Winter Survival of the Perennial Ryegrass Pathogen *Magnaporthe oryzae* in North Central Indiana

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ABSTRACT

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Winter survival of *Magnaporthe oryzae* in north central Indiana was investigated in response to reports and observations of sporadic disease incidence. Survival of the fungus in perennial ryegrass residue was assessed. Time course studies were designed to assess the conidia production potential of infested perennial ryegrass residue exposed to ambient and predetermined treatments. Approximately 50,000 conidia per gram dry weight were produced initially on infested residue. In all years of the study, ambient winter conditions in Lafayette, IN, reduced conidia production on residue to fewer than 60 conidia per gram by spring. Unless residue was dried prior to treatment, storage of residue at all temperatures tested reduced conidia production potential. Airborne *M. oryzae* conidia, over the plot of perennial ryegrass where the winter survival studies were conducted, were estimated from particles collected with a volumetric air sampler. The stand of perennial ryegrass was inoculated with residue infested with *M. oryzae* in the summer of 2000 and late fall of 2000 and 2001. The stand of ryegrass was not inoculated in the summers of 2001 or 2002. Conidia were captured with the air sampler each year in early July, before outbreaks had been observed in north central Indiana, but capture peaked in September when outbreaks most often occur. Cumulative conidia capture followed disease severity in 2000; however, no disease was observed in 2001 or 2002. Results of this study suggest poor survival of *M. oryzae*, and inadequate populations of viable pathogen limit disease development in north central Indiana. Disease risk assessment in north central Indiana should include an estimate of viable inoculum.

Gray leaf spot of perennial ryegrass is caused by *Magnaporthe oryzae* Couch (anamorph *Pyricularia oryzae* Cavara) (3). The fungus was recognized as a pathogen of perennial ryegrass in Japan in the 1950s (11), but the first published report of a serious disease outbreak in the United States was in 1992 (8). Researchers have since reported the disease in the Mid-Atlantic, Midwest, and New England regions of the United States (4,8,12,16,17). In Indiana, *M. oryzae* was first isolated in 1999.

M. oryzae also causes the blast disease of rice. Isolates of *M. oryzae* from rice and other grass hosts survive as mycelium in infested host residue or in seed (6,7,11,18). Pathogen populations were found to be reduced significantly by winter conditions in temperate regions (6,7,11,18). Poor winter survival of the pathogen in temperate regions limits surviving inoculum and disease development in such areas when

no other source of primary inoculum exists (7,11).

Environmental conditions that generally favor the establishment and spread of gray leaf spot (10,15) regularly occur throughout Indiana each summer, but disease appears with greater frequency in the southern part of the state. Outbreaks of gray leaf spot have not been confirmed in Indiana north of Rensselaer (40.937°N latitude).

Air sampling techniques were evaluated for the estimation of the aerial density of *M. oryzae* conidia in rice production regions (13). Volumetric air samplers were found to be more efficient than passive deposition devices and methods, especially at low aerial conidia concentrations (13). A relationship between the number of conidia collected with a volumetric air sampler and rice blast disease progress was observed all years the study was conducted (13).

The effects of low temperature conditions on the survival of *M. oryzae* in infested perennial ryegrass residue had not been explored. The quantities of airborne *M. oryzae* conidia available in summer to cause disease in north central Indiana have not been investigated previously. The relationship between the incidence and severity of gray leaf spot and the concentration of airborne conidia of *M. oryzae* over a stand of perennial ryegrass has not been considered previously.

The objectives of this research were to (i) document gray leaf spot outbreaks on golf courses in Indiana over a 3-year period and to document environmental conditions for both the winter prior and summer of the recorded outbreaks, (ii) investigate the survival of *M. oryzae* on infested perennial ryegrass residue exposed to a range of environmental conditions over time as measured by the number of conidia produced on samples of the residue when removed from treatment and incubated in moist chambers, and (iii) utilize a volumetric air sampler to quantify *M. oryzae* inoculum (conidia) over a stand of perennial ryegrass before, during, and after favorable conditions for gray leaf spot disease development.

MATERIALS AND METHODS

Gray leaf spot occurrence. Symptomatic perennial ryegrass samples from Indiana were collected during the summers of 2000 through 2003. Samples were incubated in near-saturated environments for 24 to 48 h then inspected for *M. oryzae* conidia. If *M. oryzae* conidia were identified, the fungus was isolated onto V8 juice agar. Gray leaf spot outbreaks reported here are limited to those that ultimately led to isolations of *M. oryzae*. Communication with turf managers through university extension activities facilitated location and collection of turf with gray leaf spot symptoms. Not all turfgrass managers in Indiana were involved with this study, and no statewide systematic survey of perennial ryegrass turf was conducted.

Records of environmental conditions from weather stations in Lafayette, IN, for the summers of 2000 through 2002 and the winters of 1999 to 2000 through 2001 to 2002 were obtained from the Indiana Climate Page online database (Purdue University, Department of Agronomy, Applied Meteorology Group, Indiana Climate Page [database on the Internet], West Lafayette, IN. Updated 26 August 2003; cited 22 October 2003). The website is maintained by the Indiana State Climatologist with the Purdue Applied Meteorology Group, Department of Agronomy, Purdue University.

Hourly air temperatures, relative humidity percentages, precipitation totals, and precipitation durations were obtained for Lafayette for the time period 1 July through 30 September of 2000 through 2002. An hour of leaf wetness was recorded if the relative humidity was greater

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than 96% or if any precipitation was measured during the hour. Daily records of maximum and minimum air temperatures and heating degree days (HDD) were obtained for 1 October through 31 March of 1999 to 2000, 2000 to 2001, and 2001 to 2002 in Lafayette. Daily HDD units were calculated by summing the difference between 65°F (18.3°C) and the average air temperature in degrees Fahrenheit for each day. Cumulative HDD values refer to the sum of daily HDD units for a given time period. Comparatively higher HDD values indicate lower winter temperatures.

***M. oryzae*-infested residue.** Perennial ryegrass (cv. Legacy II) was grown in the greenhouse in plastic flats (30 × 61 cm), or in plastic pots (15 cm diameter) filled with calcine clay (Turface MVP, Profile Products LLC, Buffalo Grove, IL) or a 2:1 pasteurized sand-soil mixture. The ryegrass was seeded at a rate approximately equivalent to 2.5 kg per 100 m² and allowed to grow for 3 weeks. The ryegrass pots and flats were watered with a dilute solution (0.2%, wt/vol) of 20-20-20 (N-P-K, Peter's Professional Plant Food, Peters Fertilizer Products, Fogelsville, PA) at 14 and 21 days after planting.

Inoculum was prepared from an isolate (IN9931) of *M. oryzae* collected during the summer of 1999 from infected perennial ryegrass in central Indiana. The isolate was maintained on V8 juice agar in (9 cm diameter) petri dishes (14). Juvenile perennial ryegrass periodically (approximately every 6 weeks during the winter of 1999 and spring of 2000) was inoculated with the isolate; the pathogen was reisolated to maintain pathogenicity and sporulation capacity. The isolate was stored on desiccated filter paper disks at 4°C (9). Sporulation was induced by incubating cultures at 24°C under continuous fluorescent light (two General Electric F40W white fluorescent bulbs, 118 V, 60 Hz). Sterile distilled water (5 ml) was added to each petri dish culture, and conidia were dislodged with a small metal spatula or rubber policeman. The spore suspension was filtered through two layers of cheesecloth to remove large mycelial fragments. The concentration of the initial suspension was determined microscopically with a hemacytometer, and then adjusted with distilled water to 1 × 10⁶ conidia per ml for inoculations.

An atomizer was used to inoculate 3-week-old perennial ryegrass plants with the conidial suspension. Approximately 75 ml of the suspension was uniformly applied to each flat of plants. The inoculated ryegrass plants were then placed in a growth chamber (Model 1-35DLM, Percival Scientific, Perry, IA) maintained at 28°C, 100% relative humidity, and an 18-h photoperiod. Inoculated flats were incubated in the chamber for 96 h before being returned to a greenhouse bench where temperature ranged from 25 to 30°C. The diseased perennial ryegrass was cut at the

crown. A portion of the harvested residue was air-dried before it was thoroughly mixed by hand (>10 min) and exposed to various environmental regimes. The portion of the residue that was not dried was thoroughly mixed by hand (>10 min) and introduced into the various temperature regimes (treatments) as fresh residue.

Pathogen survival 2000 to 2001. Dried, infested perennial ryegrass residue was exposed to three different environmental regimes from November 2000 through May 2001. Samples were collected at approximately monthly intervals to assess the extent of pathogen survival in each of these regimes. The experiment was initiated 10 November. Sample dates were 9 December, 13 January, 14 February, 14 March, 17 April, and 30 May.

The LAB regime was a constant, dry environment (23°C, stored on a laboratory bench). This residue was stored in a sealed plastic bag and was expected to survive well and maintain a high level of sporulation capacity, i.e., the ability of the fungus to produce conidia from infested residue when incubated at 23°C in a near-saturated environment. Residue exposed to the VAR regime was maintained in an incubator (Model 307, Fisher Scientific, Suwanee, GA) where temperature was adjusted at approximately monthly intervals to mimic ground temperatures during a typical November–May time span in north central Indiana. The incubator was initially set to 4°C and adjusted on each sample date in this order: 0°C, –4°C, 4°C, 8°C, 12°C.

Fig. 1. Perennial ryegrass samples were collected from golf courses and athletic fields in 2000, 2001, and 2002. Filled square icons on the Indiana maps are positioned approximately where gray leaf spot samples were collected and are labeled with the collection date. An icon over Lafayette, IN, is included on each map for reference.

Fig. 2. Cumulative heating degree days (HDD) calculated for Lafayette, IN, during the time periods between 1 October and 31 March before the summers of 2000, 2001, and 2002 (Purdue University, Dept. of Agronomy, Applied Meteorology Group. Indiana Climate Page [database on the Internet]. West Lafayette, IN. Updated 26 August 2003; cited 22 October 2003).

The 2001 field regime (DAN01) was defined by the conditions that occurred at the Daniel Center in West Lafayette from November 2000 through May 2001. Residue samples (3 g each) were placed in 12 × 15 cm envelopes of fine mesh fiberglass screening. The individual sample envelopes were then placed in a 1 × 1.2 m fiberglass screen envelope in a single layer. This larger envelope was put in the canopy of a 4-year-old perennial ryegrass stand on 12 November 2000. The ryegrass stand at the experimental site under the envelope was clipped to the crowns to ensure that the mesh envelope containing the residue was at or slightly below canopy level.

Sporulation capacity was used to evaluate survival of *M. oryzae* on infested residue. A residue sample (3 g) was collected from each of the three environmental regimes on each sample date. The residue samples were placed in sealed plastic containers lined with three paper towels and moistened with 25 ml of sterile distilled water, then incubated at 23°C for 48 h to induce sporulation of *M. oryzae*. After incubation, each residue sample was placed in a 500-ml beaker and vigorously rinsed in 100 ml of sterile distilled water. The suspension was then filtered through two layers of cheesecloth to remove the debris. Samples of the filtrate were examined microscopically with a hemacytome-

ter to assess the amount of sporulation from the infested leaf blade samples. The distinct, three-celled, pear-shaped conidia of *M. oryzae* were counted. Conidia concentrations of filtrates were calculated from the mean of six hemacytometer fields. The conidia recovered per gram of residue was calculated.

Pathogen survival 2001 to 2002. Fresh infested perennial ryegrass residue (prepared as described above) was exposed to four environmental regimes from November 2001 through May 2002. The four treatments were identified as -20°C, 4°C, ALT, and DAN02. Residue in the -20°C and 4°C treatments were sealed in plastic containers and maintained in dark incubators at -20°C and 4°C, respectively. Residue in the ALT regime was exposed to alternating 24-h periods of 4°C and -20°C in dark incubators. The field regime (DAN02) was defined by the conditions that occurred in a stand of perennial ryegrass at the Daniel Center from 4 November 2001 through 25 April 2002.

Sample dates were 4 November (initial), 14 November, 24 November, 4 December, 19 December, 28 January, 9 March, and 25 April. For each sample date, residue was washed in 100 ml of sterile distilled water before incubation. The residue samples were placed in sealed plastic containers lined with three paper towels and moistened with 25 ml of sterile distilled water, then incubated at 23°C for 48 h to induce sporulation of *M. oryzae*. Samples were smaller in this experiment (2 g) to allow for five samples per treatment per date.

Samples were washed after incubation, and filtrate was examined microscopically with a hemacytometer for the distinct conidia of *M. oryzae*. The concentrations of conidia in the filtrates were calculated from observations of six hemacytometer fields for each of five 2-g samples from each treatment. The conidia recovered per gram of residue were calculated.

Data were analyzed after a square-root transformation of the mean of five replications. Two-factor analysis of variance was run with the general linear model in SAS statistical software.

Pathogen survival 2002 to 2003. Infested perennial ryegrass residue was prepared as described above. Residue was dried and divided into 10-g samples. Samples were exposed to one of two environmental regimes, LAB or DAN03. Dried residue subjected to the LAB regime was stored at constant 23°C on a laboratory bench. The field (DAN03) regime was defined by the environmental conditions that occurred at the Daniel Center from 10 December 2002 through 18 June 2003.

One residue sample from each treatment on each date was placed in sealed plastic containers lined with three paper towels and moistened with 25 ml of sterile distilled water, then incubated at 25°C for 72 h to induce sporulation of *M. oryzae*. After

Fig. 3. Cumulative biweekly hours of leaf wetness from 1 July to 30 September of 2000 through 2002 for Lafayette, IN, were calculated from weather data obtained from the Indiana Climate Data Website (Purdue University, Dept. of Agronomy, Applied Meteorology Group, Indiana Climate Page [database on the Internet]. West Lafayette, IN. Updated 26 August 2003; cited 22 October 2003). An hour of leaf wetness was recorded if relative humidity was greater than 96% or precipitation was measured during the hour.

Fig. 4. Biweekly average air temperatures during the hours of leaf wetness in Lafayette, IN (Fig. 3) were calculated from weather data obtained from the Indiana Climate Data Website (Purdue University, Dept. of Agronomy, Applied Meteorology Group, Indiana Climate Page [database on the Internet]. West Lafayette, IN. Updated 26 August 2003; cited 22 October 2003).

incubation, the residue was washed in 100 ml of sterile distilled water. The suspension was filtered through two layers of cheesecloth, and the conidia concentrations of filtrates were calculated from microscopic observations of six hemacytometer fields for each 10-g sample from each treatment. The conidia recovered per gram of residue were calculated.

Conidial density determination by air sampling. On 7 July 2000, approximately 350 g of dried infested perennial ryegrass residue (produced as described above) was spread in a 4 × 4 m plot of perennial ryegrass at the Daniel Center. Prior to this inoculation, gray leaf spot had not been identified on this site. Inoculum was not applied during summer in subsequent years. Gray leaf spot severity within the plot was assessed visually using the Horsfall-Barratt scale (5). Values from eight evaluation dates were then converted into disease percentages (2).

A Burkard continuous volumetric air sampler (Burkard Manufacturing Co. Limited, Woodcock Hill Industrial Estate, Rickmansworth, Hertfordshire, England) with wind orientation and a single reverse flow miniature cyclone with an air throughput of 16.5 liters/min was located in the center of the plot on 1 July 2000. The sampling device operated from 1 July 2000 to 1 October 2000, from 29 May 2001 to 5 October 2001, and from 23 April 2002 to 2 October 2002. The air sampler was powered by one of two deep-cycle, 12 V trolling motor batteries (Part 27DC-6, EverStart Battery, Princeton, NJ). The batteries were changed and charged weekly. Airborne particles, including spores, were collected directly into 1.5-ml Eppendorf vials. The vials were exchanged at intervals of 1 to 5 days during the sampling periods of 2000, 2001, and 2002. The vials were labeled and stored for later microscopic examination of the contents. Vial contents were suspended in distilled water (0.3 or 0.5 ml), and *M. oryzae* conidia were quantified with a hemacytometer. Six hemacytometer fields were visually inspected for the distinct conidia of *M. oryzae*. The number of collected *M. oryzae* conidia was calculated for the period during which the sampler deposited particles into each tube. From these data and the known volume throughput of the sampler, conidia per liter of air was calculated. Cumulative conidia collected through the summer of 2000 also was calculated.

RESULTS

Gray leaf spot occurrence. Incidence of gray leaf spot in the state of Indiana during the summers of 2000, 2001, 2002, and 2003 was documented. Confirmed reports of gray leaf spot in 2000, 2001, and 2002 are plotted on state maps with the dates the samples were collected (Fig. 1). There were no confirmed outbreaks of gray leaf spot in Indiana in 2003. In 2001,

only two occurrences (both in southern Indiana) of gray leaf spot were confirmed, but in 2000 and 2002, seven and nine outbreaks, respectively, were confirmed.

Environmental data for Lafayette. Cumulative HDD values were plotted for the winters of 1999 to 2000, 2000 to 2001, and 2001 to 2002 in Lafayette (Fig. 2). The 6-month period prior to the summer of 2001 was colder (higher cumulative HDD values) than the period prior to the summers of 2000 and 2002. Biweekly cumulative hours of leaf wetness for Lafayette from 1 July through 31 September of 2000, 2001, and 2002 were calculated (Fig. 3). Biweekly average temperatures during hours of leaf wetness also were calculated (Fig. 4). There were fewer hours of leaf wetness in 2000 and 2002 than in 2001. Throughout all three summers, there was no obvious pattern regarding temperatures during the leaf wetness periods.

Pathogen survival 2000 to 2001. Although each treatment was represented by only one replicate, numerically far fewer conidia of *M. oryzae* were recovered from perennial ryegrass residue exposed to ambient conditions (DAN01) compared with the LAB treatment (Fig. 5). An additional sample of residue from the DAN01 treatment was processed on 30 May 2001, but no *M. oryzae* conidia were recovered (data not shown). Conidia produced per gram of infested residue for both the LAB and VAR treatments were greater than for the DAN01 treatment on each sample date and were relatively constant on all sample dates.

Pathogen survival 2001 to 2002. The number of conidia recovered per gram of infested residue declined over time for all treatments (Fig. 6). Conidia recovered

from residue exposed to ambient conditions at the Daniel Center (DAN02) declined from an initial estimation of 42,000 conidia per gram of residue to 60 conidia per gram. The greatest decline in conidia recovered occurred for residue exposed to 24-h periods of alternating 4°C and -20°C (ALT). Conidia produced in residue stored at 4°C and in the ALT treatment were reduced below levels of detection by 25 April 2002.

Survival of *M. oryzae* in residue stored at -20°C declined compared with the initial estimation, but conidia were recovered at all sample times. Although conidia were recovered from residue exposed to ambient conditions at the Daniel Center (DAN02) at all sample times, only one conidium was observed in 30 hemacytometer fields of the five samples collected on 25 April.

Mean separations according to the Waller-Duncan *k*-ratio *t* test within each date are included in Figure 6. An analysis of variance indicated that the interaction effects between date and treatment were significant, but residuals were normal. Analyzing each date separately, the estimated residual variances among dates were found to be statistically different using Hartley's *F*_{max} test.

Pathogen survival 2002 to 2003. Although each treatment was represented by one replicate, sporulation capacity declined steadily for residue exposed to ambient conditions at the Daniel Center to less than 100 conidia per gram by May 2003 (Fig. 7). No conidia were recovered from the DAN03 treatment on 18 June. Conidia were consistently recovered from residue in the LAB treatment.

Conidial density determination by air sampling. Airborne conidia of *M. oryzae*

Fig. 5. Conidia of *Magnaporthe oryzae* produced per gram of infested perennial ryegrass residue during the winter and spring of 2000 to 2001. Residue subjected to the LAB regime was dehydrated and stored at 23°C. Residue subjected to the VAR regime also was dehydrated, but temperatures were maintained between -4°C and 8°C to mimic ground temperatures in Indiana between November and May. Residue in the DAN01 treatment was exposed to ambient conditions in a perennial ryegrass research plot at the Daniel Center in West Lafayette, IN. Initial conidiation was approximately 50,000 conidia per gram. The experiment was initiated on 10 November 2000.

were collected from early July through September of 2000, 2001, and 2002. Conidial densities were calculated from conidia counts and the volume of air sampled for each time period (Fig. 8). No conidia were found before 1 July in any year the experiment was conducted. Conidia were collected by the air sampler in the summer of 2000 approximately 1 week after inoculation of the experimental site with pathogen-infested residue. The quantities of

observed conidia were small then gradually increased to a peak level of 0.056 conidia per liter of air between 30 August and 1 September 2000. Cumulative conidia captured during the summer of 2000 were calculated from conidia count data (Fig. 9).

The first gray leaf spot symptoms in the inoculated plot surrounding the air sampler were observed 11 days after inoculation in 2000. Gray leaf spot severity increased in

July and August and peaked at approximately 20% in mid-September (Fig. 9).

In 2001, plots were not inoculated in the summer with infested residue. Infested residue was placed in the plot the previous winter (2000). Conidia were captured beginning on 6 July. Fewer than 1,700 conidia were collected throughout the season. A peak capture of 0.0028 conidia per liter of air was calculated after examination of contents collected between 2 and 7 August 2001 (Fig. 8). In 2002, a similar trend occurred, with approximately 5,000 conidia recorded throughout the season. The peak capture of 0.015 conidia per liter of air occurred between 10 and 15 September 2002. Gray leaf spot symptoms were not evident in 2001 and 2002, although the site was inoculated with infested residue in November 2000 and December 2001. Gray leaf spot was not observed anywhere on the Daniel Center, and the pathogen was not isolated from perennial ryegrass in the experimental plot in 2001 or 2002.

DISCUSSION

Results indicate that the pathogen can survive the winter in north central Indiana in infested residue, but the magnitude of the surviving population may be insufficient to serve as effective primary inoculum for summertime gray leaf spot epidemics.

Incubator experiments using dehydrated host residue in 2000 (LAB, VAR) showed that temperature was not the single factor determining the extent of pathogen survival. It was not unexpected that the pathogen survived these treatments, since isolates are commonly stored at a range of temperatures without loss of pathogenicity or viability on a range of media (9). However, when fresh (undried) residue was maintained at controlled temperatures, pathogen viability was reduced substantially. Repeated freezing and thawing by alternation between 24-h periods of 4°C and -20°C was quite detrimental to pathogen viability, probably due to cell membrane disruption by ice crystal formation. Similar results have been reported on survival of the rice blast pathogen (11). Although the pathogen recovered from infested residue in the field remained viable during March and April, the quantities of conidia recovered indicated that populations were greatly reduced from initial levels. By May or June in each of the 3 years, conidia could not be recovered from residue. Typically, during the months of May and June, perennial ryegrass grows rapidly and microbial activity increases in the soil and thatch as soil temperatures approach optima for cool-season grass growth (1). Because *M. oryzae* is a poor saprophytic competitor (11), it is likely that the already reduced populations were further diminished below the method of detection during the spring months. It is also likely that *M. oryzae* conidia produc-

Fig. 6. Conidia of *Magnaporthe oryzae* produced on infested perennial ryegrass during the winter and spring of 2001 to 2002. Letters within each date represent means separation according to the Waller-Duncan *k*-ratio *t* test. Residue was exposed to 4°C, -20°C, and alternating (ALT) 24-h periods of 4°C and -20°C. Residue in the DAN02 treatment was exposed to ambient conditions from October to June in a perennial ryegrass research plot at the Daniel Center in West Lafayette, IN. The initial conidiation of the residue prior to exposure to different environmental regimes was recorded at 42,000 conidia per gram on 4 November 2001.

Fig. 7. *Magnaporthe oryzae* conidia recovered from infested perennial ryegrass after incubation during the winter and spring of 2002 to 2003. Residue was dried and stored at 20°C (LAB). Residue in the DAN03 treatment was exposed to ambient conditions from December to June in a perennial ryegrass research plot at the Daniel Center in West Lafayette, IN. An initial assessment of 51,000 conidia per gram of residue was taken prior to exposure to the two environmental regimes on 13 December 2002.

tion on fresh (undried) residue maintained at 4°C was decreased due to competition with other microorganisms (Fig. 6).

Results differed among the 3 years of air sampling for *M. oryzae* conidia. In 2000, the number of conidia observed after inoculation of the research plot increased over time, disease symptoms developed, and the pathogen was isolated from perennial ryegrass surrounding the air sampler. Disease progress closely followed cumulative conidia capture data (Fig. 9).

In 2001 and 2002, conidia were collected at approximately the same time of year as in 2000. If conditions were equally conducive for the production of conidia in early July of these 3 years, and the captured conidia were produced locally from the pathogen on perennial ryegrass, one would expect similar amounts of disease to develop if environmental conditions were similar for the remainder of the summer. However, gray leaf spot was not observed in the research plot in 2001 or 2002. Two important environmental variables for gray leaf spot development, leaf wetness duration and temperature during the period of leaf wetness, were compared for Lafayette in 2000, 2001, and 2002 (15). Temperature and moisture conditions in Lafayette in 2001 appear to have been similar or slightly more favorable for gray leaf spot development than in 2000 (Figs. 3 and 4) when compared with published optima (15). However, disease developed at the experimental site only in 2000. Also, temperature and moisture conditions in 2002 in Lafayette appear to have been similar or slightly less favorable for gray leaf spot development than in 2001, but there were many confirmed outbreaks of gray leaf spot throughout central and north central Indiana in 2002 and few in 2001 (Fig. 1). If summer environmental conditions were the primary determinant of gray leaf spot development in north central Indiana, then one would expect the greatest disease incidence in 2001. It is most likely that disease did not develop because effective primary inoculum was absent.

The winter temperatures prior to the summer of 2001, a summer with only two confirmed gray leaf spot outbreaks, in Indiana were lower (higher HDD) than temperatures prior to 2000 or 2002, two years with many confirmed outbreaks of gray leaf spot. These observations and data suggest that when winter temperatures are low, disease outbreaks are less common the following summer and are consistent with the hypothesis that pathogen populations, reduced by low winter temperatures, limit disease development in north central Indiana. An especially low level of surviving inoculum in 2001 might also explain why only two outbreaks of disease in Indiana were recorded that summer despite apparently favorable summer conditions.

In 2001 and 2002, conidia captured in the air sampler could have been those of

M. grisea or *M. oryzae* from other hosts (11). Cultures of the pathogen were not obtained (despite efforts) from the conidia collected in the air sampler, so molecular genetic comparisons that might confirm the identity of the conidia were not applied. Additionally, if the observed conidia did not originate in the research plot, and were exotic to the Daniel Center, then it is possible that infection efficiency of the

spores was adversely affected in transit or that many of those conidia collected were nonviable as a result of long-distance dissemination. Similar observations have been made in rice blast literature (11).

The frequency and extent of *M. oryzae* survival and apparently inefficient and irregular introduction of conidia from exotic locations limit the amount of initial inoculum available each summer and con-

Fig. 8. Densities of *Magnaporthe oryzae* conidia collected during three summers. Conidia were collected with a Burkard volumetric air sampler located on a perennial ryegrass research plot at the Daniel Center in West Lafayette, IN.

Fig. 9. Cumulative *Magnaporthe oryzae* conidia collected in a volumetric air sampler in 2000 (spore capture) and gray leaf spot disease severity recorded in a 4 × 4 m research plot surrounding the volumetric air sampler in 2000.

tribute to the more sporadic disease occurrence and severity observed in north central Indiana.

Results suggest that there exists a transition zone for the survival of *M. oryzae*. Gray leaf spot has not been observed or reported north of Rensselaer, IN (40.937°N latitude), which is located approximately 65 km north of Lafayette. It is likely that the poor overwinter survival of *M. oryzae* limits the threat of this disease in northern Indiana. In north central Indiana, an often inconsequential pathogen population and intermittent disease-favorable conditions result in sporadic disease occurrence. In southern Indiana, gray leaf spot is a more persistent and serious threat to perennial ryegrass because of the more constant supply of effective primary inoculum and a longer period of environmental conditions that are conducive to disease development.

This information is valuable to turfgrass managers because considerable fungicide expenditure is required to protect perennial

ryegrass from this disease, and a severe epidemic can result in substantial losses of turf. Considering this risk and additional cost, some turfgrass managers have been persuaded to convert their golf course fairways and athletic fields to alternative grass species such as creeping bentgrass, Kentucky bluegrass, and bermudagrass. These species, although not affected by gray leaf spot, have other significant disease and agronomic limitations that should be carefully considered. While conversion to other grass species because of gray leaf spot concerns may indeed be reasonable in southern Indiana, gray leaf spot may be managed effectively and efficiently with fungicides in north central Indiana.

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