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EPIEMIOLOGICAL STUDIES OF MELTING-OUT OF KENTUCKY BLUEGRASS AND DEVELOPMENT OF A FUNGICIDE BIOASSAY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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The Ohio State University

1980

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Diseases of turfgrasses.

Taxonomy and nomenclature of Drechslera, Bipolaris, and Exserohilum spp.
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LITERATURE REVIEW

Etiology and Epidemiology of Melting-Out

Melting-out caused by *Drechslera poae* (Baudys) Shoem. (*=Helminthosporium poae* Baudys, *Helminthosporium vagans* Drechs., *Drechslera vagans* (Drechs.) Shoem.) has been recognized as one of the most important diseases of Kentucky bluegrass (*Poa pratensis* L.) in the United States (10,11,12,13,15,24,31,48,59). Melting-out can cause severe problems on Kentucky bluegrass throughout the growing seasons (10). Two distinct phases of melting-out, the leaf spot and crown rot phase, were recognized by Drechsler (12,13). Generally, the leaf spot phase is most prominent during the cool, moist periods in the spring and fall while the crown rot phase predominates during the warm summer months (10,11). However, the severe if sporadic incidence of the crown rot phase has been reported occurring from late April to mid-June rather than later in the summer (24,58). In addition, *D. poae* may also cause severe infections of roots and panicles of Kentucky bluegrass (6,20).

In Minnesota, *Drechslera dictyoides* has been reported as the most important pathogen of Kentucky bluegrass (5,6,18,19,63). However, Morrison (58) failed to isolate *D. dictyoides* from field collections but frequently isolated *D. poae*. Morrison (58) concluded that *D. poae* was misidentified as *D. dictyoides* as a result of misinterpretation of conidium morphology at low temperatures (7-10°C). Therefore
reference to the above mentioned reports (5, 6, 18, 19, 63) will be assumed to relate to \textit{D. poae} and not \textit{D. dictyoides}.

Leaf lesions initially appear as small water-soaked spots which enlarge into purple-brown ovals with tan centers (10, 11, 13). Lesions developing on the crowns of Kentucky bluegrass plants are often diffuse brown discolorations (10, 11). Substantial reductions in leaf density in a Kentucky bluegrass stand are the result of crown lesion girdling the leaf sheaths (10, 11).

\textit{Bipolaris sorokiniana} (Sacc. ex. Sorok.) Shoem. (=\textit{Helminthosporium sorokinianum} Sacc. ex. Sorok.) causes leaf spot lesions on Kentucky bluegrass which closely resemble those produced by \textit{D. poae}. Typically, \textit{D. poae} is most frequently collected from infected plant tissue during the spring and fall while \textit{B. sorokiniana} is almost exclusively isolated during the summer (5, 24, 58, 59). \textit{Drechslera poae} is more common in the Northern states east of the Mississippi which receive ample amounts of rainfall throughout the growing season while \textit{B. sorokiniana} is the predominant pathogen of Kentucky bluegrass in the western half of the country where drought stress is a severe problem (5, 12, 24, 30, 57, 58, 59, 66).

\textit{Drechslera poae} overwintered in lesions on host tissue as well as plant debris in the form of vegetative hyphae or conidia (11, 31). No perfect state of \textit{D. poae} has been identified, but immature ascocarps have been found in culture (11, 22, 31). Conidium production of \textit{D. poae} occurred in the spring and fall when the weather is cool and moist (11, 24). Couch (10) reported that \textit{D. poae} conidia were carried to the leaf surface by splashing water. However, other factors could be involved in the release and dispersal of \textit{D. poae} conidia. Meredith (54) using a
Hirst spore trap situated 1 m above Bermudagrass (Cynodon dactylon) infested with Drechslera gigantea (Heald & Wolf) Ito (-Helminthosporium giganteum Heald & Wolf) noted that the incidence of airborne conidia occurred approximately at 1000 hours. The incidence of airborne conidia was also highest on days following rainfall or very humid nights (54). Rapid infection of leaf and crown tissue was also favored by cool moist weather in the spring and fall (11,24,58). Poor sporulation of D. poae on infested leaves collected during the summer indicates that little inoculum was available during the summer to initiate new infections (24).

Germination of D. poae conidia was rapid on excised Kentucky bluegrass leaves at 24 to 30 C (59). Horsfall (31) observed that at temperatures between 3-35 C, 98% of the D. poae conidia on agar had germinated within 24 hours. At 24 C, germ tube development of D. poae was nearly completed 12 hours after inoculation and only small increases in length were noted (59). Well-differentiated appressoria developed on the tips of approximately 55% of the germ tubes on Kentucky bluegrass leaves within 18 hours of inoculation (59). After 18-24 hours, penetration and primary hyphal development was noted within leaves of common and 'Merion' Kentucky bluegrass (59). Penetration of leaf tissue occurred most frequently at the junctures between epidermal or guard cells but was rare through open stomates (59). Primary hyphae which developed at the site of penetration in the epidermal cell or intercellular space consisted of greatly expanded, thin walled, hyaline and regularly septate filaments (59). Both primary and secondary hyphae were usually restricted to the area of a developing lesion although hyphae may be found in healthy tissue
immediately after penetration (59). No information was available concerning possible toxin production by D. poae in host tissue.

In a moist environment, sporulation of D. poae began within five days of inoculation on excised Kentucky bluegrass leaves (59). Halisky and Funk (24) have reported that D. poae sporulation occurs less frequently on naturally infected leaves collected from May to October. The poor sporulation of D. poae on leaf tissue collected during the warmer months of the year was attributed to the presence of a heat induced metabolite in the plant tissue (24). Colbaugh and Beard (8) reported that Helminthosporium spp. seldom sporulated on infected plant tissue remaining attached to the plant.

Difficulties have been encountered inducing abundant sporulation of D. poae on artificial media (10,13,24,31,49,59). Several authors observed that poor sporulation of D. poae was due to an antisorpulation factor in the media (24,49). Subsequent work has shown that abundant sporulation of D. poae can be induced on artificial media or excised leaf tissue by incubating material at 15-18 C with 12-14 hours of fluorescent illumination followed by 10-12 hours of darkness (22,58,63).

Temperature has a substantial effect on conidium development (58,63). Morrison (58) has illustrated that D. poae conidia formed at 8-10 C were substantially longer and had twice as many septa as conidia formed at 18-20 C. As incubation temperatures increased, conidium length and number of septations continued to decrease (58,63). At temperatures above 21 C, conidiophore development also became atypical with the production of enlarged cells or germ tubes at the conidiophore apex (59,63).
Turf management practices have an influence on the development and severity of melting-out (11). Excessively close clipping of Kentucky bluegrass and application of high rates of nitrogen rich fertilizers have been linked to increases in the severity of melting-out (11,13,19,24,48,51). Colbaugh and Beard (8) have observed substantial increases in the population of *Helminthosporium* spp. conidia on bermudagrass turf maintained at low cutting heights with high fertility levels than on turf maintained at low cutting heights with low fertility levels. However, Gibbs et al (19) have noted that disease development was greater at higher cutting heights than at the lower cutting heights. Conversely, the application of nitrogen to diseased turf has been recommended as a means of offsetting some disease damage (11,16,48,57).

Moisture may also be an important factor influencing the development of melting-out and similar turf diseases (11). Irrigation of 'Merion' Kentucky bluegrass seed fields increases the severity of foliar symptoms as well as the percentage of seeds infested with *D. poae* (20). Colbaugh and Endo (9) using a mineral oil flotation technique developed by Ledingham and Chinn (47), have observed substantial increases in *Bipolaris sorokiniana* conidium production in leaf litter collected from drought-stressed Kentucky bluegrass turf than from irrigated turf. Increases in conidium production was attributed to increases in the saprophytic activity of *B. sorokiniana* caused by substantial releases of nutrients from remoistened leaf litter (9).

Lukens (50) observed that the incidence of melting-out increases proportionally to the amount of shade over Kentucky bluegrass turf. The enhancement of melting-out was correlated with lower carbohydrate
levels in host tissue as a result of shading or nitrogen fertilization (51). Later work indicated that the severity of Helminthosporium - incited diseases was not linked to carbohydrate levels in host tissue (17,19).

Conidium Discharge

A number of techniques and instruments have been developed to collect fungal spores, pollen grains, and other material from air (21). Basically, sampling techniques can be broken down into two categories: gravity sedimentation and inertial methods (21). Sampling techniques involving sedimentation rely on the collection of airborne conidia on horizontal surfaces such as a slide or petri dish which may contain nutrient media or other material (21,28). A number of inertial traps, which operate by drawing air through a jet or tube separating material by centrifugation or impaction of material on rotating surface have been developed (28). The most popular and useful traps for epidemiological studies are automatic volumetric traps (17,28,37,38). Commercially available volumetric spore traps sample either continuously or intermittently (27,37,38). Automatic volumetric spore traps are useful for studying the diurnal periodicity of conidium release exhibited by a number of dry-spore fungi like Drechslera spp. (27).

Airborne conidia of Drechslera, Bipolaris, and Exserohilum generally exhibit a diurnal or circadian periodicity (3,46,54,56). Peak field concentration of D. gigantea, E. turcica, and B. maydis airborne conidia occurs sometime between 0900 and 1100 hours (3,46,54,56). The actual number of airborne conidia of different species trapped will vary considerably during an epiphytotic (46,54,65). The low incidence of D. gigantea and B. maydis conidia may be due to poor
sporulation on host tissue or inability of conidia to remain suspended in air (54,65).

The influence of weather variables on the diurnal periodicity of Drechslera, Bipolaris, or Exserohilum conidium release from conidiophores has been hotly debated (2,48). The debate has centered on whether conidium release is passive or violent (2,4,39,41,43,44, 46,53,54,55,64). Aylor and Day (2) contend that violent release of Helminthosporium spp. conidia is epidemiologically unimportant because the percentage of conidia forcibly released was often quite low (35,53,54). Leach (40) has responded by questioning whether certain important environmental conditions were monitored by Aylor and Day (2). Leach (40) does not discount the epidemiological importance of wind, but notes that wind is just one mode of conidium release which includes rainfall and violent conidium release. The relative importance of each release mechanism changes as weather variables change (40).

Passive release of conidia depends solely on wind velocity and turbulence (1,2). Experiments in wind tunnels or enclosures indicate that puffs of wind of 3–6 m/sec were required to dislodge Bipolaris maydis conidia from conidiophores (4,64). Yet, in the canopy of corn, winds with a mean speed of 1 m/sec liberated approximately 60–75% of B. maydis conidia from corn leaves (3). Closer examination of this phenomenon has revealed that conidium release occurred when puffs of wind reached speeds of 5 m/sec for several microseconds although the mean wind speed was approximately 2 m/sec (4).

Violent release of conidia is triggered by either drastic changes in relative humidity or infrared radiation (39,43,44,53,54). Meredith
(54,55) microscopically observed the release of D. gigantea or E. turcica conidia after rapid desiccation. Forcible conidium release was attributed to mechanical stress as well as production of gases within the conidium and conidiophore after desiccation (53,54,55). Kenneth (35) did not observe violent conidium release of several Drechslera spp. including D. poae after rapid conidium and conidiophore desiccation. Diurnal changes in relative humidity have been linked to the release of E. turcica (46,56). In the laboratory, Leach (39,42,43,44) has illustrated that drastic changes in relative humidity as well as infrared radiation influences the violent release of E. turcica and B. maydis conidia. Further investigation has attributed violent release of E. turcica conidia to the repulsion of unipolarly charged surface of the same polarity (41,45).

Rainfall may also influence the release of dry-spore fungi conidia from plant surfaces (32,39). Jarvis (34) noted massive releases of Botrytis cinerea conidia during heavy showers on calm nights. Conidium release was attributed to percussion waves from impacting raindrops on moldy fruit (29,32). Increases in the number of E. turcica conidia trapped following light showers were noted by Leach (46). Leach (40,46) suggests that release may involve electrostatic forces as well as mechanical forces.

Bioassays of Fungicide Residue Activity

Fungicide residues on plant foliage are constantly reduced due to weathering caused by wind, rain, temperature and light (14). Fungicide retention studies on leaf surfaces were usually done in the greenhouse with artificial rainfall serving as the primary weathering agent (7,26,62). Chemical tests or symptom expression were
used to evaluate fungicide retention rather than the direct effects of the residues on pathogen activity (7,52). Correlations between greenhouse tests failed to account for several weather variables important in field situations (62).

Several bioassays have been developed to evaluate the persistence of fungicide residues on plant foliage in the field (36,60). Neely (60) modified the cellophane transfer technique developed by Neely and Himelick (61) to study the persistence of commercial fungicides on the leaves of 12 woody plants. Leaf disks were collected from treated plants at weekly intervals after fungicide application and placed in Coors porcelain spot plates (60,61). A conidium suspension of *Monilinia fructicola* was applied to a cellophane disk placed over a leaf disk (60). Residue activity was evaluated by microscopically examining each cellophane disk for germinated *M. fructicola* conidia (60). Ko *et al* (36) applied fungicides to the foliage of passion fruit, collected treated leaves at weekly intervals, and inoculated the excised leaves with *Alternaria alternata* conidia. After 24 hours, fungicide retention was evaluated by determining the number of germinated conidia using a vertical illumination microscope (36).

The collodion technique which has not yet been used to evaluate fungicide residue efficacy has several possible advantages over the two bioassays already described (23). Like the preceding tests, pathogens applied to treated host tissue are used to evaluate fungicide efficacy (23). However, fungal structures are embedded in a collodion film which is stained and mounted for later observation rather than requiring immediate attention (23,36,60). This technique
permits not only the monitoring of conidium germination but also
germ tube elongation and appressorium formation, which are important
measures of fungicide efficacy in some instances. Hagan and Larsen
(23) have illustrated that germ tube development and appressorium
formation in addition to conidium germination were critical for
evaluating the efficacy of several turf fungicides. Iprodione
and chlorothalonil, which provided excellent disease control did not
inhibit Bipolaris sorokiniana conidium germination on Kentucky blue-
grass leaves (23). However, both materials did inhibit germ tube
development and suppressed appressorium formation (23).


Chapter I

MELTING-OUT OF KENTUCKY BLUEGRASS: SOURCE OF INOCULUM RELEASE, AND DISPERSAL OF DRECHSLERA POAE CONIDIA
ABSTRACT

Populations of *Drechslera poae* conidia in the thatch and leaf litter of Kentucky bluegrass turf were monitored in 1979 and 1980. Results indicate that leaf litter and not thatch is the primary source of inoculum in the spring. Peak populations in the leaf litter coincided with periods of heavy *D. poae* conidium release as well as substantial increases in diseases incidence on Kentucky bluegrass in May and June of 1980. During this period, thatch temperatures ranged from 6 to 20 C with peak conidium production occurring at 9 to 18 C. Moisture in the form of rainfall or heavy 8-12 hour dew periods were plentiful during this period. Low temperatures and sporadic rainfall limited conidium populations in April or early May. Sporulation in leaf litter ceased in early July, 1980 as the mean daily thatch temperature exceeded 20 C.

The diurnal release of *D. poae* conidia peaked between 1200 and 1400 hours. The incidence of airborne conidia began to increase at 0800 and substantial numbers of conidia were collected through 2000 hours. Few conidia were trapped in the late evening or early morning. Discharge of conidia was associated primarily with reductions in the moisture levels in turf microenvironment while rainfall and wind were of minor importance. Negligible releases of conidia were noted during periods of prolonged leaf wetness or high humidity.
The incidence of the leaf spot and crown rot phases of melting-out peaked in May and June when inoculum was plentiful. During the summer in 1979, disease severity remained high.

In September, a rapid decrease in disease incidence was noted when conditions for rapid plant growth were favorable. The low incidence of disease was reflected in the low population of D. poae conidia in leaf litter and generally low level of D. poae conidium discharge.

**INTRODUCTION**

Melting out which is caused by Drechslera poae (Baudys) Shoem. (=Helminthosporium poae Baudys, Helminthosporium vagans, Drechs., and Drechslera vagans (Drechs.) Shoem.) is an important disease of Kentucky bluegrass throughout the United States (6, 7, 8, 9, 10, 11, 13, 14, 33, 34). Two distinct phases of melting-out generally develop during the growing season. The leaf spot phase, which is most visible develops in the cool, humid spring and fall, while the crown rot phase is most prominent during the summer (6, 7). Although the appearance of the crown rot phase was often sporadic in Minnesota, Morrison (33) noted that the crown rot phase was most severe in the late spring rather than during the summer.

Drechslera poae overwinters on infected plant material or leaf litter as vegetative hyphae or conidia (6, 7, 14, 16). The primary source of inoculum, i.e., D. poae conidia, throughout the year may be thatch, leaf litter or lesions on diseased Kentucky bluegrass plants (3, 7). Colbaugh and Beard (4) have found that sporulation of Helminthosporium spp. is poor on intact plants but may be quite heavy on leaf litter.
Maintenance practices such as irrigation, mowing height and rate of fertilization may have a significant influence on the population of *Helminthosporium* spp. conidia in bermudagrass leaf litter (3,4). The availability of moisture in Kentucky bluegrass leaf litter also has a profound effect on the saprophytic and parasitic activity of *Bipolaris sorokiniana* (*=Helminthosporium sorokinianum*) (5). Sporulation of *B. sorokiniana* was 20 to 30X higher on Kentucky bluegrass leaf litter in drought stressed areas than in areas receiving adequate moisture (5).

Little information concerning the release and dispersal of airborne conidia of any turfgrass pathogen was available. Couch (6, 7) states that *D. poae* conidia were splashed onto the foliage by rain but no data exists to back this assertion. Meredith (30) observed the diurnal periodicity of *Drechslera gigantea* (*=Helminthosporium giganteum*) over bermudagrass turf. Peak concentration of airborne *D. gigantea* conidia occurred at approximately 1000 hours (30). The incidence of airborne conidia of *Bipolaris maydis* (*=Helminthosporium maydis*) and *Exserohilum turcica* (*=Helminthosporium turcicum*) also occurred between 0900 and 1100 hours (1, 25). Meredith (30) also observed that the incidence of airborne *D. gigantea* was highest on dry days following rainfall.

Periods when the incidence of airborne *Helminthosporium* conidia was highest coincide with substantial changes in relative humidity, temperature or wind velocity (1, 25, 30). Research has shown that changes in relative humidity, infrared radiation, and wind velocities of 3-6 m/sec influence the removal of conidia from conidiophores.
The objective of this research was to evaluate the influence of weather variables on the development of the leaf spot and crown rot phase of melting-out of Kentucky bluegrass caused by *Drechslera poae*. Emphasis was placed on determining the site of conidium production and the incidence of airborne *D. poae* conidia within a field of Kentucky bluegrass throughout the growing season.

**MATERIALS AND METHODS**

**Conidium trapping:** Airborne conidia of *Drechslera poae* were collected with a Kramer-Collins intermittent band spore sampler (G-R Electric Manufacturing Co., Manhattan, KS) or a Burkard 7 day continuous spore sampler (Burkard Scientific Ltd., Rickmansworth, Hertfordshire, England).

The Kramer-Collins spore trap was adjusted to operate approximately 23 min/hr at a flow rate of 35 l/hr through an orifice 15 cm above the ground. Particulate matter was deposited on a glass slide treated with WD-40 (WD-40 Company, San Diego, CA) penetrating oil. Samples were collected with the Kramer-Collins trap from April 10 to July 10 plus August 27 through November 12 in 1979 and from March 17 to July 10, 1980. Slides were changed daily, stained with 0.5% cotton blue in lactophenol and examined for the presence of *D. poae* conidia.

The Burkard spore trap operated at a flow rate of 10-11 liters of air/min with the orifice at 30 cm above the turf. Conidia were collected on petroleum jelly coated plastic trap tapes. Weekly samples were collected with the Burkard trap from July 27 to November 2, 1979 and April 1 to July 14, 1980. Tapes collected from the trap were cut into 48 mm strips, mounted on glass slides, stained with 0.5%
cotton blue in lactophenol, and examined microscopically at 100X magnification.

Both traps were installed in or around a 1.5 m² enclosure in an 11 acre common Kentucky bluegrass field naturally infested with *Drechslera poae* at the ChemLawn Research Facility in Milford Center, Ohio. The spore traps were situated around the enclosure to take advantage of the prevailing west-southwest winds. The bluegrass was maintained at a height of 3.8 to 5.1 cm, irrigated when necessary, and treated with broadleaf herbicides. The 11 acres of Kentucky bluegrass were not maintained at uniform fertility levels.

**Meterological equipment:** A hygrothermograph (Weather Measure Corp., Sacramento, CA) anemometer (Weather Measure Corp.), leaf wetness meter (28), pyranograph (Weather Measure Corp.) and recording rain gauge (Meterology Research, Inc., Altadena, CA) were installed in the enclosure containing the spore traps. Thatch, ambient air, and soil thatch temperature were monitored with t-type thermocouples, which were placed in Kentucky bluegrass turf 50 m from the enclosure and wired to a multipoint recorder (Leeds and Northrup Corp., North Wales, PA). All equipment except the pyranograph and leaf wetness meter were installed on April 1, 1979 and removed on November 2, 1979. The pyranograph and leaf wetness meter were installed in September and October, 1979, respectively. In 1980, all instruments were operational on March 11, 1980, except the leaf wetness meter which was installed on April 19, 1980. Collection of weather data was terminated on July 14, 1980.

**Inoculum source:** Two thatch samples were collected bi-weekly from March 26 to November 21, 1979 and March 17 to July 10, 1980 with
a 2.5 cm soil probe from a 95 m² plot divided into 64 subplots.

Leaf litter samples were collected using a clawed hand tool along with the thatch samples from 4 sub plots from August 16 to November 21, 1979 and from March 15 to July 10, 1980. Before processing, each sample was air dried under a bank of fluorescent lights.

The number of *Drechslera poae* and *Bipolaris sorokiniana* conidia in the thatch and leaf litter samples determined using the mineral oil flotation technique developed by Ledingham and Chinn (27) and further modified by Colbaugh (25). Ten grams (dry weight) of thatch or five grams (dry weight) of leaf litter were placed in a flask containing 100 ml distilled water. The flask was shaken vigorously for 3 minutes and the soil-water suspension was filtered through a 200 mesh screen to remove debris. The filtrate was poured into a graduated cylinder, brought to a volume of 100 ml distributed into 25 ml aliquots and centrifuged at 480 xg. The supernatant was discarded and the pellet was mixed with 5 ml of light mineral oil. Approximately 20 ml of double distilled water was emulsified with the mineral oil-soil suspension with a vortex mixer. The emulsion was poured into a glass petri dish and allowed to settle. A total of four plates were prepared from each 10 g thatch or 5 g leaf litter sample. Six fields at 60X magnification of each plate were examined with a dissection microscope and the number of *D. poae* conidia was recorded. The area of the microscope field and petri plate were calculated, and the total number of conidia in the four plates totaled. The population of *D. poae* conidia/g dry weight of sample was calculated by dividing the total number of conidia extracted by the weight of the sample (5
or 10 g). *Drechslera poae* conidia, as well as, those of several *Bipolaris* spp. were found at the oil-water interface rather than floating in the mineral oil as noted by Ledingham and Chinn (27).

**Disease incidence:** The incidence of the crown rot and leaf spot phase of melting out was monitored weekly from March through November in 1979 and March through July, 1980. Disease incidence was expressed as a percentage of the 25 plants inspected displaying disease symptoms.

**RESULTS**

Populations of *D. poae* in the thatch of Kentucky bluegrass turf were quite low and never approached the populations in the leaf litter (Figures 1.1-1.3). Never more than 35 conidia/g dry weight thatch were detected in any thatch sample collected in 1979 or 1980 (Figures 1.1-1.3). Although variable, populations of conidia in thatch were higher in the spring of 1979 than in the summer or fall (Figures 1.1, 1.2). In 1980, populations did not exceed 20 conidia/g dry weight thatch throughout the spring and dropped to zero by July (Figure 1.3).

**Inoculum source:** Leaf litter consisting of decomposing Kentucky bluegrass leaf fragments was apparently the primary source of *Drechslera poae* conidia in the field (Figures 1.2, 1.3). In 1979, no conidia were detected in leaf litter collected from August through most of October (Figure 1.2). A sharp increase in the population of *Drechslera poae* conidia was noted in a leaf litter sample collected in late October but the population of conidia immediately dropped to near zero in early November (Figure 1.2). High populations of *D. poae* in 1980 were noted in leaf litter collected from mid-May through late June (Figure 1.3). Conidium populations in leaf litter from March to May were highly
Figure 1.1 Populations of *Drechslera poae* conidia in Kentucky bluegrass thatch from March 26 to July 16, 1979.
Figure 1.2 Populations of *Drechslera poae* conidia in Kentucky bluegrass thatch and leaf litter from July 25 to November 2, 1979.
Conidia/g dry weight substrate

For the years 1979:

- Thatch
- Leaf litter

Data points:

- 7/25
- 7/31
- 8/7
- 8/14
- 8/21
- 8/28
- 9/4
- 9/10
- 9/17
- 9/24
- 10/1
- 10/8
- 10/15
- 10/22
- 11/2

Graph shows the conidial count per gram of dry weight substrate over time from July 25 to November 2, 1979.
variable (Figure 1.3) but lower than populations in May and June (Figure 1.3).

Thatch temperatures apparently had an important impact on the population of \textit{D. poae} conidia in Kentucky bluegrass leaf litter during the spring 1980. Populations of 400 to 700 \textit{D. poae} conidia/g dry weight leaf litter were observed at mean thatch temperatures between 9 to 18 \textdegree{}C while populations between 0 to 300 conidia/g dry weight leaf litter were noted at 5 to 9 \textdegree{}C (Figure 1.3, 1.4). Populations of conidia in leaf litter dropped to nearly zero as the mean temperature exceeded 20 \textdegree{}C in July (Figures 1.3, 1.4). Moisture in the form of rain and dew was plentiful during May and June (Figure 1.4). Substantial increases in \textit{D. poae} populations in leaf litter were even noted during the dry period from mid April through early May. Quite possibly, heavy dews during this period provided adequate moisture for the initiation of \textit{D. poae} conidium production.

Note: Information presented in Figure 1.5 to 1.9 concerning conidium formation and discharge is representative of the data collected in 1979 and 1980.

Factors influencing sporulation of \textit{Drechslera poae} in the field: It was difficult to pinpoint the minimal period of leaf wetness required for sporulation in this study. The period of leaf wetness of Kentucky bluegrass leaves, sheaths, or leaf litter varied up to several hours per day. Sporulation often followed periods of rainfall or irrigation (Figure 1.6-1.9). Moderate releases of \textit{D. poae} conidia on May 13 and 21, 1980, were preceded by rainfall which kept plants and leaf litter wet at least 36 hours prior to conidium release (Figure 1.6, 1.7).
Figure 1.3 Populations of *Drechslera poae* conidia in Kentucky bluegrass thatch and leaf litter from March 17 to July 10, 1980.
Figure 1.4 Summary of weekly total rainfall and mean thatch temperature from April 4 to July 10, 1980 at the Chemlawn Research Facility, Milford Center, Ohio.
Mean thatch temp.

Temperature

Rainfall

1980

Weekly rainfall cm
Approximately 1.3 cm of irrigation water was applied to the Kentucky bluegrass ranges at the ChemLawn Research Center on the afternoons of May 15 and June 13, 1979 (Figures 1.8, 1.9). Nearly 48 hours later, on May 17 and June 15, moderate releases of *D. poae* conidia were noted (Figure 1.8, 1.9).

In the fall of 1979, conditions were generally favorable for sporulation yet populations of conidia in the thatch were low and few airborne conidia were collected with the spore traps (Figures 1.2, 1.3). Excessive moisture in the leaf litter may have either suppressed sporulation of *D. poae* on leaf litter or prevented release of *D. poae* conidia.

**Factors influencing the release of *D. poae* conidia:** Release of *D. poae* was influenced by drying of leaf surfaces, wind and possibly irrigation. Several examples of each release pattern will be discussed.

Depletion of moisture in the turf microclimate which can be illustrated by sharp decreases in the relative humidity or drying of plant surfaces was associated with *D. poae* conidium release (Figures 1.5, 1.6). Discharge of *D. poae* conidia generally followed the drying of sheath surfaces or substantial drops in relative humidity (Figures 1.5, 1.6, 1.8). Few airborne conidia were collected on days when the duration of leaf wetness exceeded 20 hours per day (Figures 1.6, 1.7).

Conidium release often occurred during the time of day when the wind velocity was approximately 0.5 to 2.0 m/sec 15 cm above the turf (Figures 1.5-1.7). On May 13, 1980, at 2000 hours, the peak incidence of airborne *D. poae* conidia occurred when the wind velocity reached 5.8 m/sec (Figure 1.7).
Figure 1.5 Influence of weather variables on the production and release of *Drechslera poae* conidia within a Kentucky bluegrass field from June 11-13, 1980 (LW=leaf wetness).
Figure 1.6 Influence of weather variables on the production and release of *Drechslera poae* conidia within a Kentucky bluegrass field from May 20-22, 1980 (LW=leaf wetness, WS=wind velocity).
Figure 1.7 Influence of weather variables on the production and release of *Drechslera poae* conidia within a Kentucky bluegrass field from May 12-14, 1980 (LW=leaf wetness).
D. poae conidia release increased substantially following periods of irrigation. Substantial number of D. poae conidia were collected when 1.3 cm of water was applied to the Kentucky bluegrass turf at 1100 hours on May 16, 1979 (Figure 1.9). Simultaneously, a large decrease in relative humidity was also noted (Figure 1.9). The release of D. poae conidia did not increase following rainshowers in 1979 or 1980.

Diurnal release of D. poae conidia: The incidence of airborne D. poae conidia was much higher during daylight hours (0800 to 2000 hours) than at night (2000 to 0800 hours) (Figures 1.10, 1.11). Collection of D. poae conidia which often began between 0800 and 1000 was associated with rapid changes in moisture levels in the turf microclimate in the morning, wind velocities in excess of 2 m/sec, or in several cases irrigation (Figures 1.5-1.11). In 1979 and 1980, peak concentration of D. poae airborne conidia occurred between 1200 to 1400 hours (Figures 1.10, 1.11). Substantial numbers of conidia were trapped between 0800 and 1800 hours (Figures 1.10, 1.11). Discharge of D. poae conidia was low between 2000 and 0800 hours, when leaf surfaces and leaf litter were wet and wind velocities quite low (Figures 1.5-1.11).

Seasonal distribution of airborne Drechslera poae conidia: The incidence of airborne D. poae conidia was highest in the spring when ambient air and thatch temperatures were generally mild and moisture was readily available (Figures 1.4, 1.12, 1.14). In 1979, peak incidence of 30-80 airborne D. poae conidia m$^3$/day was noted between May 14 and 28 and June 4 to 24 (Figure 1.12). In late April and early
Figure 1.8 Influence of weather variables on the production and release of *Drechslera poae* conidia within a Kentucky bluegrass field from June 13-15, 1979.
Figure 1.9 Influence of weather variables on the production and release of *Drechslera poae* conidia within a Kentucky bluegrass field from May 15-17, 1979.
Figure 1.10 Diurnal periodicity of airborne Drechslera poae conidia collected within a Kentucky bluegrass field with a Kramer-Collins spore trap from April 17 to November 2, 1979.
Figure 1.11  Diurnal periodicity of airborne Drechslera poae conidia collected within a field of Kentucky bluegrass with a Burkard spore trap from April 1 to July 6, 1980.
1980 (April 1-July 6)
May, the total number of conidia collected was lower than levels noted in June (Figure 1.12). The incidence of airborne conidia remained low from July to November ranging from 8-40 airborne conidia m$^3$/day (Figure 1.13).

In 1980, the spore traps were fully operational several weeks earlier than in the spring of 1979 (Figures 1.12, 1.14). With the exception of the period between April 7 to 13, the incidence of airborne D. poae conidia was quite low in April and early May (Figure 1.14). Number of airborne conidia increased between May 12 to 18 to 100-120 D. poae conidia/m$^3$/wk (Figures 1.4, 1.14). Peak concentration of 150-300 airborne D. poae conidia/m$^3$/wk were noted between June 2 and 29 with moderate concentrations being recorded into early July (Figure 1.14). The incidence of airborne D. poae dropped sharply in mid-July to nearly zero as temperatures in the turf microenvironment regularly exceeded 20 C (Figures 1.4, 1.14). Collection of D. poae conidia with the Burkard spore trap was interrupted between June 19 and June 25.

**Disease incidence:** In 1980, a close relationship between disease incidence and periods of conidium release was observed. Lesion development was observed 5 to 14 days after a substantial conidium release (Figures 1.14, 1.16). For example, on April 7 a cloud of D. poae conidia with a density of 8 to 33 conidia/m$^3$ was detected for several hours. Approximately 14 days later, substantial increases in the incidence of the leaf spot and crown rot phase of melting out were recorded. Lesion formation was faster during May and June as temperatures in the turf microclimate increased (Figure 1.16). In mid-May, substantial increases in disease incidence occurred within 5-7 days of moderate conidium releases (Figures 1.14, 1.16).
Figure 1.12 Seasonal distribution of airborne Drechslera poae conidia collected within a field of Kentucky bluegrass with a Kramer-Collins spore trap from April 22 to July 29, 1979 (ND=no data).
Conidia/m³

10 20 30 40 50 60 70 80


1979

ND ND
Figure 1.13 Seasonal distribution of airborne Drechslera poae conidia collected within a field of Kentucky bluegrass with a Kramer-Collins and Burkard spore traps from July 30 to November 2, 1979 (ND=no data).
Figure 1.14 Seasonal distribution of airborne *Drechslera poae* conidia collected within a field of Kentucky bluegrass with a Burkard spore trap from March 31 to July 14, 1980 (Note: collection of conidia was interrupted between June 19 to June 25).
Figure 1.15 Incidence of leaf spot and crown rot phases of melting-out of Kentucky bluegrass caused by *Drechslera poae* in 1979. Disease incidence was expressed as the percentage of 25 plants inspected weekly for the presence of the leaf spot or crown rot phases of melting-out.
Disease incidence

Leaf spot

Crown rot

4/1 4/15 5/1 5/15 6/1 6/15 7/1 7/15 8/1 8/15 9/1 9/15 10/1 10/15 11/1 11/15

1979
Figure 1.16 Incidence of the leaf spot and crown rot phases of melting-out of Kentucky bluegrass caused by *Drechslera poae* from March 24 to July 10, 1980. Disease incidence was expressed as the percentage of 25 plants inspected weekly for the presence of the leaf spot or crown rot phases of melting-out.
A poor relationship was noted between disease incidence and the seasonal incidence of airborne *D. poae* conidia in the spring of 1979 (Figures 1.12, 1.15). Substantial increases in disease incidence were observed in April despite the absence of airborne *D. poae* conidia. Disease incidence did continue to increase when airborne conidia were plentiful in May and June (Figures 1.12, 1.15). Only small decreases in the incidence of the leaf spot and crown rot phases of melting out occurred during July and August when the plants were generally dormant. Sharp decreases in disease incidence were noted in September through November when inoculum levels were low (Figures 1.13, 1.15).

It is important to note that the development of the leaf spot and crown rot phase was simultaneous through the spring of 1979 and 1980 (Figures 1.15, 1.16). The crown rot phase was quite prominent and often serious during the spring and early summer in 1979 and 1980 but severe melting-out was never observed. Evidently, environmental conditions favorable for the development of severe melting-out of Kentucky bluegrass were never present during 1979 or 1980.

**DISCUSSION**

Kentucky bluegrass leaf litter was the most important source of inoculum in the spring, while thatch and lesions on diseased leaves were minor sources. High populations of conidia in leaf litter coincided with peak concentrations of airborne *D. poae* conidia and preceded substantial increases in the incidence of the leaf spot and crown rot phases of melting out (Figures 1.13, 1.15, 1.16). Colbaugh and Beard (4) and Colbaugh and Endo (5) have noted that conidium production of *Helminthosporium* spp on bermudagrass leaf litter and *B. sorokiniana* on
Kentucky bluegrass leaf litter was also quite heavy. Populations of *D. poae* which peaked between 600-700 conidia/g dry weight leaf litter did not approach the population levels of *Helminthosporium* spp. noted by Colbaugh and Beard (4) in leaf litter from tall fescue, bermudagrass, and St. Augustinegrass turf in Texas.

Couch (7) noted that thatch, i.e., crowns, roots and rhizomes of Kentucky bluegrass plants, in addition to leaf litter served as a site of inoculum production. In this study even during periods of peak conidium populations in Kentucky bluegrass leaf litter, populations of *D. poae* conidia in thatch were quite low (Figure 2.3). Conidia of *D. poae* found in the thatch probably were deposited there after being dislodged from leaf litter.

Intact diseased plants were not evaluated as a source of inoculum in the field. Colbaugh and Beard (4) observed that sporulation of *Helminthosporium* spp. rarely occurs on plants in the field. Halisky and Funk (14) observed poor *D. poae* sporulation on lesions on Kentucky bluegrass leaves collected between May and July, periods of peak *D. poae* airborne conidium incidence (Figures 1.12, 1.14). Laboratory studies have indicated that sporulation of *D. poae* is 4 to 10 times higher on excised diseased leaves than on diseased leaves collected from intact plants (Table 2.2).

It is difficult to pinpoint in this study the duration of free moisture required for *D. poae* conidium formation on infested plant material in the field. Heavy releases of *D. poae* conidia were observed following periods of rainfall or heavy dews when the leaf litter remained continuously moist for at least 36 hours (Figures 1.6, 1.7). Laboratory results confirm field observations which indicate that
24 to 36 hours of continuous free moisture are required for conidiophore development and conidium formation on excised Kentucky bluegrass leaves (Chapter II).

Conidium formation was not limited to periods of continuous free moisture in excess of 24 hours. Sporulation of *D. poae* also occurred during dews of 8 to 10 hours duration on several successive nights (Figure 1.5). The number of dew periods on successive evenings required for conidium production is unknown. Leach et al (26) noted that the presence of free moisture, i.e., dew or rainfall, for 10 to 12 hours on corn leaves at night favored *Exserohilum turcica* conidium formation. However, *E. turcica* often produces successive crops of conidia for several days on the same conidiophores thereby reducing the time required for the production of conidia on corn leaves. Production of successive crops of *D. poae* conidia on mature conidiophores may not occur. Competition between *D. poae* and other saprophytes on leaf litter probably limits conidium production.

The level of *D. poae* sporulation in the field which reflected by the conidium population in leaf litter was heaviest at mean thatch temperatures of 9 to 18 C (Figures 1.3, 1.4). Below 9 C, sporulation on leaf litter was light. As the daily mean thatch temperatures exceeded 20 C, sporulation of *D. poae* rapidly decreased in leaf litter.

Field and growth chamber data concerning influence of temperature on *D. poae* sporulation were in agreement. Sporulation of *D. poae* on excised Kentucky bluegrass leaves peaked at 12 and 18 C but was light at 6 C (Figure 2.10). Vargas and Wilcoxson (35) also noted heavy *D. poae* sporulation at 15 and 18 C *in vitro*. Sporulation of *D. poae* began to decrease at 24 C on excised leaves (Figure 2.10).
However, poor sporulation was noted by Vargas and Wilcoxson (35) at 21 °C.

Melting-out of Kentucky bluegrass has been recognized as a major disease throughout the growing season in the northern United States (6, 7, 14). Usually, the leaf spot phase developed in the spring and fall while the crown rot phase predominates during the summer (6, 7). In the spring of 1979 and 1980 simultaneous increases in the incidence of the leaf spot and crown rot phases of melting-out were noted (Figures 1.15, 1.16). Disease development followed increases in the population of *D. poae* conidia in leaf litter and incidence of airborne *D. poae* conidia. Little increase in disease incidence was observed from late May to early June when inoculum was plentiful because leaf and crown lesions were found on nearly all Kentucky bluegrass plants examined.

Severe melting-out of Kentucky bluegrass did not develop during the summer of 1979 or 1980. Although close inspection of Kentucky bluegrass plants in July and August did reveal that some were severely diseased, overall turf quality was good. Decreases in disease incidence were small in 1979 but quite large in July 1980 (Figures 1.15, 1.16). Morrison (33) noted that the occurrence of melting-out, though sporadic, was most severe in the late spring rather than during the summer.

In the fall of 1979, environmental conditions were ideal for disease development yet sporulation of *D. poae* and disease incidence were low. Other authors have noted the development of the leaf spot phase in the fall (6, 7, 14). It is quite possible that heavy rainfall throughout the fall suppressed conidium formation. Langenberg et al
have observed that prolonged periods of rain or wet leaves, cool temperatures, and high wind velocities inhibited sporulation of *Alternaria dauci* on carrot. Sporulation of *B. sorokiniana* was substantially higher on Kentucky bluegrass leaf litter from drought stressed turf than from turf receiving adequate moisture (5). Evidently alternating wet-dry cycles favored heavy sporulation of *B. sorokiniana* (5), and it is quite possible that the same pattern of wet-dry cycles influenced sporulation of *D. poae* on leaf litter.

The discharge of *D. poae* conidia follows a diurnal pattern with peak concentrations of airborne conidia occurring between 1200 and 1400 hours. This peak is several hours later than those reported for *D. gigantea* and *E. turcica* (25, 30, 32). The incidence of airborne conidia begins to rise at 0800 and discharge of conidia often continued until 2000 hours (Figures 1.10, 1.11). Few conidia were collected at night or on days when wind velocity was low and moisture levels high.

Release of *D. poae* conidia was associated with substantial drops in the level of moisture, i.e., relative humidity or leaf wetness. Discharge of conidia followed sharp drops in the ambient relative humidity by 1 to 2 hours on a number of occasions (Figures 1.5-1.9). A close relationship between conidium release and evaporation of surface moisture from plant surfaces and leaf litter was noted in 1980. Discharge of *D. poae* conidia may have been delayed until late afternoon on days following rain showers because of excessive moisture levels in leaf litter. Meredith (32) and Leach et al (25) have observed the importance of changes in the relative humidity and evaporation of free moisture on leaf surfaces as causes of conidium release. Release of *D. poae*
conidia was negligible during prolonged periods of high relative humidity or leaf wetness which occurred on rainy or cloudy days, as well as, at night. Prolonged periods of high relative humidity or leaf wetness had a similar influence on the release of _E. turcica_ and _D. gigantea_ (25, 32).

Two theories have been developed to explain the moisture related violent discharge of _E. turcica_ and _D. gigantea_ conidia (22, 24, 30, 31). Meredith (30, 31) attributed the forcible discharge of _E. turcica_, and _D. gigantea_ conidia to mechanical stress as well as production of gases within the conidium and conidiophore after desiccation. Leach (22, 24) demonstrated that forcible discharge of _E. turcica_ conidia is caused by the repulsion of unipolarly charged conidia from a charged surface of the same polarity.

It is difficult to evaluate the role of wind on _D. poae_ conidium discharge. Although light winds with velocities of 0.5 to 2.0 m/sec are sufficient to keep large conidia such as _D. poae_ airborne, it is questionable whether light winds could dislodge conidia from conidiophores (12).

Some research has centered on determining the wind velocity required to removed _Bipolaris maydis_ conidia from corn leaves (1, 2, 36). Waggoner (36) noted that wind velocities of 3 to 7 m/sec were required for substantial release of _B. maydis_ conidia from corn leaves in a wind tunnel. Further work in the laboratory by Aylor and Parlange (2) has shown that winds of less than 2 m/sec/hr removed significant numbers of _B. maydis_ conidia from corn leaves. During very brief periods of time, the velocity of wind puffs exceeded 4.5 m/sec over the conidium bearing surface although the mean wind velocity did not exceed
2 m/sec/hr (2). Aylor and Lukens (1) observed substantial releases of *B. maydis* conidia from corn leaves in the field at 0.5 to 1.4 m/sec. Since *B. maydis* conidia were collected between 0900 and 1100 hours, conidium release may have been caused by abrupt changes in ambient relative humidity which generally occurs at this time of day (1). The question of the influence of wind velocity on *D. poae* conidium discharge must be answered using wind tunnel studies rather than relying solely on field data.

Discharge of *D. poae* conidia was not observed during light showers or heavy thunderstorms although light irrigation may cause conidium release. Rapid washout of airborne *D. poae* conidia during rain showers may have prevented detection of significant discharges of conidia. Meredith (32) also noted that the incidence of airborne *E. turcica* conidia was quite low during periods of rainfall. However, later studies have indicated that rainfall may trigger significant releases of *E. turcica* conidia (25).

Little effort has been made to study the epidemiology of netting-out (6, 7, 13, 14). This study certainly does not provide answers to all epidemiologically-related questions but it does provide the groundwork for further research. Further work should be directed at identifying the influence of chemicals such as fertilizers, herbicides, and fungicides on population dynamics of *D. poae* in the turf micro-environment as well as further refining of techniques used for collecting and analyzing field data.
Melting-out is controlled primarily by using a preventive fungicide spray program in combination with cultural control procedures in the spring and fall. Integration of field data concerning environmental influences on D. poae population and disease development into preventive fungicide programs will allow the turf manager to improve the timing of fungicide applications. The ultimate objective of this research program is the development of a program integrating cultural and chemical control measures for effective control of melting-out of Kentucky bluegrass.
LITERATURE CITED


Chapter II

INFLUENCE OF TEMPERATURE ON DRECHSLERA POAE ACTIVITY AND LESION DEVELOPMENT ON KENTUCKY BLUEGRASS LEAVES
ABSTRACT

Pots of Kentucky bluegrass plants were inoculated with a suspension of *D. poae* conidia and incubated at 6, 12, 18, 24, and 30 C. Fungal pre-penetration events were monitored 2, 4, 6, 8, 13, 16, 24, 32, and 48 hours after inoculation using the collodion leaf impression technique. Penetration and colonization of leaves was observed in cleared tissue. Conidium germination began 4 hours after inoculation at all temperatures. Initially, the rates of conidium germination, germ tube elongation, and appressorium formation were often slower than the rate of development at 18 or 24 C but few differences were noted 48 hours after inoculation. Although the number of successful penetrations 24 hours after inoculation was relatively high at all temperatures, ramification of primary and secondary hyphae was poor at 6 and 30 C. Lesion expansion which was rapid at 12 to 24 C and poor at 6 and 30 C reflected the rate of hyphal development in host tissue. Conidium production of *D. poae* on excised leaves is rapid and profuse at 12 to 24 C but sparse at 6 and 30 C. The level of *D. poae* sporulation is much higher on excised leaves than on intact leaves from Kentucky bluegrass plants.

INTRODUCTION

Development of melting-out on Kentucky bluegrass caused by *Drechslera poae* (Baudys) Shoem. (*Helminthosporium poae* Baudys, *Helminthosporium vagans* Drechs., *Drechslera vagans* (Drechs,)Shoem.) is greatly influenced by temperature (3, 4, 6, 7). The leaf spot phase of melting-out is usually observed in the spring and fall while the crown
rot phase is often most severe in the late spring and summer (3, 4, 9, 16, 17, 18). Field observations of leaf spot and crown rot lesion development indicate that inoculum production and subsequent infections of bluegrass plants are favored by the cool moist conditions in the spring and fall (3, 4, 9). The activity of D. poae in plant tissue is not limited to cool temperatures. Bean and Wilcoxson (1) noted moderate reductions of 'Park' Kentucky bluegrass seedling stands inoculated with D. poae at temperatures up to 29 C.

There has been some confusion concerning the identity of the Drechslera species causing leaf spot and crown rot of Kentucky bluegrass in the midwest. Bean and Wilcoxson (1) reported that D. dictyoides and not D. poae was the most important pathogen of Kentucky bluegrass in Minnesota. As a result of his failure to isolate D. dictyoides from Kentucky bluegrass, Morrison (17) studied the etiology of melting-out of Kentucky bluegrass. Morrison (17) concluded that D. poae had been misidentified as D. dictyoides by Bean and Wilcoxson (1) and Vargas and Wilcoxson (21). Therefore, reference to the above mentioned report (1, 21) will be assumed to relate to D. poae rather than D. dictyoides.

Conidium germination of D. poae on nutrient media or leaf tissue was quite rapid (10, 18). Within 18-24 hours of inoculation at temperatures from 3 - 35 C, 85-98% of the D. poae conidia examined had germinated (10, 18). Germ tube development was nearly completed within 18 hours on tips of 55% of the germ tubes on bluegrass leaves at 24 to 30 C (18). Halisky and Funk (9) noted that penetration by D. poae of a susceptible Kentucky bluegrass cultivar 'Newport' occurred within 6 hours of inoculation while penetration of the resistant cultivar 'Dwarf' was delayed 12 hours after inoculation. Development of primary hyphae of D. poae
was noted in the substomatal cavities or epidermal cells of either susceptible or resistant Kentucky bluegrass cultivars by Mower (18) 18 to 24 hours after inoculation. The greatly expanded globose, hyaline, thin walled, branched, and septate primary hyphae were usually restricted to epidermal cells in contact with the penetration peg while thin walled, sparingly branched, and irregularly septate secondary hyphae began developing in the intercellular spaces near the point of infection 24-36 hours after inoculation (18).

A number of studies concerning the sporulation of *D. poae* have touched on the difficult problem of inducing sporulation on artificial media (7, 9, 10, 14, 17, 20). A review of this work is unnecessary since an excellent discussion has been prepared by Morrison (17). The influence of temperature on conidium production of *D. poae* has been addressed by several authors (9, 17, 21). Halisky and Funk (9) have reported that *D. poae* readily sporulated on naturally infected leaves collected from November to April but rarely on leaves collected between May and October. Poor sporulation of *D. poae* on leaves collected during the warmer seasons has been attributed to the presence of an unidentified heat-induced metabolite in host tissue (9). Colbaugh and Beard (2) have stated that sporulation of *Helminthosporium* spp. rarely occurs on intact plants in the field. On SAYA agar plugs, Vargas and Wilcoxson (21) observed that sporulation of *D. poae* was generally good at 15-18°C but quite poor above 21°C. Sporulation of *D. poae* was not observed above 27°C (21).

Ambient temperature at the time of conidium formation has an influence on conidium development (17). Conidium length as well as the number of septations in a *D. poae* conidium are greatest at 8-10°C but
as temperatures increased, the length and number of septations decreased (17, 21). At temperatures above 21 C, atypical swellings of conidiophore cells and germination of apical conidiophore cells have also been observed (17, 21).

Field studies have raised questions concerning the influence of temperature on Drechslera poae and disease development which have yet to be answered. The objective of this project was to study the influence of temperature on the behavior of D. poae on leaf surfaces, penetration and colonization of leaf tissue, lesion enlargement and sporulation on host tissue.

MATERIALS AND METHODS

Plant cultivation: 'Park' Kentucky bluegrass (Poa pratensis L.) was seeded at a rate of 80 to 100 seeds/pot in 8.8 cm styrofoam cups filled to within 1 cm of the top with a 1:1:1 steamed soil, sand, and vermiculite potting mix. Pots of plants were maintained in the greenhouse, routinely clipped to 10 to 15 cm and treated with 200 mg/L of a 15-15-15 water soluble fertilizer. Pots of plants were 6-8 months old when used in experiments.

Inoculum: A isolate of Drechslera poae was obtained from Richard H. Morrison of Northrup King Co., Eden Prairie, MN. Stock cultures of fungus were maintained on silica gel to insure a consistant source of inoculum (20). Cultures of Drechslera poae was produced on V-8 juice agar at 18 C with 12 hours fluorescent illumination (4 Klux).

Inoculation: Drechslera poae conidia were harvested from 21 day-old cultures by placing the media from several petri plates in a 1-liter flask with 50 ml of double distilled water, plus one drop of Tween 20 (Emulsion Engineering, Inc., Elk Grove Village, IL). The
The suspension was shaken and then strained through one layer of 80 mesh cheese cloth. A Sedgwick-Rafter counting cell (Hausser Scientific, Blue Bell, PA) was used to adjust the concentration of the conidium suspension to 10,000 conidia/ml. Conidium suspensions were uniformly applied to Kentucky bluegrass foliage with an artist's air brush at .7 kg/cm² for 10 sec. Inoculated plants were then sealed in plastic bags for incubation.

**Influence of temperature on pre-penetration:** Inoculated pots of Kentucky bluegrass were incubated at 6, 12, 18, 24 and 30 C with 12-hour fluorescent illumination (4 Klux) in plastic bags which served as a moist chamber. Leaf samples were collected from pots 2, 4, 6, 8, 12, 16, 24, 32 and 48 hours after inoculation. After removal of the leaf samples, the pots were placed back at the same temperature after removal of the plastic bag.

The leaf samples were taped to 15 x 22 mm index cards. Collodion solution (Fisher Scientific Co., Pittsburgh, PA) was applied to the excised leaves with a tapered glass rod and allowed to dry. Dried collodion strips were removed from the leaves with forceps, placed on a glass slide and stained with 0.5% cotton blue in lactophenol. Germination, germ tube elongation and appressorium formation of 50 D. poae conidia per treatment on collodion strips was determined at 100X and 400X magnification. Conidia were considered germinated when the germ tubes exceeded 2 um.

**Disease severity:** Pots were held at the initial incubation temperature for 5 days after inoculation. All foliage was removed for disease evaluation by clipping each pot 1 cm above the lip of the styrofoam cup.
The incidence and severity of the leaf spot phase was evaluated on 25 randomly selected leaves using the Horsfall and Barratt rating system (11, 12). The Horsfall and Barratt grading system (11, 12) was based on the percent leaf area diseased and was as follows: 1 = 0%, 2 = 0-3%, 3 = 3-6%, 4 = 6-12%, 5 = 12-25%, 6 = 25-50%, 7 = 50-75%, 8 = 75-87%, 9 = 87-94%, 10 = 94-97%, 11 = 97-100%, 12 = 100%.

**Leaf lesion enlargement:** 'Park' Kentucky bluegrass leaves were collected five days after inoculation from pots held in moist chambers for 16, 24, 36, and 48 hours at 6, 12, 18, 24, and 30 C. After several leaves were taped to a glass slide, the length and width of 20 lesions were measured with an eyepiece micrometer at 40X and 100X magnification. Since many lesions were elliptical in shape, lesion area was determined using the formula for the area of an ellipse (A = \( \pi ab \) where \( a \) = length and \( b \) = width).

**Penetration and colonization of leaf tissue:** Leaf samples were collected from pots of plants incubated at 6, 12, 18, 24, and 30 C after 16, 24, 32, and 48 hours incubation in a moist chamber, steamed in 0.5% trypan blue in lactoglycerine for 10 minutes, destained in lactoglycerine and mounted in lactoglycerine on glass slides for examination.

A second set of leaf samples were collected from all the 24 hour treatments, steamed in 0.5% cotton blue in lactophenol for 10 minutes, destained in clear lactophenol for 2 minutes, and mounted on glass slides for examination.

Leaf pieces were examined microscopically for *D. poae* conidia, appressoria, and the presence of primary or secondary hyphae in host tissue. The number of successful penetrations was determined using
leaf material from the 24 hour treatment by noting the association between appressoria and the development of primary hyphae in leaf tissue.

**Infection efficiency:** Pots of 'Park' Kentucky bluegrass which were inoculated with a *D. poae* conidium suspension were incubated at 6, 12, 18, 24, and 30°C in sealed plastic bags to provide a moist environment with 12 hour fluorescent illumination (4 Klux). After 24 hours, the plastic bags were removed and the pots were incubated for five days to allow lesion development. Leaf sections of 10-15 cm were collected from each treatment and examined with a dissecting microscope at 30 to 80X magnification. Infection efficiency, i.e., the proportion of conidia successfully giving rise to a lesion was expressed as the number of lesions per leaf section divided by the total number of conidia observed on the section (8).

**Sporulation of *D. poae* on excised leaves:** Diseased leaves were collected from pots of 'Park' Kentucky bluegrass inoculated with a suspension of 10,000 *D. poae* conidia/ml, incubated at 18°C in plastic bags for 24 hours, removed from the plastic bags, and held another 4 days at 18°C. Excised diseased bluegrass leaves were placed on sterile filter paper in petri dishes and allowed to air dry for 24 hours to halt fungal activity. Double distilled water was added to remoisten the dried leaves and the petri dishes were sealed with parafilm to provide a moist environment. The petri plates were incubated at 6, 12, 18, 24, and 30°C with 12 hour fluorescent illumination (4 Klux).

Sporulation of *D. poae* was monitored after 2, 4, and 6 days incubation. Approximately 5-7 leaves were placed in small vials containing 2 ml of 1% CuSO₄ and shaken vigorously to remove conidia from conidio-
phores. The leaves were removed from the vials, folded in a piece of aluminum foil, dried at 80°C for 24 hours, and weighed. The concentration of conidia in the CuSO₄ solution was determined with a Sedgwick-Rafter counting cell. The number of conidia/mg dry weight of leaf tissue was calculated by dividing the total number of D. poae conidia in the 2 ml CuSO₄ solution by the weight (mg) of leaf tissue.

Comparison of D. poae sporulation on intact plants and excised leaves:
Pots of 'Park' Kentucky bluegrass were inoculated with a conidium suspension of 10,000 D. poae conidia/ml, incubated 24 hours at 18°C in plastic bags, and held another 5 days at 18°C. Leaves from half the pots were harvested, placed in petri plates and dried while the remaining pots were held an additional day at 18°C. After 24 hours, the leaves in the plates were remoistened and the remaining pots of Kentucky bluegrass were sealed in plastic bags. Petri plates and pots of Kentucky bluegrass were incubated six days at 12, 18, and 24°C with 12 hour fluorescent illumination (4 Klux). After six days, leaf samples were collected from both treatments, placed in vials containing 2 ml 1% CuSO₄, and the concentration of D. poae conidia was determined using the technique described in the previous section.

Influence of temperature on conidium and conidiophore morphology:
Small leaf pieces were collected from excised Kentucky bluegrass leaves incubated for six days at 6, 12, 18, 24 and 30°C. The samples were mounted on glass slides in 0.5% cotton blue in lactophenol. Conidium length and width at the apical, mid spore, and basal cell, conidiophore length and width as well as the number of septa in a conidium were measured or determined at 400X magnification.
RESULTS

Influence of temperature on conidium germination, germ tube elongation and appressorium formation of Drechslera poae on Kentucky bluegrass leaves: Conidium germination of D. poae was first observed on Kentucky bluegrass leaves four hours after inoculation at all temperatures (Figure 2.1). Initially conidium germination was most rapid at 18 and 24 C, moderate at 30 C, and slow at 6 and 12 C (Figure 2.1). At 18, 24, and 30 C, D. poae conidium germination was nearly completed within 24 hours of inoculation. Approximately 32 hours after inoculation, the percentage of germinated conidia on Kentucky bluegrass leaves reached the 90% level at 6 and 12 C.

Shortly after germination, germ tube development was quite rapid at 18, 24, and 30 C while only modest increases in length were noted at 6 and 12 C (Figure 2.2). The bulk of germ tube development occurred within 24 hours of inoculation at 18, 24, and 30 C with moderate increases being observed in the following 24 hours. At 6 and 12 C, germ tube development although slower than at the other temperatures, steadily increased throughout the 48 hour period following inoculation. After 48 hours, the mean lengths of D. poae germ tubes formed at 12, 18, and 24 C were significantly greater than the mean lengths of germ tubes developing at 6 and 30 C (Figure 2.2). Appressorium formation was most rapid at 18 and 24 C within 24 hours of inoculation but only moderate increases in the number of appressoria were noted during the following 24 hour period (Figure 2.3). The rate of appressorium formation of D. poae initially was slower at 6, 12, and 30 C than at 18 or 24 C. However, after 48 hours, no substantial differences in appressorium
Figure 2.1 Influence of temperature on the germination of *Drechslera poae* conidia on leaves of 'Park' Kentucky bluegrass. Leaf samples were collected from pots of Kentucky bluegrass incubated at 6, 12, 18, 24, and 30 C for 2, 4, 6, 8, 12, 16, 24, 32, and 48 hours after inoculation, collodion leaf impressions were made, stained and mounted. A total of 100 conidia/treatment were checked for the presence of germ tubes. Conidia were considered germinated when germ tubes exceeded 2 μm in length.
% germination vs. Period of incubation (hr) in a moist environment.

- 6C
- 12C
- 18C
- 24C
- 30C

The graph shows the percentage of germination over time at different temperatures.
Figure 2.2 Influence of temperature on the elongation of *Drechslera poae* germ tubes on leaves of 'Park' Kentucky bluegrass. Leaf samples were collected from pots of Kentucky bluegrass incubated at 6, 12, 18, 24, and 30 °C for 2, 4, 6, 8, 12, 16, 24, 32, and 48 hours after inoculation, collodion leaf impressions were made, stained and mounted. The germ tubes of a total of 100 conidia/treatment were measured with an eyepiece micrometer at 100X and 400X magnification.
Mean germ tube length

Period of incubation (hr) in a moist environment

- 6°C
- 12°C
- 18°C
- 24°C
- 30°C

Mean germ tube length (µm)

20 40 60 80 100 120 140 160 180
Figure 2.3 Influence of temperature on the formation of appressoria of *Drechslera poae* on leaves of 'Park' Kentucky bluegrass. Leaf samples were collected from pots of Kentucky bluegrass incubated at 6, 12, 18, 24, and 30 °C for 2, 4, 6, 8, 12, 16, 24, 32, and 48 hours after inoculation, collodion leaf impressions were made, stained, and mounted. The germ tubes of 100 conidia/treatment were examined for the presence of appressoria.
formation were observed on Kentucky bluegrass leaves at 12 to 30 °C. Only at 6 °C were significant reductions in appressorium formation observed.

**Influence of temperature on the penetration and colonization of 'Park' Kentucky bluegrass leaves:** Incubation temperature had no apparent effect on the site of appressorium formation. Appressorium formation and subsequent penetration of Kentucky bluegrass leaves by *D. poae* generally occurred at the juncture of the walls of two adjacent epidermal cells. Few penetrations through stomatal apertures were noted although the formation of appressoria over stomates was not uncommon at any temperature. Rather, the subsidiary cells surrounding the guard cells were penetrated and colonized. *Drechslera poae* rarely penetrated through the epidermal cells to the intercellular spaces surrounding the palisade layer but colonized epidermal cells prior to movement between the palisade cells.

The number of successful penetrations of Kentucky bluegrass leaves was significantly greater at 18 and 24 °C than at 6, 12, and 30 °C (Table 2.1). However, it must be noted even at 6 and 30 °C, nearly 50% of the appressoria on Kentucky bluegrass leaves were associated with infection sites (Table 2.1).

Successful penetration of epidermal cells was indicated by the formation of a large globose cell known as the primary hyphal initial (Figure 2.4 A,B). Shortly after development of the primary hyphae initial, primary hyphal filaments which were often thin walled, expanded and regularly septate began to grow from the primary hyphal initial (Figure 2.5 A,B.) Formation of primary hyphal initials and hyphae were commonly associated with appressoria on the leaf surface at 18 and 24 °C.
Table 2.1 Influence of temperature on the penetration of Kentucky bluegrass leaves by *Drechslera poae*.

<table>
<thead>
<tr>
<th>Temperature C</th>
<th>% successful penetrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>46 b^z</td>
</tr>
<tr>
<td>12</td>
<td>50 b</td>
</tr>
<tr>
<td>18</td>
<td>67 a</td>
</tr>
<tr>
<td>24</td>
<td>65 a</td>
</tr>
<tr>
<td>30</td>
<td>41 b</td>
</tr>
</tbody>
</table>

^xInoculated pots of 'Park' Kentucky bluegrass were incubated 24 hours at 6, 12, 18, 24, and 30 C in a moist environment and held a further four days at the same temperature. Leaves were collected and cleared by steaming the leaves in 0.5% lactophenol cotton blue. Six to eight leaves/treatment were mounted on slides in lactoglycerine. Examination of the leaves focused on the association between appressoria and primary hyphae in leaf tissue.

^yPenetration was successful when primary hyphae began to develop in leaf tissue.

^zValues in the column followed by the same letter are not significantly different from one another at the P=0.05 level according to Duncan's Multiple Range Test.
Figure 2.4 Pre-penetration events of *Drechslera poae* on leaves of Kentucky bluegrass. A) germinated conidia (c) approximately 24 hours incubation, note extensive germ tube (gt) development and several appressoria(a). B) germinated conidium with short germ tube tipped with an appressorium. C) appressoria sometimes formed over stomates (s) but D) most often at the cell wall juncture of two epidermal cells.
and uncommon at 12 C approximately 16 hours after inoculation. At 6 and 30 C, penetration and development of hyphal initials were not noted until 24 hours after inoculation. Primary hyphae in epidermal cells continued to expand for several hours prior to movement into the palisade layer. The length of primary hyphae varied from 2 μm to 200 μm in some epidermal cells. The rate of elongation of primary hyphae in epidermal cells was more rapid at 12, 18, and 24 C than at 6 or 30 C. Hyphae of 40 μm or more were present in epidermal cells within 24 hours when incubated at 12, 18, and 24 C but were not observed in epidermal cells incubated at 6 or 30 C until 32 to 48 hours after inoculation.

Infrequently, appressoria-like swellings on the lateral walls of the colonized epidermal cells were observed developing at 12, 18, 24, and 30 C. A distinct pore was formed in the cell wall between the two cells and primary hyphae began to develop in the newly colonized cell. It was impossible to determine whether the cell was alive when penetrated or what effect penetration had on cellular components.

Development of secondary hyphae in the intercellular spaces of bluegrass rapidly followed primary hyphae formation at 18 and 24 C. Secondary hyphae filaments which were thin walled, narrow, and irregularly septate, were present 16 hours after inoculation. Extensive ramification by secondary hyphae occurred within 24 to 32 hours of inoculation. Development of secondary hyphae in leaf tissue at 12 C was not observed until 24 hours after inoculation. At 6 and 30 C, secondary hyphal development was sparse 48 hours after inoculation. Direct penetration and colonization of palisade cells by secondary hyphae was not observed.
Palisade cells often did not visibly react to the presence of *D. poae* hyphae in epidermal cell layer. Lesion development coincided with the ramification of secondary hyphae and plasmolysis of the surrounding cells in the palisade layer (Figure 2.5 C). However, at 6 C, moderate ramification of *D. poae* secondary hyphae in the palisade layer was not accompanied by lesion formation. Based on lesion size, the ramification of *D. poae* hyphae was much more extensive at 12, 18, and 24 C than at 6 or 30 C.

Once lesion formation had begun, secondary hyphae were not observed outside the lesion margin. Lesion expansion was limited by the presence of vascular bundles in leaf tissue which limited secondary hyphal development (Figure 2.5D). Inhibition of secondary hyphal ramification by vascular bundles may cause the formation of characteristic or oval leaf lesions (Figure 2.5D).

**Influence of temperature on the development of the leaf spot phase of melting-out:** Initially, temperature had a substantial effect on the incidence of the leaf spot phase of melting-out caused by *D. poae*. Penetration of leaf tissue and subsequent leaf lesion formation began within four hours of inoculation at 18 C (Figures 2.6, 2.7). At 12 and 24 C, penetration occurred within eight hours of inoculation (Figure 2.6). Once penetration began, the leaf spot incidence rapidly increased to the point where nearly all leaves were diseased at 12, 18, and 24 C (Figure 2.6). Leaf spot initiation was inhibited at 6 and 30 C until 12 hours after inoculation (Figure 2.6). As the duration of incubation of inoculated plants continued, leaf spot incidence greatly increased. After 32 hours incubation, no substantial differences in disease incidence were noted between the five treatments (Figure 2.6).
Figure 2.5 Penetration and colonization of Kentucky bluegrass leaves by *Drechslera poae*. A) the primary hyphal initial (phi) forms immediately after penetration in host epidermal cells or intercellular spaces. Primary hyphae (ph) develops from the primary hyphal initial in epidermal cells or intercellular spaces (here) (320X). B) primary hyphal initial and primary hyphae in epidermal cell (1000X). C) secondary hyphae (sh) ramifying between palisade cells (pc) (1000X). D) extensive secondary hyphal development near a vascular bundle (1000X).
Figure 2.6 Influence of temperature and moisture on the incidence of the leaf spot phase of melting-out of Kentucky bluegrass caused by *Drechslera poae*. Five days after inoculation, the foliage of each pot was harvested and disease incidence determined. Disease incidence was based on the percentage of leaves with leaf lesions per 200 leaves examined for symptom development.
Figure 2.7 Influence of temperature and moisture on the severity of the leaf spot phase of melting-out of Kentucky bluegrass caused by *Drechslera poae*. Five days after inoculation, the foliage of each pot was harvested and disease severity determined. Disease severity ratings were based on the Horsfall and Barratt grading system which is as follows: 1=0%, 2=0-3%, 3=3-6%, 4=6-12%, 5=12-25%, 6=25-50%, 7=50-75%, 8=75-87%, 9=87-94%, 10=94-97%, 11=97-100%, 12=100% of the leaf area was diseased.
Disease severity

Period of incubation (hr) in a moist environment

- 6C
- 12C
- 18C
- 24C
- 30C

Disease severity

1.0 2.0 3.0 4.0 5.0 6.0

2 4 6 8 12 16 24 32 48
Figure 2.8 Influence of temperature on leaf lesion enlargement in a moist environment. Inoculated pots of 'Park' Kentucky bluegrass were removed from plastic bags 16, 24, 32, and 48 hours after inoculation. Lesion dimensions were measured five days after inoculation and lesion area was calculated using the formula for the area of an ellipse.
Figure 2.9 Influence of temperature on the infection efficiency of *Drechslera poae* on leaves of 'Park' Kentucky bluegrass. Inoculated pots of bluegrass were incubated 24 hours in a moist environment at 6, 12, 18, 24, and 30°C. Infection efficiency which was calculated five days after inoculation was determined by dividing the number of lesions observed by the total number of germinated conidia in that sample.
Incubation temperature

Infection efficiency

6C 12C 18C 24C 30C

* Incubation temperature
Disease severity, which is a logarithmic scale based on the percent area diseased rather than percentage of leaves diseased, provided a different view of disease development. Disease severity increased slower than disease incidence rating reflecting the gradual expansion of lesions on Kentucky bluegrass leaves maintained in a moist environment (Figure 2.7). Increases in disease severity were comparable at 12, 18 and 24 C until 16 hr then slightly higher at 18 C from 16-48 hr (Figure 2.7). Lesion development was noticeably inhibited at 6 and 30 C even after 48 hours incubation in a moist environment.

Moisture also plays an important role in leaf lesion formation. Besides being required for conidium germination and infection, moisture is also necessary for lesion expansion (Figures 2.7, 2.8). Lesion area increases in proportion to the length of the incubation period in a moist environment (Figures 2.7, 2.8). Lesion expansion occurred at approximately the same rate at 12, 18, and 24 C (Figure 2.8). At 6 and 30 C, the low disease severity ratings reflected in the slow rate of increase of leaf lesion area (Figure 2.8).

Influence of temperature on infection efficiency: The efficiency of infection of bluegrass leaves infected with D. poae peaked at a temperature of 18 C (Figure 2.9). Although efficiency values were significantly different from one another, infection efficiency values at 12, 24, and 30 C were moderate. Only at 6 C, was lesion development effectively suppressed.
Sporulation of *Drechslera poae* on Kentucky bluegrass leaves:

Conidiophore development began on excised leaves after 24-32 hours incubation in a moist environment. Sporulation of *D. poae* was quite light at all temperatures after two days (Figure 2.10). Tremendous increases in the level of *D. poae* sporulation at 12, 18, and 24 C were observed after four days incubation. This increase in conidium production closely followed extensive conidiophore development on the surface of excised Kentucky bluegrass leaves (Figures 2.10, 2.11). At 6 and 30 C, conidiophore development was confined to areas within the lesion margin. Sporulation of *D. poae* at 6 C, though greatly inhibited, did continue to increase slowly in intensity while at 30 C sporulation was sparse after six days incubation (Figure 2.10). Saprophytic activity of *Aspergillus* and *Alternaria* spp. was quite high at 24 C and especially at 30 C. Very few saprophytic fungi were observed colonizing the excised leaves at 6, 12, or 18 C.

Sporulation of *D. poae* was not as profuse on intact plants as on excised leaves (Table 2.2). The level of sporulation of *D. poae* on excised leaves was 4 times greater at 12 and 18 C to 10 times greater at 24 C than on leaves from intact plants. Temperature did not significantly influence sporulation on leaves of intact plants (Table 2.2).

**Influence of temperature on the morphological characters of *Drechslera poae* conidia and conidiophores:** Ambient temperatures had a substantial influence on the length and number of septae in a conidium as well as the conidiophore length (Table 2.3, Figure 2.12). Mean conidium length and number of septate was highest at 12 C and
Figure 2.10 Sporulation of Drechslera poae on excised, diseased 'Park' Kentucky bluegrass leaves at five temperatures. Conidium production was monitored after 2, 4, and 6 days incubation at 6, 12, 18, 24, and 30 C by dividing the number of D. poae conidia washed from leaves by the dry weight of the leaves (mg).
Period of incubation (days)

No. conidia / mg dry leaf weight

10

100

1000

6C

30C

24C

12C

18C
Table 2.2 Sporulation of *Drechslera poae* on diseased, excised leaves and intact 'Park' Kentucky bluegrass plants after six days incubation in a moist environment at three temperatures.

<table>
<thead>
<tr>
<th>Temperature C</th>
<th>excised leaves</th>
<th>intact plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1408 a²</td>
<td>280 bc</td>
</tr>
<tr>
<td>18</td>
<td>1267 a</td>
<td>329 bc</td>
</tr>
<tr>
<td>24</td>
<td>438 b</td>
<td>48 c</td>
</tr>
</tbody>
</table>

*Pots of 'Park' Kentucky bluegrass were inoculated with 10,000 conidia/ml, incubated 24 hours at 18 C, and held a further five days at 18 C. Foliage from half the pots was harvested and dried for 24 hours. Simultaneously, the dried leaves and remaining pots were placed in a moist environment for six days at 12, 18, and 24 C with 12 hours fluorescent illumination. Leaves were collected from both treatment sets, places in vials containing 2 ml of 1% CuSO₄ solution, and shaken. The concentration of *D. poae* conidia in the 1% CuSO₄ solution was determined with a Sedgewick-Rafter cell. Leaf material was collected from the vials, dried and weighed.*

*The number of conidia/mg dry leaf weight was calculated by dividing the total number of conidia in 2 ml of 1% CuSO₄ solution by the dry weight (mg) of the leaves.*

*Values followed by the same letter are not significantly different from one another at the P=0.05 level according to Duncan's Multiple Range Test.*
Figure 2.11 Profuse sporulation of *Drechslera poae* on a diseased, excised Kentucky bluegrass leaf (80X).
Figure 2.12 Influence of temperature on *Drechslera poae* conidium morphology. Note the differences in length and number of septations on these *Drechslera poae* conidia collected from excised Kentucky bluegrass leaves after six days incubation at A) 12 C, B) 18 C, C) 24 C, and D) 30 C (all 500X).
Table 2.3 Influence of temperature on the morphological characters of *Drechslera poae* conidia and conidiophores developing on lesions on 'Park' Kentucky bluegrass.

<table>
<thead>
<tr>
<th>Morphological Characters</th>
<th>6 C</th>
<th>12 C</th>
<th>18 C</th>
<th>24 C</th>
<th>30 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>118.9</td>
<td>141.1</td>
<td>122.5</td>
<td>85</td>
<td>50</td>
</tr>
<tr>
<td>Range</td>
<td>71-173</td>
<td>103.5-181.7</td>
<td>86.9-156.9</td>
<td>50.6-115</td>
<td>25.3-75.9</td>
</tr>
<tr>
<td>Basal septum width (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16.8</td>
<td>18.3</td>
<td>17.8</td>
<td>15.3</td>
<td>15.2</td>
</tr>
<tr>
<td>Range</td>
<td>12.4-20</td>
<td>11.5-23.5</td>
<td>14.3-23</td>
<td>11.5-18.4</td>
<td>9.2-20.9</td>
</tr>
<tr>
<td>Mid-spore width (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>19.7</td>
<td>20.5</td>
<td>20.5</td>
<td>18.3</td>
<td>17.2</td>
</tr>
<tr>
<td>Range</td>
<td>17.9-21.9</td>
<td>16.1-23.2</td>
<td>16.6-25.3</td>
<td>14.3-23</td>
<td>13.1-23</td>
</tr>
<tr>
<td>Apical septum width (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>15</td>
<td>14.7</td>
<td>14</td>
<td>12</td>
<td>16.8</td>
</tr>
<tr>
<td>Range</td>
<td>11.5-19.3</td>
<td>11.5-18.4</td>
<td>11.5-18.9</td>
<td>9.2-14.3</td>
<td>11.7-25.8</td>
</tr>
<tr>
<td>Septae (no.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.7</td>
<td>10.4</td>
<td>8.6</td>
<td>6</td>
<td>3.2</td>
</tr>
<tr>
<td>Range</td>
<td>5-11</td>
<td>7-14</td>
<td>5-12</td>
<td>3-9</td>
<td>1-5</td>
</tr>
</tbody>
</table>
Table 2.3 (cont.) Influence of temperature on the morphological characters of *Drechslera poae* conidia and conidiophores developing on lesions on 'Park' Kentucky bluegrass.$^2$

<table>
<thead>
<tr>
<th>Conidiophore Morphological Characters</th>
<th>6 C</th>
<th>12 C</th>
<th>18 C</th>
<th>24 C</th>
<th>30 C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length μm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>86.6</td>
<td>92.3</td>
<td>114.2</td>
<td>186.5</td>
<td>256.8</td>
</tr>
<tr>
<td>Range</td>
<td>43.7-161.5</td>
<td>55-144.9</td>
<td>82.8-154.1</td>
<td>135.7-269.1</td>
<td>128.8-384</td>
</tr>
<tr>
<td><strong>Width μm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.1</td>
<td>9.7</td>
<td>9.5</td>
<td>9.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Range</td>
<td>6.9-11.3</td>
<td>8.7-11.5</td>
<td>7.2-13.8</td>
<td>8.6-11.5</td>
<td>8.1-13.8</td>
</tr>
</tbody>
</table>

$^2$Conidium and conidiophore dimensions were measured with an eyepiece micrometer at 400X magnification. Each value is the mean of 25 different observations.
Figure 2.13 Influence of temperature on *Drechslera poae* conidium initial formation. A) At 18°C, conidium development is porogenous, i.e., conidium initials develop through pores or a protrusions of the conidiophore wall. B) At 24°C, conidium initials develop as extensions of the conidiophore tip (500X).
the values of both parameters decreased in magnitude as the temperature increased or decreased (Table 2.3, Figure 2.12). Substantial differences in conidium width at the apical, mid-spore or basal septum were not observed (Table 2.3). Murogenous conidium development, i.e., conidium initial developing as an extension of the conidiophore tip rather than through pores or as a protrusion in the conidiophore wall, was common at 24°C but very unusual at other temperatures (Figure 2.13).

Mean conidiophore length increased as the incubation temperature increased from 6 to 30°C while conidiophore width was unaffected by changes in temperature (Table 2.3). At 30°C, the apical cell of the conidiophore never differentiated to allow conidium formation and continued to divide and elongate.

**DISCUSSION**

Results of this study confirm the field observation of several authors that *Drechslera poae* activity on leaf surfaces, penetration of leaf tissue and subsequent lesion development is greatest at temperatures from 12, to 24°C with peak activity occurring at 18°C (3,4,9,17,21). The level of *D. poae* conidium germination, germ tube development, and appressorium formation on Kentucky bluegrass leaves was moderately to severely restricted at 6, 12, and 30°C for 16 to 24 hours after inoculation (Figures 2.1 - 2.3). Although *D. poae* conidium germination was noted at all temperatures after 4 hours, substantial differences in the level of germination were quite evident. Horsfall (10) noted that approximately 98% of the *D. poae* conidia examined at temperatures as low as 3°C had germinated within 24 hours. In this study that level of germination at 6 and 12°C did not occur until 32 to 48 hours after inoculation (Figure 2.1).
The level of *D. poae* conidium germination 24 hours after inoculation at temperatures between 18 to 20 C reached the same levels noted by Horsfall (10) and Mower (18). Germ tube development was nearly completed within 24 hours of inoculation at 18 to 30 C (Figure 2.2). However, Mower (18) noted at 24 to 30 C germ tube development was completed within 12 hours of inoculation. Probably, the method of observation used to monitor these phenomena account for the difference between those observations. Although appressorium formation was inhibited at 6 and 12 C, appressorium formation was generally steady for up to 48 hours after inoculation (Figure 2.3). It is difficult to determine whether the levels of appressorium formation in this study were comparable to those of Mower's (18) because different methods for monitoring appressorium formation were used.

The sequence of events pertaining to penetration and colonization of Kentucky bluegrass by *D. poae* were nearly the same in this study as those outlined by Mower (18). Mower (18) stated that primary hyphae did not colonize adjacent cells by the development of intracellular hyphae. However, formation of appressorium-like structures in colonized epidermal cells preceded the penetration and colonization of adjacent cells by primary hyphae at temperatures from 12 to 30 C.

It must be noted prior to further discussion that the study completed by Mower (18) was done at temperatures between 24 to 30 C, temperatures generally higher than those encountered in the field when Kentucky bluegrass is being penetrated and colonized by *D. poae*. In this study, I have attempted to observe phenomena at temperatures above and below field optimums.
Temperature extremes did reduce the rate of penetration and colonization of Kentucky bluegrass leaves by *D. poae*. Development of primary hyphae indicating successful penetration of an epidermal cell was commonly observed at 18 to 24°C approximately 16 hours after inoculation. Mower (18) first noted primary hyphae developing after 18 hours. However, disease incidence data from this study and one completed by Halisky and Funk (9) indicate that penetration of Kentucky bluegrass leaves may begin approximately 6 hours rather than 16-18 hours after inoculation. Penetration follows appressorium formation by several hours rather than 8 to 10 hours at optimum temperatures (18). Primary hyphal development at 12°C was sparse while at 6 and 30°C development of primary hyphae was severely restricted.

Disease incidence data again indicate penetration of leaves at 6 and 30°C began in 12-16 hours of inoculation yet no successful penetrations were observed on the cleared leaf material incubated 16 hours at either 6 or 30°C (Figure 2.6). Quite possibly, successful penetrations at 6 and 30°C were rare after 16 hours but not uncommon 24 hours after inoculation (Table 2.1).

As observed by Mower (18), ramification of secondary hyphal filaments into the palisade layer began 6-8 hours after colonization of epidermal cells 12-24°C. Secondary hyphal development was uncommon at 6 and 30°C.

Data concerning disease severity and lesion expansion is a good indicator of secondary hyphae development in host tissue (Figures 2.8, 2.9). Development of primary hyphal filaments often stopped after several cell divisions. Lesion expansion at 12 to 24°C indicates that ramification of hyphae in the palisade layer was quite rapid.
Hyphae of *D. poae* may be quite sensitive to moisture levels in host tissue. Lesion expansion on intact plants continued even at optimum temperatures for *D. poae* only if leaves are held in a moist environment. If the leaves were removed from the moist environment, lesion expansion and presumably hyphal growth ceased (Figures 2.8, 2.9). A similar effect was noted by Halisky and Funk (9).

The infection efficiency of temperature on infection has not been widely studied. Work has concentrated on the influence of inoculum levels on lesion development (5, 8, 19, 22). Peak efficiency occurred at 18 °C and decreased significantly with increases or decreases with temperature. At 18 °C, the peak efficiency rating of *D. poae* on Kentucky bluegrass was slightly larger than that of *Bipolaris maydis* (*Helminthosporium maydis*) on corn. Infection efficiency of other fungi such as *Septoria lycopersici* and *Alternaria solani* on tomato were much lower than the values for either *D. poae* or *B. maydis* (15). Given losses due to dispersal and germination, there was a 25-30% chance that a conidium will penetrate and produce a lesion under optimal conditions (22).

Conidiophore and conidium formation of *D. poae* on diseased, excised Kentucky bluegrass plants began approximately 24 to 36 hours at 18 °C in a moist environment. Mower (18) reported that conidiophore and conidium formation began five days after inoculation of excised leaves with *D. poae* conidium suspension. In Mower's (18) study, inoculated leaves were incubated continuously in a moist environment while the leaf material used in this study was held in a moist environment only 24 hours at 18 °C to allow penetration and colonization, held for days to permit symptom development, and then remoistened for
Temperature has a tremendous impact on the level of *D. poae* sporulation on excised leaves of intact plants (Figure 2.10). Sporulation occurred most rapidly at temperatures between 12-24 C with peak conidium production occurring at 18 C (Figure 2.10). At 6 and 30 C, conidium production was light and sparse, respectively, after six days incubation on excised leaves (Figure 2.10). Vargas and Wilcoxson (21) noted a different pattern of *D. poae* sporulation on SAYA agar. Although 18 C was recognized as the temperature optimum for sporulation in both studies, Vargas and Wilcoxson (21) noted tremendous drops in the level of sporulation at 15 and 21 C. On excised leaves, sporulation of *D. poae* was profuse from 12 to 24 C, a much wider range than indicated by Vargas and Wilcoxson (21).

It was difficult to pinpoint the maximum temperature for *D. poae* sporulation. In this study, the level of sporulation at 24 C did vary considerably on excised leaves (Figure 2.10, Table 2.2). Evidently, *D. poae* was quite sensitive to temperatures between 21 to 24 C but it was difficult to predict how it will respond to these temperatures at any time.

Conidium production on intact plants was 4 to 10 times lower than on excised leaves depending on the incubation temperature (Table 2.2). At 12 to 24 C, conidiophores developed over the entire surface of excised Kentucky bluegrass leaves within four to six days indicating the rapid colonization of the leaf by *D. poae*. Colonization of leaves on intact plants appeared to progress at a slower rate. After six days, approximately 50% of the area of the leaves collected from Kentucky bluegrass plants was healthy. The level of sporulation on
Murogenous *B. sorokiniana* conidia also formed at temperatures above optimum for conidium formation (13).

Abnormal development of *D. poae* conidiophores has been observed in several studies (18,21). Twisting and curling as well as germination of conidiophores has been noted at temperatures above 21 to 24 C (18). Conidiophore length also increased significantly as temperature increased to 20 C (Table 2.3). It was quite possible that the apical cell of the conidiophore was no longer sensitive to wavelength of radiation greater than 480 mm which directed its differentiation and initiation of conidium formation (21).


Chapter III

BIOASSAY OF FUNGICIDE RESIDUE PERSISTANCE
ON KENTUCKY BLUEGRASS LEAVES
ABSTRACT

Iprodione, anilazine, and cycloheximide were applied to 1.5 m² replicated field plots containing a blend of 'Park' and 'Delta' Kentucky bluegrass. Plugs of turf 12 cm in diameter were removed from each treatment plot 0, 1, 3, 5, 7, 14, and 21 days after fungicide application. Plugs were inoculated uniformly with a suspension of 10,000 Drechslera poae conidia/ml and incubated at 18 C for 24 hours. At that time, conidium germination, germ tube elongation, and appressorium formation of D. poae on leaves of Kentucky bluegrass were evaluated using the collodion leaf impression technique. Initially, all fungicides effectively suppressed the pre-penetration events of D. poae. Residues of iprodione and anilazine effectively inhibited D. poae on Kentucky bluegrass leaves for three weeks while little evidence of cycloheximide residue was noted seven days after fungicide application. The collodion leaf impression technique proved to be a simple means of assaying fungicide residue activity on leaf surfaces under field conditions.

INTRODUCTION

Weather variables such as rain, wind, temperature, and light play a significant role in the inactivation of fungicide deposits on plant surfaces (5). Greenhouse tests employing artificial weathering agents such as simulated rainfall have proved useful for the evaluation of fungicide persistence however poor correlations between greenhouse and field data may occur (13). Bioassays have been specifically developed to monitor fungicide weathering from foliage in the field. Neely (11)
evaluated fungicide activity by placing a cellophane disk over a leaf disk collected from fungicide treated plants, pipetting a suspension of *Monilinia fructicola* on the cellophane disk, and microscopically examining each cellophane disk for germinated conidia. Ko *et al* (8) collected an inoculated treated leaves with *Alternaria alternata* conidia with a vertical illumination microscope.

Hagan and Larsen (6) used a collodion technique that is a simple and useful method for evaluating the effect of fungicides on the conidium germination, germ tube elongation, and appressorium formation of *B. sorokiniana* on Kentucky bluegrass leaves. The major advantages of the collodion technique over the bioassay described above are that the fungal thallus is permanently mounted for later observation and no special equipment is required.

Melting-out caused by *Drechslera poae* (Baudys) Shoem. (=*Helminthosporium poae* Baudys, *Helminthosporium vagans* (Drechs.) Shoem.). is a serious disease wherever Kentucky bluegrass (*Poa pratensis* L.) is grown in the United States (1,3,7,9,10). Little is known of the direct toxic effects of registered fungicides on *D. poae* pre-penetration events on leaf surfaces or the rate of fungicide deposit degradation on Kentucky bluegrass leaf surfaces. Generally, fungicide efficacy is evaluated on the basis of inhibition of symptom development.

The objective of this study was to determine the usefulness of the collodion technique as a method for evaluating the weathering of fungicide deposits on Kentucky bluegrass under field conditions by monitoring conidium germination, germ tube elongation, and appressorium formation of *D. poae*. 
MATERIALS AND METHODS

Fungicide application - Iprodione (Chipco 26019, 50WP, Rhone-Poulenc, Inc., Mommouth Junction, NJ) anilazine (Dyrene, 50WP, Mobay Chemical Corp., Kansas City, Mo), and cycloheximide (Acti-dione, 2.1 WP, Upjohn Co., Kalamazoo, Mich.) were applied at two label rates at a dilution rate of 11.4 L of water/93m² (3 gal/1000 ft²) using a CO₂-powered sprayer at 2.1 Kg/cm² to 1.5 m² replicated and randomized field plots containing a blend of 'Park' and 'Delta' Kentucky bluegrass on October 3, 1979. Twelve cm diameter plugs were collected from each replicate plot 0, 1, 3, 5, 7, 14, and 21 days after fungicide application.

Inoculation - An isolate of Drechslera poae was obtained from Richard H. Morrison of Northrup King Co., Eden Prairie, MN. Stock cultures of D. poae were maintained on silica gel to insure a consistent source of inoculum (11). Abundant sporulation was induced by culturing D. poae on V-8 juice agar at 18 C with 12 hours fluorescent illumination (4 Klux).

Conidia were harvested from 21 day old cultures by placing the media from petri plates in a 1-liter flask with 50 ml of double distilled water plus one drop of Tween 20 (Emulsion Engineering, Inc., Elk Grove Village, IL), vigorously shaking the flask, and straining through one layer of cheese cloth. The conidial adjustment of the concentration in the filtrate was adjusted 10,000 conidia/ml with a Sedgwick-Rafter counting cell using a Howard eyepiece micrometer. The conidial suspensions were uniformly applied to the Kentucky bluegrass foliage with an artist's air brush at .7 kg/cm² for 10
sec. Inoculated plugs were sealed in plastic bags and incubated at 18°C with 12 hr. fluorescent illumination (4 Klux).

After 24 hours incubation, the plastic bags were removed and leaf samples were collected from each treatment. Four leaves from each treatment were taped to 15 x 22 cm index cards. Collodion solution (Fisher Scientific Co., Pittsburgh, PA) was applied to the leaves with a tapered glass rod and allowed to dry. Dried collodion strips were removed from the leaves with a pair of forceps, placed on glass slides and strained with 0.5% cotton blue in lactophenol for microscopic examination.

Fifty randomly selected conidia on the collodion strips of each treatment were evaluated for conidium germination, germ tube elongation and appressorium formation at 100X and 400X magnification. Conidia were considered germinated when the germ tubes exceeded 2 μm.

**Meterological data** - Rainfall and turf microclimate temperature data were collected at a site approximately 50 m from the field plot with a tipping bucket rain gauge and T-type thermocouples, respectively. A summary of the meterological data is contained in Figure 3.1.

**RESULTS**

The level of inhibition of *Drechslera poae* conidium germination, germ tube elongation, and appressorium formation on Kentucky bluegrass leaves provided by iprodione and anilazine which was greater than that provided by cycloheximide completely inhibited conidium germination, germ tube elongation and appressorium formation of *D. poae* on leaves treated with each fungicide (Figures 3.2 - 3.4). Cycloheximide efficacy decreased at a much faster rate than that observed for either iprodione or anilazine (Figures 3.2 - 3.4). Within seven days of fungicide application,
Figure 3.1 Maximum and minimum daily turf microclimate temperature and daily rainfall from October 3-24, 1979 at the OSU Turf Plots, Columbus, Ohio.
Figure 3.2 Effect of fungicide residues on Kentucky bluegrass leaves on *Drechslera poae* conidium germination. Fungicides were applied to 1.5 m² replicated field plots of Kentucky bluegrass on October 3, 1979. Plugs were collected 1, 3, 5, 7, 14, and 21 days after fungicide application, inoculated with a *Drechslera poae* conidium suspension and incubated 24 hours at 18 C. Pre-penetration events were monitored using the collodion leaf impression technique (m = slope of the regression line).
Germination

Control

Cycloheximide

Iprodione

Anilazine

Days between fungicide application and inoculation

% Germination

$\frac{m}{1.5}$

$\frac{m}{2.9}$

$\frac{m}{1.3}$

$m = 0.4x$
Figure 3.3 Effect of fungicide residues on Kentucky bluegrass leaves on *Drechslera poae* germ tube elongation. Fungicides were applied to 1.5 m² replicated field plots of Kentucky bluegrass on October 3, 1979. Plugs were collected 1, 3, 5, 7, 14, and 21 days after fungicide application, inoculated 24 hours at 18 C. Pre-penetration events were monitored using the collodion leaf impression technique (m=slope of the regression line).
Days between fungicide application and inoculation

Mean germ tube length µm

- Control: $m = 0.6x$
- Cycloheximide: $m = 5.3x$
- Iprodione: $m = 3.2x$
- Anilazine: $m = 2.7x$
Figure 3.4 Effect of fungicide residues on Kentucky bluegrass leaves on *Drechslera poae* appressorium formation. Fungicides were applied to 1.5 m² replicated field plots of Kentucky bluegrass on October 3, 1979. Plugs were collected 1, 3, 5, 7, 14, and 21 days after fungicide application, inoculated with a *Drechslera poae* conidium suspension and incubated 24 hours at 18°C. Pre-penetration events were monitored using the collodion leaf impression technique (m=slope of regression line).
Days between fungicide application and inoculation

- Control
  - \( m = 0.3x \)
  - \( m = 1.5x \)
- Cycloheximide
- Iprodione
  - \( m = 0.7x \)
  - \( m = 0.3 \)
- Anilazine

# of Appressoria

0 1 3 5 7 14 21
Table 3.1 Correlation between the time from fungicide application to inoculation with the pre-penetration events of *Drechslera poae* on Kentucky bluegrass leaves treated with fungicides.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (g a.i./93m²)</th>
<th>Correlation Coefficient (r)</th>
<th>Conidium germination</th>
<th>Germ tube elongation</th>
<th>Appressorium formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iprodione</td>
<td>56</td>
<td><strong>Z .83</strong></td>
<td>.56**</td>
<td>.61**</td>
<td></td>
</tr>
<tr>
<td>Anilazine</td>
<td>112</td>
<td>** .77**</td>
<td>.51**</td>
<td>.67**</td>
<td></td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>1.2</td>
<td>** .63**</td>
<td>.65**</td>
<td>.60**</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>.11</td>
<td>.25</td>
<td>.19</td>
<td></td>
</tr>
</tbody>
</table>

*Asterisks: **=statistically significant at the P=0.01 level.*
no significant differences in conidium germination, germ tube elongation or appressorium formation of D. poae were noted between the control and cycloheximide treatment values whereas these processes were inhibited below control levels for at least three weeks for iprodione and anilazine (Figures 3.2 - 3.4).

Although well below control values, levels of conidium germination, germ tube elongation, and appressorium formation of D. poae on leaves treated with each fungicide increased significantly over three weeks (Table 3.1). The rate of increase of conidium germination, germ tube elongation, and appressorium formation was much faster on leaves treated with cycloheximide than those treated with either iprodione or anilazine (Figures 3.2 - 3.4). In general, the activity of D. poae increased at a slightly faster rate on the iprodione treatments than on the anilazine treatments (Figures 3.2 - 3.4). Since no significant differences were noted between the results of the two rates of each fungicide evaluated, the data of the lower rate of each fungicide has been excluded from all tables and figures.

DISCUSSION

The collodion technique proved to be a simple method for evaluating activity of fungicide residues on leaf surfaces of Kentucky bluegrass. Evaluation of fungicide activity is based on the ability of fungicidal residues on the leaf surface to inhibit the pre-penetration events of D. poae, conidium germination, germ tube elongation, and appressorium formation. Similar tests of fungicide activity have monitored only conidium germination (8, 11). Evaluation of residual activity of a fungicide based solely on conidium germination may be sufficient for
most materials, but some fungicides will selectively inhibit germ tube
development or appressorium formation while having little influence on
conidium germination (4, 6). Aside from permitting the evaluation of
several phenomena on the leaf surface, the easily mounted collodion
strips provide a permanent record of fungicide performance. The versa­
tility of this technique allows the simultaneous evaluation of several
different fungicides at different rates outdoors.

Apparently, routine mowing and weather variables such as rainfall
did not greatly affect persistance of iprodione and anilazine of Ken­
tucky bluegrass leaves. Initially, cycloheximide performed nearly as
well as iprodione and anilazine in controlling pre-penetration events
of D. poae (Figures 3.2 - 3.4). However, within several days of
fungicide application, significant reductions in cycloheximide
residue activity were noted (Table 3.1). It was not determined whether
the cycloheximide residues were photochemically inactivated or washed
from leaf surfaces by rain (Figure 3.1).

The fungitoxicity of iprodione and cycloheximide to Drechslera poae
and Bipolaris sorokiniana varies on Kentucky bluegrass leaves (6). Both
materials provided excellent control of all D. poae pre-penetration events
immediately after application of either fungicide while neither material
effectively suppressed B. sorokiniana conidium germination (6). Anila­
zine suppressed all pre-penetration activity of D. poae and B. sorokiniana
on Kentucky bluegrass leaves (6).
LITERATURE CITED


