates at 15 C and declined rapidly above 28 C. The pathogen failed to grow only at the lowest osmotic potential evaluated. Its growth was not slowed by 50% until the potential was about −2.2 MPa. Therefore, this pathogen appears capable of growth at higher temperatures and lower water potentials than those that impede the growth of Kentucky bluegrass roots (Fig. 6). In our growth chamber study, *L. korrae* did not incite detectable levels of disease at 14, 21, or 29 C. Moisture was never limiting to plant growth in this study. At 14 C, the replacement of roots appears to be favored more than growth of the pathogen. At 21 C, the reverse is true, but the level of plant stress in our study was
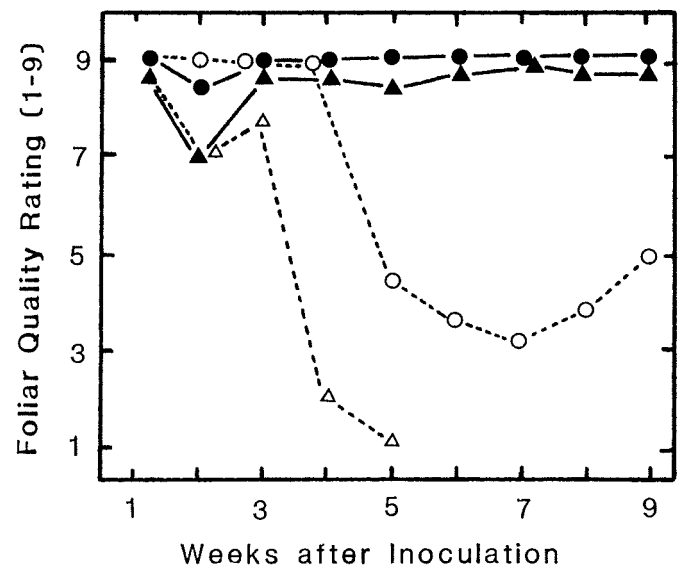

Fig. 5. Turfgrass quality ratings (scale of 1–9; 1 = dead, 4 = acceptable quality, and 9 = healthy) in pots of Merion Kentucky bluegrass inoculated (open symbols) or uninoculated (solid symbols) with *Phialophora graminicola*, and mowed (△, ▲) each week at 2-cm height or left unmowed (o, ●). Coefficients of variation for each datum point varied from 0 to 0.85 (mean = 0.42) in the inoculated, unmowed treatment and from 0 to 0.10 (mean = 0.02) in the other three treatments.
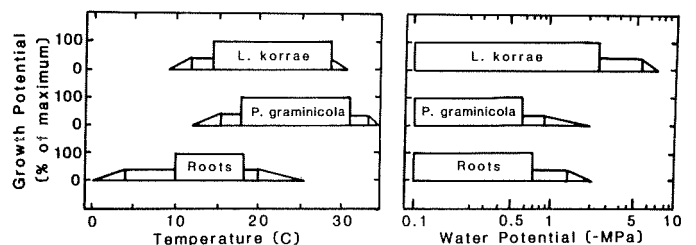

Fig. 6. Comparison of temperature and water potential effects on relative growth rates of Kentucky bluegrass roots (from references 2 and 16), and of *Phialophora graminicola* and *Leptosphaeria korrae* (tallest bars depict maximum relative growth rates, shorter bars depict moderate growth potential, and sloped bars depict minimal to nil growth).

apparently insufficient to favor disease expression. Smith (32) found that the temperature for maximum growth of *L. korrae* on agar was clearly 25 C for young cultures, and shifted to a broad peak from 10 to 25 C for older cultures. He also studied its growth rate through unsterile soil in the absence of plants, and found that the greatest rate of growth decreased from 20 C early in the test to 15 C after a 2 wk period. Smith (32) attributed this shift in temperature optimum to higher levels of microbial antagonism or competition at the higher temperatures. It is not known whether shifts in temperature optima, as reported by Smith (32), occurred in our pot study in the growth chamber. If so, this process presumably would have reduced the capacity for this pathogen to kill roots more rapidly than the plant could replace them at the cooler temperatures. This may also explain why we, in an earlier study (28), succeeded in establishing foliar symptoms of necrotic ring spot only when the infected turf was moved outdoors and exposed to intense sunlight, wind, and drying, even though plants that were devoid of most roots remained asymptomatic for several weeks in the greenhouse. At 29 C, the growth of roots and *L. korrae* appear equally restricted by high temperature. These results indicate that disease progress would be optimal at about 23 to 26 C, when root replacement is minimal and growth of foliage and *L. korrae* are rapid. Conditions would be especially favorable for disease if soil water potentials are also limiting to plant growth. Unfortunately, this hypothesis was not evaluated in our study.

F. crookwellense is adapted to temperatures and osmotic potentials similar to those for *F. culmorum* (4,19). This fungus is also adapted to a wider range of osmotic potentials and lower range of temperatures than *P. graminicola*. Although the ecological significance of these characterizations remains to be determined, it is interesting that *F. crookwellense* and the ectotrophic pathogen each colonize cortical cells (*unpublished*) and that the latter is then also capable of penetrating the endodermis and colonizing the vascular system (22,28). If cortical cells are first colonized by *Fusarium* spp., it is unlikely that they would also be available for colonization by the ectotroph. Plants surviving in patch-affected turfs are nearly always heavily colonized by species of *Fusarium*. We found that the *Fusarium* quickly halted the growth of the ectotrophs on agar media, and did not contribute to the severity of disease expression. The broader range of adaptation to environmental extremes by *F. crookwellense* than by *P. graminicola* may help to explain the occurrences of inverse relationships between the proportion of crowns colonized by *Fusarium* and the incidence of summer patch at two research sites in New York (23,29).

These studies indicate that refinements in summer patch disease control strategies should be possible by targeting fungicide applications to periods when the pathogen is growing most actively rather than when disease symptoms appear. Late spring and early summer applications of fungicides would seem most efficient for controlling summer patch, whereas applications to control necrotic ring spot would seem necessary in late summer through autumn and in the spring and early summer. Similarly, changes in irrigation and mowing practices may be possible on some turfgrasses to minimize the stress on the grass when temperatures are more favorable for pathogenesis than for replacement of roots on affected plants.

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