

## V. DEVELOPMENT AND VALIDATION OF A BROWN PATCH FORECAST MODEL FOR PERENNIAL RYEGRASS

### SYNOPSIS

This three-year field investigation monitored the microclimate in perennial ryegrass (Lolium perenne L. cv. 'Caravelle') and non-uniformed disease development to identify environmental parameters associated with brown patch (Rhizoctonia solani Kühn) infection events, and to develop a weather-based forecast model. Micrometeorological conditions measured were ambient air temperature, relative humidity, leaf wetness duration, precipitation, soil temperature, soil moisture, and solar irradiance. Brown patch outbreaks or infection events were confirmed by visual assessment of foliar R. solani mycelium. An environmental favorability index (EFI) was developed to forecast R. solani infection events from correlation and univariate statistical procedures, and validated by chi-square and multiple regression ( $r^2 = 0.70$ ) analyses. The EFI was based on relative humidity (RH  $\geq 95\%$  for  $\geq 8$  hrs; mean RH  $\geq 75\%$ ), leaf wetness duration ( $\geq 6$  hr) or precipitation ( $\geq 12$  mm), and minimum air ( $\geq 16^\circ\text{C}$ ) and soil ( $\geq 16^\circ\text{C}$ ) temperatures. Using these data, brown patch outbreaks were predicted with 85% accuracy over a three year period. All major infection events were predicted. R. solani foliar mycelium first appeared in perennial ryegrass between 200 and 300 accumulated degree-days determined from soil temperature with a base temperature of  $16^\circ\text{C}$ . In 1993, the forecast model was used to field evaluate fungicide performance in perennial ryegrass and colonial bentgrass (Agrostis tenuis Sibth. cv. 'Bardot'). There were

equivalent levels of blighting between forecast-based sprays and a 14-day calendar-based spray schedule. The EFI-based schedule, however, provided a 29% reduction in fungicide applications.

## INTRODUCTION

Brown patch is caused by Rhizoctonia solani (Kühn) and is a major disease problem of turfgrasses worldwide (Burpee and Martin, 1992; Schumann et al., 1994). In cool-season turfgrasses, brown patch typically occurs during the summer, and is associated with high temperature and prolonged periods of high humidity and leaf wetness conditions (Smiley et al., 1992).

Weather-based plant disease prediction systems are utilized to identify critical periods when epidemics are apparent, thereby assisting with disease management decision-making strategies (Berger, 1977). Weather-based turfgrass disease forecast models were developed for anthracnose (Colletotrichum graminicola [Ces.] Wils.) (Danneberger et al., 1984), dollar spot (Sclerotinia homoeocarpa Bennett) (Hall, 1984), and Pythium blight (Pythium aphanidermatum [Edson] Fitzp.) (Nutter et al., 1983).

Dickinson (1930) conducted the earliest investigation on environmental conditions associated with brown patch. On putting green turf, Dickinson (1930) observed that brown patch symptoms coincided with afternoon irrigation when the daytime air temperature ranged from 26.4 to 34.6°C then decreased to 15.4 to 20.9°C at night. The first reported brown patch prediction system, however, was based on air temperature (Dahl, 1933). Dahl (1933) observed that brown patch occurred on 82% of days

when the minimum air temperature was  $\geq 21^{\circ}\text{C}$ . Unfortunately, Dahl (1933) did not publish any meteorological records or brown patch incidence data.

Rowley (1991) monitored weather conditions on creeping bentgrass over a two-year period. She (1991) compared observed brown patch outbreaks with environmental data to determine weather-based disease risk thresholds (i.e., low, moderate, high, or very high). The threshold levels were compared with air and soil temperatures, periods of high relative humidity, and precipitation (Rowley, 1991). These disease risk thresholds were integrated into a model by Schumann et al. (1994). The Envirocaster<sup>®</sup> (Neogen Corporation, Lansing, MI) is a commercially available weather station that is programmed to forecast brown patch based on Rowley's (1991) thresholds. Schumann et al. (1994) assessed the Envirocaster<sup>®</sup> accuracy in predicting brown patch outbreaks on creeping bentgrass (Agrostis palustris Hudson) in Massachusetts, New Jersey, and Georgia. They (1994) concluded that fungicide applications could be reduced and acceptable brown patch control achieved by combining weather-based disease forecasts with confirmation of pathogen levels through an enzyme-linked immunosorbent assay (Reveal<sup>®</sup> Turf Disease Detection Kit, Neogen Corporation, Lansing, MI).

Early observations indicated that poorly drained putting greens were more susceptible to brown patch than well drained turf (Piper and Oakley, 1921). No differences in brown patch severity, however, were observed in greenhouse grown creeping bentgrass subjected to five soil moisture levels, ranging from field capacity to the permanent wilting point (Couch and Bloom, 1958; Bloom and Couch, 1960).

Several researchers have attempted to document the influence of leaf wetness on brown patch. Shurtleff (1953) concluded from field observations that colonial bentgrass (*Agrostis tenuis* Sibth.) must experience a leaf wetness period of at least 15 hr for *R. solani* to penetrate leaf blades and cause injury. In a greenhouse study, Rowley (1991) observed that 8 to 12 hr leaf wetness duration were required for *R. solani* to infect creeping bentgrass at 12°C.

Brown patch management has relied on the use of fungicides since Bordeaux was first applied to putting greens in 1917 (Carrier, 1923; Monteith and Dahl, 1932). On high value turf, with a very low tolerance for disease injury, current brown patch management strategies involve the use of repeated, preventive fungicide applications (Watschke et al., 1994). Urban environmental concerns and pest management costs have contributed to increased pressure to reduce or limit pesticide use (Wallace, 1993). Integrated pest management (IPM) is considered a broad, multidisciplinary, and ecological approach to managing turf pests and considers many strategies (i.e., cultural, chemical, and biological) (Shurtleff et al., 1987). For turfgrass disease management, knowledge of the pathogen, environment, and host are critical to implementing successful IPM programs (Bruneau et al., 1992; Smiley et al., 1992). Fungicide applications are justified when timed to coincide with environmental conditions favorable for disease development (Schumann et al., 1994). Strategically timed and targeted fungicide applications, as demonstrated by Hall (1984) and Schumann et al. (1994), may facilitate a reduction in fungicide use while maintaining acceptable disease control.

The objectives of this study were as follows: (1) to identify environmental conditions associated with brown patch outbreaks or R. solani infection events in 'Caravelle' perennial ryegrass (Lolium perenne L.); (2) to statistically corroborate the environmental parameters associated with brown patch outbreaks; and (3) to develop and field validate a brown patch forecast model. To satisfy the first two objectives, an environmental monitoring system was used to measure and record air and soil temperatures, relative humidity, leaf wetness duration, soil moisture, solar irradiance, and precipitation. Unlike the model developed by Schumann et al. (1994), environmental data were validated statistically to verify the specific conditions associated with R. solani infection events. To satisfy the third objective, the brown patch forecast model was evaluated on 'Caravelle' perennial ryegrass and 'Bardot' colonial bentgrass for its ability to assist in reducing fungicide applications. The goal was to determine if commercially acceptable brown patch reduction could be achieved with fungicide applications based on an environmental favorability index (EFI).

## MATERIALS AND METHODS

Environmental Monitoring. Micrometeorological conditions and occurrence of R. solani infection events were monitored and measured in a mature stand of perennial ryegrass from June through August 1991 to 1993. Data from 1991 and 1992 were used to develop an EFI, which was field tested in 1993. The study site was located at the University of Maryland Cherry Hill Research and Education Facility in Silver Spring, MD. Soil was a Chillum silt loam (fine-silty, mixed mesic Typic Hapludult) with a pH of 6.0

(measured in distilled water) and 16 mg organic matter g<sup>-1</sup> soil. The turfgrass plots were mowed to either a height of 1.7 or 4.5 cm three times weekly with a reel mower from May through August, and twice weekly in the spring and fall. Turf clippings were removed.

The summer macroclimate at the study site consisted of warm temperatures and humid or high atmospheric moisture, and is classified as temperate continental (Turgeon, 1985). The region is considered the transition zone, which is a suboptimal turfgrass adaptability and growth zone between temperate and subtropical climates (Turgeon, 1985).

Air temperature and relative humidity were measured with two probes at a height of 30 cm above the plant canopy with a Model HMP35C Temperature and Relative Humidity Probe (Campbell Scientific, Logan, UT). To ensure measurement accuracy, the instruments were housed in a 12 plate, louvered radiation shield and protected from direct sunlight and rain. Air temperature was measured with a thermistor, which consisted of a semiconductor made of ceramic materials that determines temperature from electrical resistance (Sutton et al., 1984, 1988). Relative humidity was determined from a Vaisala® capacitive sensor (Campbell Scientific, Logan, Utah). Air temperature and relative humidity data were used to estimate dew duration and vapor pressure deficit from meteorological equations described in Unwin (1980) and Rosenberg et al. (1983). To prevent measurement error, the sensors were compared with instruments of known performance prior to starting the investigation (Coakley, 1985). Sensor calibration results are presented in Appendix E, Tables 50 and 51.

Leaf wetness duration was measured with a Model 237 Leaf Wetness Sensor (Campbell Scientific, Logan, UT). Leaf wetness sensors and their

relevance in plant disease epidemiology were reviewed by Huber and Gillespie (1992). The sensors used in this study were essentially electrical impedance grids. Leaf wetness duration was estimated from two sensors placed horizontally on the surface of the turf canopy at both mowing heights. A total of four sensors were used to monitor leaf wetness conditions. The sensors were coated with flat white latex paint to increase measurement precision and accuracy (Davis and Hughes, 1970; Gillespie and Kidd, 1978). The sensors were field calibrated to record periods of leaf wetness derived from the electrical resistance of the sensor observed at the wet/dry transition point (Gillespie and Kidd, 1978; Huber and Gillespie, 1992). The mean electrical resistance for the four sensors at the wet/dry transition point was 185 kohms. The sensors therefore were programmed to record leaf wetness conditions as either 'wet' or 'dry'. Sensor calibration results are described in Appendix E, Table 52.

Soil temperature was measured with a Model 107 Temperature Probe (Campbell Scientific, Logan, UT), consisting of a thermistor housed in a water resistant casing. Four sensors each were placed in plots mowed to a height of 1.7 or 4.5 cm for a total of eight sensors. The sensors were buried to a depth of  $2.5 \pm 0.5$  cm, as modified from the technique for soil temperature sensor placement described by Fraser (1968). Sensor calibration was performed and these data are shown in Appendix E, Table 53.

Soil moisture was measured with a Model 227 Delmhorst Cylindrical Soil Moisture Block (Campbell Scientific, Logan, UT). The sensor consists of two electrodes embedded in the interior of a porous gypsum block. Soil water potential was calculated from electrical resistance (Kneebone et al., 1992). The gypsum blocks were installed with a capacitor, as recommended

by the manufacturer, to ensure current flow would not cause rapid deterioration of the blocks. The acidifying action of soils tend to degrade gypsum and therefore reduce the longevity and performance of sensors (Kneebone et al., 1992). The blocks were therefore removed from the study site at the end of each summer. Prior to installing the sensors in the field, the blocks were subjected to two wetting and drying cycles necessary to improve uniformity and accuracy in measurements, as recommended by the manufacturer. A small strip of sod was removed, and the blocks were placed in soil at a depth of  $2.5 \pm 0.5$  cm to coincide with the depth of the soil temperature sensors. Next, a slurry of soil and water were mixed and placed around the block, thereby forcing the soil to surround the sensor and ensuring uniform contact. The hole was filled with soil and the turf was replaced. Four sensors were used for each mowing height, for a total of eight sensors.

Precipitation in the form of rainfall visually was measured with a funnel-type gauge. Total rainfall amount was measured daily at 1.5 m above the soil surface. Water from irrigation also was measured. Solar irradiance was measured with one LI200S Pyranometer (LI-COR, Lincoln, NE). The sensor consists of a silicon photocell pyranometer, and was placed 30 cm above the plant canopy. The pyranometer was calibrated and certified by the manufacturer.

Data Collection and Management. All environmental monitoring instruments were connected to a CR10 Measurement and Control Module (Campbell Scientific, Logan, UT), or datalogger encased in a weather-proof aluminum box and powered by a 12v lattern battery. Since the number of required sensors for this study exceeded the number of CR10 datalogger



input channels, an AM-416 Multiplexer (Campbell Scientific, Logan, UT) was used to accommodate the additional probes. All instruments were programmed to measure environmental conditions at 5 min intervals and average data every 60 min. Environmental data stored in the datalogger were transferred periodically into a Sharp MZ-200 Personal Computer (Sharp Corporation, Mahwah, NJ). The personal computer was connected to the datalogger with a Model SC32A optically isolated RS232-type interface connector (Campbell Scientific, Logan, UT). Further data management and analyses were conducted on Statistical Analysis Software (SAS Institute, 1985).

The environmental conditions measured (i.e., air and soil temperatures, relative humidity, leaf wetness duration, soil moisture, precipitation, and solar irradiance) provided 17 environmental variables that were tested for use in a brown patch forecast model. All measurements were determined from a 24 hr interval prior to 6 AM. The 17 environmental variables were: mean percent relative humidity (RHmean); hrs of relative humidity  $\geq 90\%$  (RH90) or  $\geq 95\%$  (RH95); hrs of leaf wetness duration (LWD); hrs of dew duration (DEW); mean vapor pressure deficit in millibars (VPD); total precipitation (mm) during the 24 hr (RAIN24) or 48 hr (RAIN48) period prior to 6 AM; minimum, mean, and maximum air temperatures ( $^{\circ}\text{C}$ ) (AIRmin, AIRmean, or AIRmax); minimum, mean, and maximum soil temperatures ( $^{\circ}\text{C}$ ) (SOILmin, SOILmean, or SOILmax); mean soil water potential in MPa (SWPmean); and mean and maximum solar irradiance ( $\text{W m}^{-2}$ ) (SOLmean and SOLmax).

Soil temperature was used to compute cumulative degree-days prior to the first observed infection events, according to the method reviewed in Ritchie and NeSmith (1991). Accounting of degree-day units began on 1 June, and were calculated by the following degree-day equation:  $\sum_{i=1}^n [(T_{\max} - T_{\min})/2] - [T_b]$ , where  $T_{\max}$  and  $T_{\min}$  equal maximum and minimum soil temperature, respectively,  $T_b$  is the base soil temperature, and  $n$  is the number of days elapsed since 31 May (Ritchie and NeSmith, 1991). A base soil temperature of 16°C was used because R. solani infection events were not observed below that temperature.

Development Of The Brown Patch Forecast Model. An infection event, also referred to as a disease outbreak, was determined visually by the presence of R. solani mycelium on the turfgrass foliage. From June through August 1991, 1992, and 1993, the study site was monitored daily between 7 and 8 AM for the presence of foliar mycelium. Infection event data were collected daily, and consisted of either a 'yes' or 'no' for the presence or absence of R. solani mycelium, thus indicating a visual confirmation of an infection event. While the presence (yes) or absence (no) of mycelium was used in developing the model, the relative amounts (i.e., low, moderate, and high) were also recorded. Reference to minor infection events indicate that little foliar mycelium were observed; whereas, the presence of copious amounts of mycelium were described as a major or severe infection event.

Environmental data from 1991 and 1992 were subjected to correlation analysis to identify key variables associated with brown patch outbreaks and to identify those variables that were highly correlated with each other. Correlation coefficients were computed to determine the strength of the associations among the environmental variables measured. Simple

correlation coefficients also were used to identify variables that were highly intercorrelated (i.e., condition of multicollinearity) and therefore are of concern when selecting model parameters (Draper and Smith, 1981). Multicollinearity can occur when too many variables are included in a model, and a number of different variables may in fact measure similar phenomena (Freund and Littel, 1991).

The distributions of the environmental variables were compared to distinguish between specific environmental conditions that occurred during periods when brown patch outbreaks were noted versus those conditions observed when infection events did not occur. A comparison was made between the distributions of each group (i.e., weather conditions on days with versus days without infection events) for each of the 17 environmental variables. Data were subjected to univariate analysis, a procedure that combines frequency distributions and descriptive statistics (Cody and Smith, 1991). The statistical procedure used was modified from Scherm and van Bruggen (1994). Since the distributions of all 17 environmental variables tested were intercorrelated, a Bonferroni correction factor (i.e., Type I error adjustment) was used to ensure that the distributions were compared at the 5% significance level (SAS Institute, 1985). Since there were 17 environmental variables, the corrected probability level was calculated as follows:  $0.05/17 = 0.0029$ .

Next, an environmental favorability index (EFI) was developed using those environmental variables that were correlated with and exhibited a strong relationship to *R. solani* infection events. To determine the validity of the EFI, observed infection events were compared with the predicted or expected EFI values. Chi-square ( $\chi^2$ ) analysis was used to test the null

hypothesis that there was no relationship between the EFI and brown patch outbreaks (Witte, 1980). This statistical procedure was used to develop a *Pythium* blight forecast model for putting green turf (Nutter et al., 1983). The value of chi-square was calculated by the following equation:  $\chi^2 = \sum [(f_o - f_e)/(f_e)]$ , where  $f_o$  equals the number or frequency of observed outcomes and  $f_e$  represents the expected outcomes, as expressed by a 'yes' or 'no' infection event (Witte, 1980). Once an acceptable EFI was developed, the data also were subjected to multiple regression analysis in an attempt to develop a simplified mathematical model that could describe the relative weighting of environmental conditions associated with the EFI.

Field Validation Of The Brown Patch Forecast Model. In summer 1993, the brown patch forecast model was tested on mature stands of 'Bardot' colonial bentgrass and 'Caravelle' perennial ryegrass mowed to a height of 1.3 and 2.2 cm, respectively. These test sites were juxtaposed to the original study area, and had the same soil type. The plots were mowed twice weekly with a reel mower, and clippings always were removed.

To test the forecast model, chlorothalonil [2,4,5,6-tetrachloroisophthalonitrile] was applied based on a 14-day calendar schedule or according to the forecast model (i.e., the EFI). Forecast-based fungicide sprays were applied on a 14-day interval (i.e., fungicide was not generally re-applied within 14 days of the previous application, regardless of a brown patch forecast). Due to a severe infection event on 14 July 1993, a forecast-based spray was applied on 15 July (i.e., 13 rather than 14 days after forecast-based application on 2 July). Calendar sprays were initiated before any observed blighting by *R. solani* on 1 June. A non-fungicide-treated check also was included. Treatments were arranged as a randomized complete

block design with four replications, and individual plots measured 1.5 by 1.5 m. Chlorothalonil was applied at a standard rate of 9.1 kg ai ha<sup>-1</sup> from June through August 1993. The fungicide was applied with a CO<sub>2</sub> pressurized (262 kPa) sprayer equipped with an 8010 fan fan nozzle, and calibrated to deliver 1018 L ha<sup>-1</sup> water.

As previously mentioned, the plots were inspected daily for the presence of foliar R. solani mycelium. The plots also were evaluated visually for blight using the Horsfall-Barratt scale (Horsfall and Cowling, 1978; Schumann and Wilkinson, 1992). According to the scale, visual blight ratings were divided into 12 classes reflecting a range of percent plot area blighted as follows: 0 (0%), 1 (1-2%), 2 (3-5%), 3 (6-11%), 4 (12-24%), 5 (25-49%), 6 (50-74%), 7 (75-87%), 8 (88-93%), 9 (94-96%), 10 (97-99%), and 11 (100%). Averaging class values, where percent disease is desired, would biased results thus negating the advantages of the disease assessment scale (Campbell and Madden, 1991). Since percent area diseased was the desired variable, each class value was converted to a percentage according to the midpoint rule prior to statistical analysis (Campbell and Madden, 1991). The plots were rated weekly for area blighted during periods conducive for disease development. Blight ratings > 2 and > 5 % were subjectively judged as unacceptable in commercially maintained "high quality" turf or in sites managed in an IPM program, respectively.

All disease severity data were subjected to analysis of variance using Statistical Analysis Software (SAS Institute, 1985). Mean separation was provided by Fisher's protected least significant difference test at  $P \leq 0.05$  (Steel and Torrie, 1980; Gilligan, 1986). Brown patch severity data over time were used to calculate area under the disease progress curve (AUDPC),

which represents the course of the disease epidemic over time (Waggoner, 1981) as is expressed as percent disease X day. The AUDPC data were calculated by the following formula:  $\sum[(y_i + y_{i+1})/2][t_{i+1} - t_i]$ , where  $i = 1, 2, 3, \dots, n-1$ ,  $y_i$  is the amount of disease and  $t_i$  is the time of the  $i$ th rating (Shaner and Finney, 1977; Berger, 1988). Mean values of AUDPC were analyzed as previously described. The AUDPC values for the colonial bentgrass and perennial ryegrass sites represent data collected from 9 June to 27 September and 6 July to 27 September, respectively.

## RESULTS AND DISCUSSION

Environmental data collected from June through August 1991 to 1993 are presented in Appendix E, Table 48. Soil temperature, soil moisture, and leaf wetness duration data were statistically similar in turf subjected to both mowing heights (data not shown), and data therefore were averaged over mowing heights. Due to a sensor malfunction in 1991, soil moisture only was measured in 1992 and 1993. All data collected were summarized and statistically analyzed to identify key environmental conditions associated with R. solani infection events. Since brown patch outbreaks typically occurred overnight, data reflect environmental conditions measured during a 24-hr period prior to 6 AM.

Environmental data from 1991 and 1992 were used to develop the brown patch EFI, which was tested in 1993. R. solani infection events were observed 6 and 12 times in 1991 and 1992, respectively (Appendix E, Table 48). The presence or absence of foliar R. solani mycelium was statistically more reliable than blight ratings for developing the EFI (data not shown).

**Brown Patch Forecast Model Development.** Environmental data were pooled from 1991 and 1992 ( $n = 174$  evaluation days) and subjected to statistical analyses. Soil moisture measurements from 1992 only were considered in developing the model, therefore  $n = 87$  evaluation days were used for that environmental variable. The 17 environmental variables were correlated among themselves to determine those variables that were highly associated with each other. While correlations among the 17 environmental variables tested were generally low, there were a few notable exceptions (Appendix E, Table 49). High correlation coefficients were observed between SOLmean and SOLmax ( $r = 0.909$ ); VPD and RHmean ( $r = -0.888$ ); minimum, mean, and maximum soil temperature measurements ( $r \geq 0.838$ ); and between DEW and RH90 ( $r = 0.809$ ). These observations were not surprising since periods of high relative humidity often are used to indicate the presence of dew (Wallin, 1963), or to estimate dew duration (Gleason et al., 1994). Simple correlation coefficients were moderately high among relative humidity parameters (RHmean, RH95, and RH90) ( $r \geq 0.665$ ) and among minimum, mean, and maximum air temperature measurements ( $r \geq 0.538$ ).

Correlation coefficients between each of the 17 environmental variables versus brown patch outbreaks were weak (Appendix E, Table 49). The highest correlation coefficients between environmental variables and observed infection events were associated with RH95 ( $r = 0.420$ ) and RHmean ( $r = 0.419$ ). Inspection of the environmental data show that prolonged periods of high relative humidity generally coincided with brown patch outbreaks (Appendix E, Table 48). The brown patch forecast model developed by Schumann et al. (1994) also relied on periods of high relative

humidity ( $\geq 10$  hrs of relative humidity  $\geq 95\%$ ) that coincided with specific air (minimum  $15^{\circ}\text{C}$  and mean  $20^{\circ}\text{C}$ ) and soil (minimum  $18^{\circ}\text{C}$  and mean  $21^{\circ}\text{C}$ ) temperature conditions. Severe disease outbreaks also were associated with  $\geq 2.54$  mm precipitation within 36 hr of a high relative humidity period (Schumann et al., 1994).

Since the correlation data did not provide evidence of strong associations between the environment and brown patch, the distributions of the 17 environmental variables were determined on days with or without infection events. Distribution data are presented as box-plots in Figure 1. Among most variables, there were no distinct statistical separations between days with or without infection events. Best separation was noted with RHmean, LWD, DEW, and VPD. The statistical distributions of those variables were significant at  $P < 0.0029$ , which was equivalent to a significance level of  $P \leq 0.05$ . The environmental variables with significant data distributions may indicate their potential use to predict brown patch outbreaks.

Based on correlation data between the environmental conditions and brown patch outbreaks (Appendix E, Tables 48 and 49), and examination of statistical distributions (Figure 1), it was apparent that R. solani infection events were influenced by a combination of microclimate factors. To summarize the influence of several environmental variables on infection events, the environmental favorability index (EFI) was developed.

The EFI is an indicator of the complex interaction of several important environmental variables and their association with the appearance of foliar R. solani mycelium. Several combinations of environmental conditions were used to compute the EFI and they were RH95, RHmean, LWD and/or



RAIN48, AIRmin and SOILmin (Table 14). Arbitrary point values shown in Table 14 were assigned to specific environmental conditions, which reflect their importance in predicting infection events. The use of point values and decision rules for use in plant disease prediction were reviewed in Krause and Massie (1975) and Fry and Fohner (1985). Analyses of the environmental data distributions (Figure 1) and observed microclimate conditions associated with brown patch outbreaks (Appendix E, Table 48), were used to determine the specific environmental conditions assigned to each value. The point values used were those that resulted in the highest chi-square value, thereby indicating the strongest association between R. solani infection events and the EFI. The individual point values assigned to the specific environmental conditions were added, and this resulted in a daily cumulative index or EFI (Table 14).

An  $EFI \geq 6$  indicated that the environment was highly favorable for an infection event, therefore, brown patch risk was considered high. An EFI of 5 was equivalent to moderate disease risk, in which environmental conditions potentially favored an infection event. An  $EFI \leq 4$  indicated that environmental conditions were not conducive for disease development.

Environmental Favorability Index Assessment, 1991. Prior to the initiation of data collection in 1991, an early brown patch outbreak occurred on 3 June. Chlorothalonil was applied ( $6.3 \text{ kg ai ha}^{-1}$ ) to slow the disease, and therefore prevented further blighting on 17 and 18 June when an  $EFI \geq 6$  was observed (Figure 2A). These early brown patch forecasts, however, were disregarded because of the application of chlorothalonil. In July and August, all 6 brown patch outbreaks coincided with an  $EFI \geq 6$  (Figure 2A).

The most severe disease outbreak occurred on 2 July, and the EFI was 6 (Figure 2A). The 24 hr period prior to 2 July was associated with prolonged periods of high relative humidity (RH95 = 14 hr) and leaf wetness (LWD = 13 hr), minimum nighttime air and soil temperatures were  $\geq 23^{\circ}\text{C}$ , and precipitation totalled 16 mm. Environmental conditions favorable for brown patch persisted through 10 July, and 2 subsequent, but less severe infection events (i.e., small amounts of foliar mycelium noted) were observed on 7 and 10 July. These were considered major infection events, however, and were associated with high disease risk forecasts (i.e., EFI = 6). From 11 to 26 July, prolonged periods of high humidity and leaf wetness were not observed. Rainfall events on 22 through 25 July preceded high humidity (RH95 = 9 hrs) and leaf wetness (LWD = 7 hrs) conditions observed on 26 July (i.e., EFI = 6 on 26 July). A major infection event was observed 48 hr afterward on 28 July.

Favorable environmental conditions for an infection event were not observed again until 8 and 9 August (EFI = 6), and trace amounts of foliar mycelium were observed afterward on 11 August (Figure 2A). A high risk forecast on 15 August preceded a minor infection event on 18 August. Brown patch was forecast on 20 and 29 August (i.e., EFI = 6), but no foliar mycelium was observed within 72 hrs, and these forecasts were considered false predictions.

In summary, all six brown patch outbreaks coincided with or were preceded by an  $\text{EFI} \geq 6$ . The infection event forecasts of 17 and 18 June were disregarded because of the chlorothalonil application of 3 June. On two dates (i.e., 20 and 29 August), however, brown patch was forecast, but

no foliar R. solani mycelium or subsequent increase in blighting were observed.

Environmental Favorability Index Assessment, 1992. In 1992, brown patch was first predicted (i.e.,  $EFI \geq 6$ ) on 5, 6, and 7 June (Figure 2B). At that time, there were precipitation events (55 mm total), and prolonged leaf wetness ( $LWD = \geq 15$  hrs) and high humidity ( $RH95 \geq 8$  hrs) periods. Minimum air temperatures, however, were  $\leq 13^{\circ}\text{C}$  on four consecutive days prior to 5 June, and the  $EFI$  was  $\leq 2$  on 1 to 4 June. The forecasts of 5, 6, and 7 June were considered false, however, since no R. solani foliar mycelium was observed within 72 hrs of the forecast. From 11 to 14 June, minimum air temperature again decreased below  $\leq 16^{\circ}\text{C}$ , and the  $EFI$  ranged from 0 to 5 on those days. These cooler temperatures may have prevented disease development at that time. Periods of high humidity ( $RH95 \geq 8$  hrs), leaf wetness ( $LWD \geq 14$  hrs), and precipitation (19 mm total) again were favorable for brown patch on 25 and 26 June, however, minimum air temperatures were  $\leq 15^{\circ}\text{C}$  on those dates. Therefore, infection events were not observed on 25 or 26 June, and the  $EFI$  ( $EFI = 3$ ) did not forecast brown patch on those dates. Additionally, minimum air temperatures ranged from 9 to  $18^{\circ}\text{C}$  on five consecutive days prior to 25 June. These observations show the importance of minimum temperatures  $\geq 16^{\circ}\text{C}$  in influencing R. solani infection events. Temperature data also suggested that there may be a soil temperature-based degree-day requirement prior to the onset of disease.

The first major infection events, observed on 1 and 2 July, coincided with an  $EFI \geq 6$  (Figure 2B). A minor brown patch outbreak on 8 July was preceded with an  $EFI \geq 6$  on 4 July. Trace amounts of R. solani mycelium were observed on 14 and 15 July, and were associated with 19 mm irrigation

from the previous 48 hr. Relative humidity conditions necessary for foliar mycelium development did not occur at that time, and therefore the EFI did not predict those minor infection events. Trace amounts of foliar mycelium again were observed on 22 July, followed by an EFI of 6 on 24 July. The minor infection event on 22 July therefore was not predicted. Brown patch outbreaks on 24, 28 and 29 July coincided with or were preceded by high risk warnings (i.e.,  $EFI = 6$ ) on 24 and 25 July. Favorable environmental conditions for brown patch were the result of extended periods of high humidity ( $RH_{95} \geq 5$  hrs;  $RH_{mean} = 93\%$ ) and natural precipitation (102 mm total) at that time.

From 30 July to 12 August, periods of low humidity and low air temperatures occurred, and brown patch was not a threat. On 12 and 14 August, high humidity ( $RH_{95} \geq 12$  hrs) and leaf wetness ( $LWD \geq 23$  hrs) conditions corresponded to high risk forecasts (Figure 2B). The high disease risk warnings (i.e.,  $EFI \geq 6$ ) on 12 and 14 August, however, were considered a false forecast because *R. solani* foliar mycelium did not appear within 72 hrs following the predictions. An EFI of 6 also was recorded from 16 to 19 August, and *R. solani* foliar mycelium was observed on 18 and 19 August. A minor infection event on 26 August was preceded by a high risk forecast (i.e.,  $EFI \geq 6$ ) one day earlier.

In summary, 9 of 12 brown patch outbreaks were associated with an  $EFI \geq 6$ , and 3 of 12 infection events were considered minor and occurred without an EFI-based forecast. All major brown patch infection events, however, were predicted in 1992. Five, false high risk forecasts were issued in 1992, however, 3 of the 5 false forecasts occurred in early June when low temperature conditions were observed.

**Degree-Days Prior To Appearance Of Foliar Mycelium.** Because of the false predictions for brown patch outbreaks in early June 1992, degree-day data were calculated beginning 1 June to determine if there were a heat accumulation influence on the first visible infection event of the year. Degree-day units were not calculated in 1991 due to the major infection event on 3 June. Degree-day totals of 214 and 310 were associated with the first severe infection events in perennial ryegrass on 1 July 1992 and 3 July 1993, respectively. Minor infection events were observed in colonial bentgrass on 9 and 20 June 1993, corresponding to 58 and 177 degree-days, respectively. The first major infection event in colonial bentgrass, however, also was observed on 3 July. Degree-day accumulation data based on soil temperature may provide an indirect measure of R. solani inoculum potential. The use of degree-days may provide a more accurate measure for predicting the first infection event in each year, and therefore warrants further study. Future investigations should initiate degree-day data collection on an earlier date (i.e., about 1 May in Maryland).

**Validation Of The Environmental Favorability Index By Chi-Square Analysis.** Chi-square analysis was performed to determine the strength of the observed association between the EFI and the R. solani infection events. The result was a significant chi-square value ( $X^2 = 53.27$ ;  $P < 0.001$ ) using 1991 and 1992 data and therefore the null hypothesis (i.e., that there was no relationship between the EFI and brown patch) was rejected. This analysis showed that the EFI-based predicted infection events were not due to random chance.

**Validation Of The Environmental Favorability Index By Multiple Regression.** Multiple regression analysis was used to identify the most

important and significant environmental conditions that predict the EFI. Results of this analysis may provide useful information that reduces or simplifies the disease prediction model. Data from the 17 variables measured in 1991 and 1992 were subjected to stepwise regression analysis (SAS Institute, 1985; Butt and Royle, 1990). Several environmental variables were identified as potential model parameters (data not shown), however, air temperature and relative humidity data provided the simplest and best-fit model (data not shown). A suitable second-order regression model was developed for relating the EFI to minimum air temperature and mean relative humidity. The model formula was as follows:

$$EFI = - b_0 + b_1 RH + b_2 T - b_{22} T^2 + e$$

in which EFI = environmental favorability index; T = minimum daily air temperature (°C); and RH = mean daily relative humidity (%) for a 24-hr period prior to 6 AM. The *b* values (i.e., *b*<sub>0</sub>, *b*<sub>1</sub>, *b*<sub>2</sub> and *b*<sub>22</sub>) are least squares estimates of the partial regression coefficients. The *e* represents a normally distributed random variable with a mean of zero and a constant variance. All estimated coefficients were highly significant at *P* = 0.0001, and the regression model accounted for 70% of the observed variation of the EFI. The actual regression equation was:

$$EFI = - 21.467 + 0.146RH + 1.358T - 0.033T^2.$$

The relationship of minimum daily air temperature and mean daily relative humidity to EFI is represented in a computer-generated surface graph from the 1991 and 1992 data (Figure 3A). A comparative graph derived from the regression equation (Figure 3B) indicated a reasonable fit for minimum air temperature range of 10 to 25°C and mean relative humidity range of 60 to 98%. Examination of the residuals (i.e., the difference

between the original data and those data predicted by the regression equation) supports the assumption that errors were independent and normally distributed, have a mean of zero, and a constant variance (Appendix E, Figure 5).

Examination of the regression model may provide insight into the association of minimum air temperature and mean relative humidity during a 24-hr period prior to 6 AM, and the appearance of R. solani foliar mycelium (Figure 3B). The model suggested that minimum air temperatures of 16 to 17°C or 24 to 25°C and a mean relative humidity  $\geq 95\%$  were required for the appearance of R. solani mycelium (Figure 3B). The regression model also showed that the appearance of R. solani mycelium was associated with minimum air temperatures from 19 to 23°C and at least a mean relative humidity of  $\geq 92\%$ .

When comparing the regression model-based EFI to observed infection events, 6 of 6 and 9 of 12 brown patch outbreaks were forecast in 1991 and 1992, respectively (Appendix E, Table 48). The accuracy of the EFI developed from the regression model was equal to the EFI determined from Table 14. Therefore, the EFI developed from the regression model (i.e., minimum air temperature and mean relative humidity) would provide comparable disease forecasts, but utilize fewer environmental variables.

The regression model developed from 1991 and 1992 data were re-analyzed by adding environmental and brown patch data from 1993. The regression equation developed from all three years of data was similar to the model previously described for 1991 and 1992, and resulted in a coefficient of determination ( $r^2$ ) of 0.64.

EFI Forecast Model Validation, 1993. Disease risk predicted by EFI (calculated from Table 14) was tested at two sites adjacent to the original study area. The study area in 1993 had similar soil and microclimate conditions to the 1991 and 1992 sites. From June through August 1993, a combined total of 22 infection events were observed on both the perennial ryegrass and colonial bentgrass test sites (Figure 4). Using the brown patch forecast model, infection events were forecast on 19 of the 22 occasions in 1993. The predominate R. solani biotypes were AG-1 IA and AG-2-2 IIIB on the perennial ryegrass and colonial bentgrass sites, respectively.

The EFI provided warnings for the first or most severe brown patch outbreaks in perennial ryegrass (3 and 4 July) and colonial bentgrass (9 and 20 June, and 3 and 4 July) (Figure 4). Subsequent brown patch outbreaks of 14 and 15 July and 2 through 17 August also were predicted by the EFI. Environmental conditions did not warrant a brown patch forecast from 8 to 13 July, yet trace amounts of active R. solani mycelium were observed on 8 and 11 July. Perhaps the fungus remained viable on leaves from the 3 and 4 July outbreaks, and required three to four consecutive days of AIRmin  $\geq 16^{\circ}\text{C}$  and RHmean  $\geq 80\%$  to re-develop the foliar mycelium observed between 8 and 11 July. A minor infection event again was observed only in colonial bentgrass on 19 July, which corresponded to a moderate disease risk warning (i.e., EFI = 5). Favorable environmental conditions for brown patch outbreaks persisted in August. On both test sites in 1993, an EFI  $\geq 6$  was associated with all major R. solani infection events.

Perennial Ryegrass. Chlorothalonil was applied on 1 June, and plots were first rated for foliar blighting on 6 July after the first major infection event of 3 July (Table 15). Fungicide-treated plots were disease free until 16 July.



With both fungicide treatments (i.e., calendar- versus forecast-based), unacceptable blight levels were observed from 21 July through 24 August. The blighting was not due to a forecast failure, but was attributed to the inability of chlorothalonil to provide effective control for 14 days during the high disease pressure period. Commercially acceptable brown patch control may have been achieved if chlorothalonil were applied on 10-day intervals. The greatest amount of blighting on fungicide-treated turf was observed on 3 August. Turf recovered and blight levels in fungicide-treated turf were acceptable (i.e.,  $\leq 2\%$  plot area blighted) by 27 September. The amount of blighting, however, was equivalent between both schedules, but two less sprays were applied using the forecast-based schedule.

When comparing all treatments over time, significantly greater AUDPC values were observed in non-fungicide-treated plots (Table 15). The AUDPC values between fungicide-treated plots were similar. Hence, blight levels were similar between spray treatments, but the forecast model resulted in two less fungicide applications compared to calendar-based sprays.

Colonial Bentgrass. Chlorothalonil was applied on 1 June. On 9 June, R. solani mycelium was first observed on the colonial bentgrass site, but only in non-fungicide-treated check plots within the study area. Unacceptable ( $\geq 5\%$  plot area blighted) injury was first observed on 21 June in non-fungicide-treated plots (Table 16). Disease severity peaked on 21 July and declined thereafter. All fungicide-treated plots had acceptable thresholds of turf injury ( $\leq 1\%$  plot area blighted) throughout the evaluation period.

When comparing all treatments over time, significantly greater AUDPC values were observed in non-fungicide-treated plots, while no blight

differences were observed between the two fungicide treatments (Table 16). Use of the EFI model again resulted in two less fungicide applications, when compared to calendar-based sprays.

## SUMMARY AND CONCLUSIONS

The analyses of environmental conditions monitored in 1991 and 1992 revealed that a combination of environmental factors influenced the occurrence of *R. solani* infection events in perennial ryegrass. An index (i.e., EFI) was developed to compile and summarize the environmental conditions associated with the infection events. The EFI, which employed six environmental variables, effectively predicted 85% (34 of 40) of all infection events in 1991 to 1993. All major infection events were successfully predicted. Minimum environmental conditions associated with major infection events were as follows:  $\geq 8$  to 10 hr relative humidity  $\geq 95\%$  (or mean relative humidity  $\geq 85$  to 90%);  $\geq 6$  to 8 hr leaf wetness duration (or  $\geq 12$  mm precipitation); and minimum air and soil temperatures  $\geq 16^{\circ}\text{C}$ .

The brown patch thresholds developed by Rowley (1991) and formulated into a model by Schumann et al. (1994) employed  $\geq 10$  hrs of relative humidity  $\geq 95\%$ , precipitation ( $\geq 2.54$  mm), minimum ( $15^{\circ}\text{C}$ ) and mean ( $20^{\circ}\text{C}$ ) air temperatures, and minimum ( $18^{\circ}\text{C}$ ) and mean ( $21^{\circ}\text{C}$ ) soil temperatures to predict brown patch. The success of their model was confirmed by its ability to assist in reducing fungicide applications. The high-risk disease warnings of their model were associated with increases in brown patch severity, and not the appearance of *R. solani* foliar mycelium. Since the brown patch forecast model developed at the University of

Maryland was based on R. solani infection events (i.e., the appearance of foliar mycelium), it is difficult to compare the accuracy of the both models. The model developed by Schumann et al. (1994), however, initially had greater false forecasts (average > 6 false or missed predictions yr<sup>-1</sup> site<sup>-1</sup>), which were attributed to a decrease in air temperatures below 15°C immediately following the forecasts. They corrected this deficiency by recommending that the user cancel a disease prediction if air temperatures fall below 15°C following a forecast.

Multiple regression analyses showed that the EFI was related to minimum daily air temperature and mean relative humidity with 70% of the variability explained by the model. When an EFI was developed based on just two variables (i.e., minimum daily air temperature and mean relative humidity), it was shown to be as accurate in forecasting R. solani infection events as the six variable EFI (Table 14).

This investigation also found that leaf wetness duration or precipitation were important elements in the EFI, but not soil moisture. Leaf wetness duration (minimum LWD ≥ 6 hrs or LWD ≥ 8 to 10 hrs for severe disease outbreaks) was an important factor in encouraging the development of foliar mycelium. This information was particularly useful when viewed in combination with temperature and humidity data. Precipitation events also were associated with brown patch outbreaks. Precipitation events provided for longer periods of high humidity and leaf wetness conditions (i.e., at least ≥ 6 hrs LWD) and severe infection events generally coincided with ≥ 12 mm of rainfall. The influence of soil moisture on brown patch occurrence was inconclusive, since periods of high humidity and precipitation events often corresponded to soil moisture levels near field capacity. A field study in

which soil moisture levels are manipulated, possibly through controlled irrigation, may clarify the effect of soil moisture on R. solani infection events.

Krause and Massie (1975), state that the accuracy of a disease prediction system is dependent on its ability to interpret the meteorological and biological relationships that precede infection or disease development. In 1991 and 1992, 15 of 18 R. solani infection events in perennial ryegrass coincided with an EFI  $\geq 6$ . Except for an early June 1991 outbreak that preceded the initiation of data collection, all major infection events were forecast accurately. On nine occasions in 1991 and 1992, an EFI  $\geq 6$  was observed, but there was no visible evidence of foliar R. solani mycelium within 72 hr. Two false forecasts in 1991 were disregarded because chlorothalonil was applied, and three false forecasts in 1992 may be discounted because of low ( $\leq 13^{\circ}\text{C}$ ) air temperatures preceding the forecast period. While these three false 1992 forecasts were accompanied by an EFI = 6, no disease may have developed because of insufficient degree-day accumulation for inoculum production. Degree-day totals, as determined from soil temperature, between 200 and 300 were associated with the first brown patch outbreaks in 1992 and 1993 in perennial ryegrass. Further research, however, is needed to fully characterize the influence of degree-days on the appearance of R. solani foliar mycelium. In 1993, the forecast model predicted 19 of 22 brown patch infection events on the colonial bentgrass and perennial ryegrass model validation sites. Three minor infection events were not predicted, and there were no false predictions in 1993.

The EFI field validation in both perennial ryegrass and colonial bentgrass employed seven calendar-based fungicide sprays compared to

five forecast-timed applications. Calendar- and forecast-based fungicide applications provided equivalent levels of brown patch control. Hence, there was a 29% reduction in fungicide application frequency at both sites using the forecast-based spray schedule.

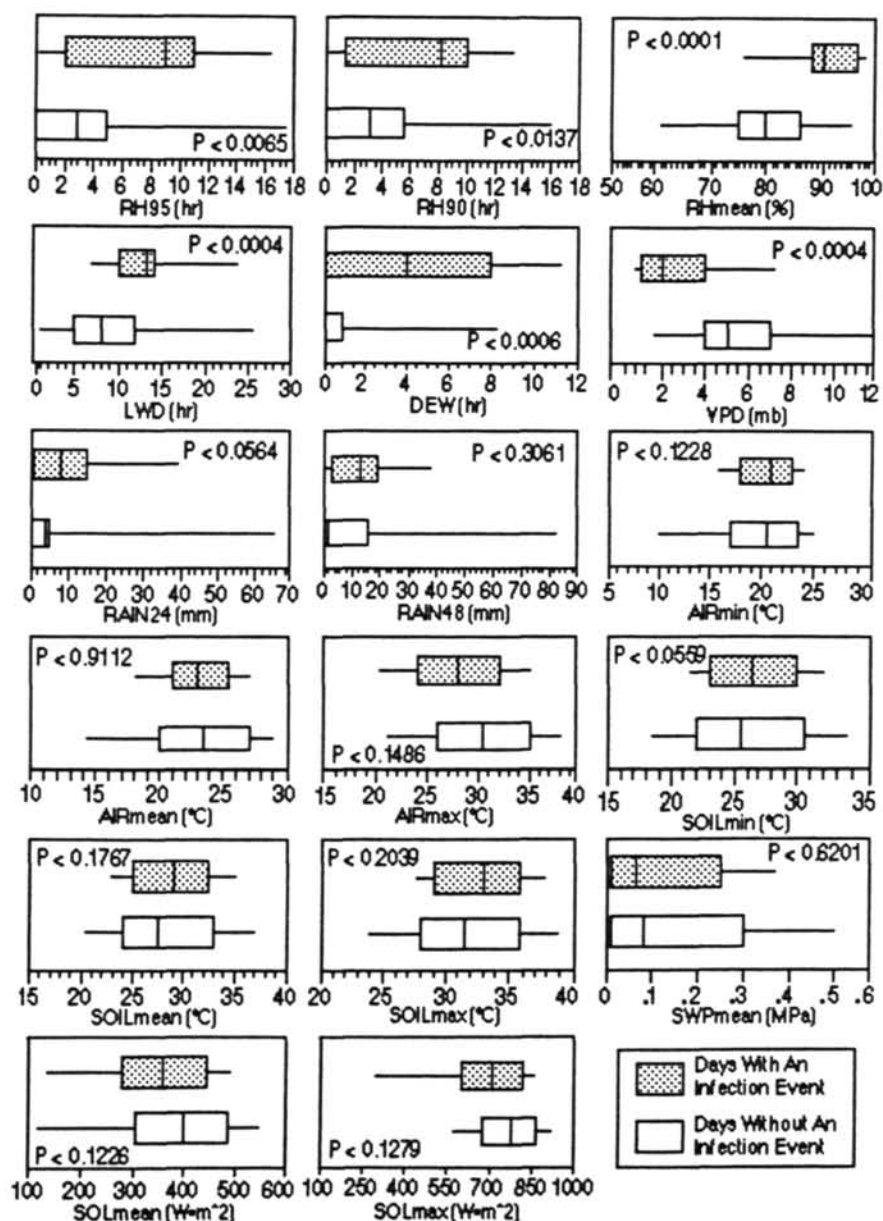


Figure 1. The distributions of the environmental variables during days when *R. solani* infection events occurred and days when infection events were not observed. Data represent 25% (left side of box), 50% (center line), and 75% (right side of box) of the observations recorded in 1991 and 1992 ( $n = 174$ ). Horizontal lines extending away from the box indicate extreme measurements recorded. The distributions were significantly different at the 5% level where  $P < 0.0029$  ( $0.05 / 17 = 0.0029$ ).

Table 14. Variables used to calculate the environmental favorability index (EFI) for predicting *R. solani* infection events.

Variable <sup>†</sup>	Condition	Value <sup>‡</sup>	Variable <sup>†</sup>	Condition	Value <sup>‡</sup>
RH95	≤ 4 hr	0	AIRmin	< 16°C	-2
	5 - 7 hr	1		≥ 16°C	1
	≥ 8 hr	2			
RHmean	< 75 %	0	Soilmin	< 16°C	-2
	≥ 75 %	1		≥ 16°C	1
LWD or RAIN48	≥ 6 hr ≥ 12 mm	1	Rain48	≥ 40 mm	1

<sup>†</sup>Variables measured in a 24 hr period prior to 6AM for all days where: RH95 = hr relative humidity ≥ 95%; RHmean = mean percent relative humidity; LWD = hr leaf wetness duration; RAIN48 = precipitation (mm) during the prior 48 hr period; AIRmin = minimum air temperature (°C); and SOILmin = minimum soil temperature (°C).

<sup>‡</sup>Point values are added to calculate the EFI, where EFI ≥ 6 equals high risk (environmental conditions favorable for brown patch outbreaks); EFI = 5 indicates moderate risk (environmental conditions potentially favorable for brown patch); and EFI ≤ 4 equals low risk (environmental conditions not conducive for brown patch).

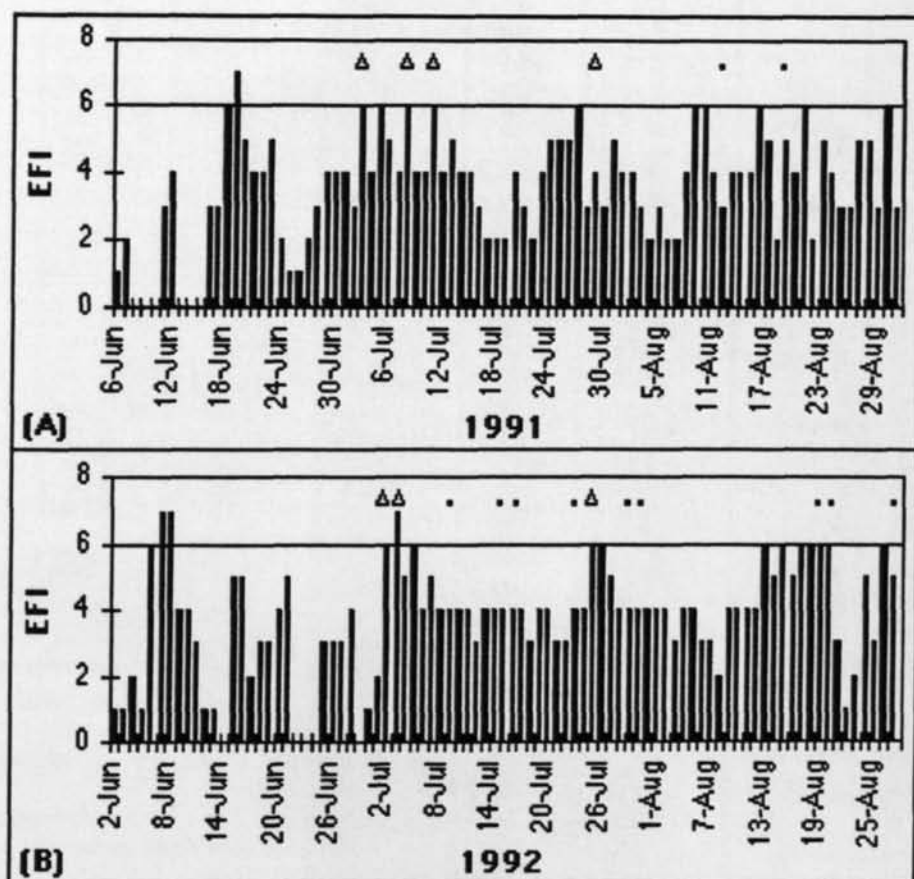


Figure 2. Environmental favorability index (EFI) predicted infection events versus observed brown patch outbreaks ( $\Delta$  = major and  $\bullet$  = minor *B. solani* infection events, respectively) in perennial ryegrass in 1991 (Figure 2A) and 1992 (Figure 2B).



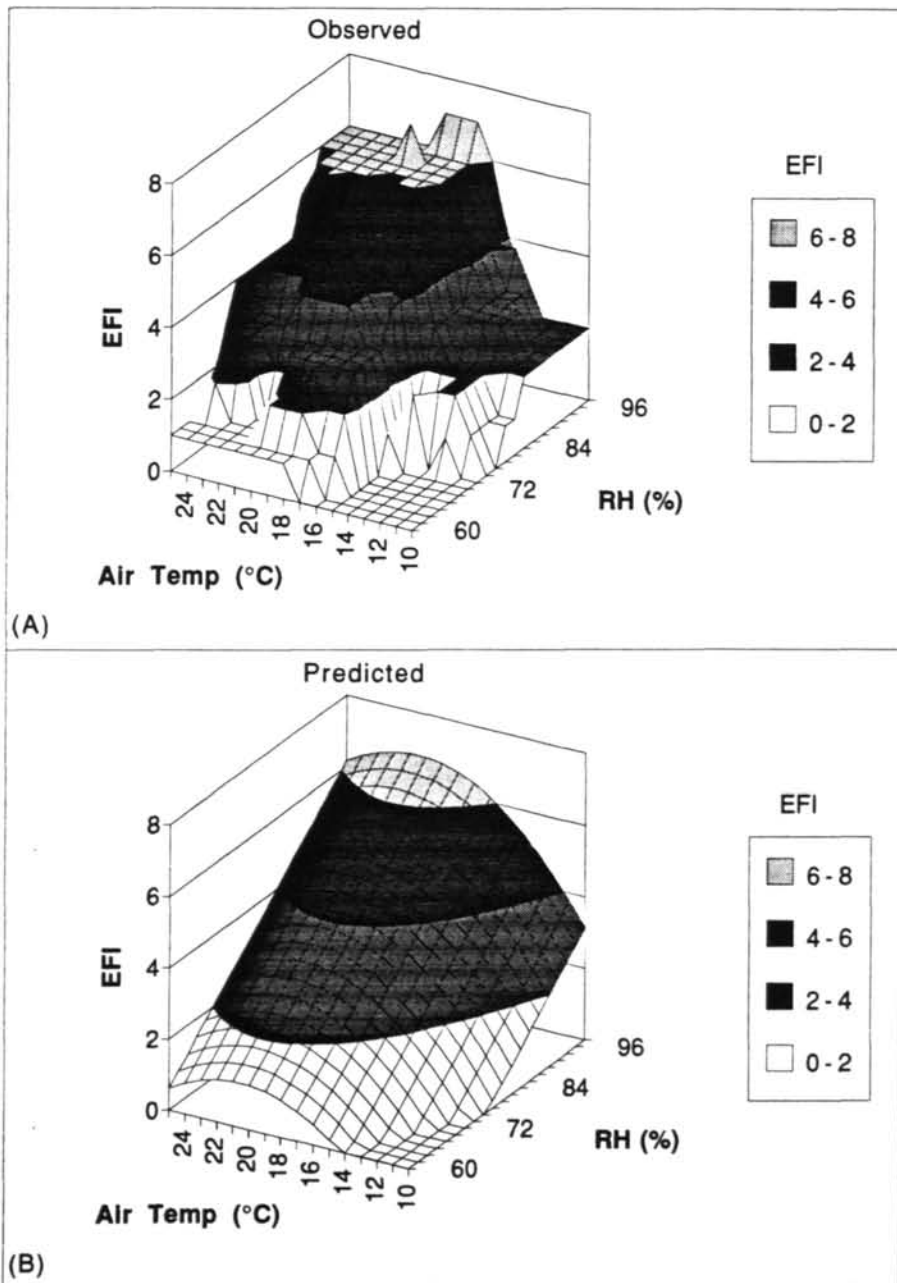


Figure 3. Relationship of minimum daily air temperature (°C) and mean daily relative humidity (%) to the environmental favorability index (EFI). Data in Figure 3A are from actual field observations and Figure 3B were predicted from the regression equation.

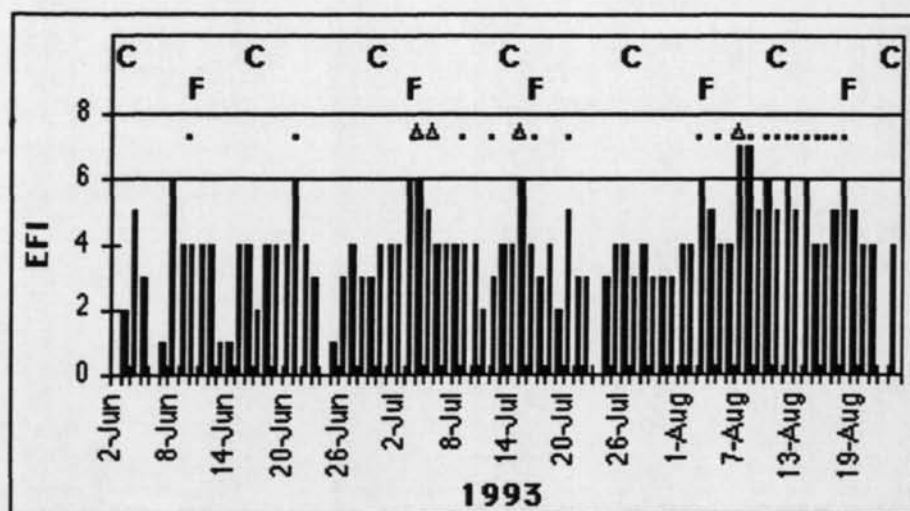


Figure 4. Environmental favorability index (EFI) predicted infection events versus observed brown patch outbreaks ( $\Delta$  = major and  $\bullet$  = minor *R. solani* infection events, respectively) in perennial ryegrass and colonial bentgrass in 1993. Infection events only were observed in colonial bentgrass on 9 and 20 June, and 19 July, and only in perennial ryegrass on 4 August; whereas, infection events occurred in both sites on all other dates. The spray dates are noted where C = calendar fungicide application on 14-day intervals and F = forecast-based sprays (minimum 14-day interval).

Table 15. Brown patch forecast model field validation on perennial ryegrass in 1993.

Treatment	Fungicide applications	Percent plot area blighted											AUDPC <sup>§</sup>
		July6	July11	July 16	July21	July 27	Aug3	Aug10	Aug18	Aug24	Sept 10	Sept27	
		(%)											
Calendar <sup>†</sup>	7	0 b <sup>¶</sup>	0 b	3 b	7 b	9 b	20 b	18 b	12 b	15 b	4 b	3 b	621 b
BP Model <sup>‡</sup>	5	0 b	0 b	4 b	11 b	10 b	18 b	15 b	10 b	17 b	3 b	2 b	641 b
Untreated	–	24 a	49 a	59 a	83 a	69 a	72 a	59 a	65 a	69 a	59 a	23 a	5023 a

<sup>†</sup>Calendar = fungicide was applied on a 14-day interval on the following dates: 1, 15, and 29 June, 13 and 27 July, and 10 and 24 Aug, 1993.

<sup>‡</sup>Brown patch (BP) Model = fungicide was applied on the following dates: 8 June, 2 and 15 July, and 2 and 17 Aug, 1993.

<sup>§</sup>Area under disease progress curve (AUDPC) data were collected between 6 July and 27 Sept, 1993.

<sup>¶</sup>Means followed by same letter in column are not significantly different according to Fisher's protected least significant difference test at  $P \leq 0.05$ .

Table 16. Brown patch forecast model field validation on colonial bentgrass in 1993.

Treatment	Fungicide applications	Percent plot area blighted												AUDPC <sup>§</sup>	
		June9	June15	June21	June28	July6	July11	July16	July21	Jul27	Aug10	Aug18	Aug24		Sept27
Calendar <sup>†</sup>	7	0 a <sup>¶</sup>	0 a	< 1 b	< 1 b	< 1 b	0 b	0 b	0 b	< 1 b	< 1 b	0 b	0 b	0 b	12 b
BP Model <sup>‡</sup>	5	0 a	< 1 a	< 1 b	0 b	< 1 b	0 b	0 b	< 1 b	< 1 b	0 b	0 b	0 b	0 b	14 b
Untreated	-	1 a	1 a	5 a	10 a	19 a	29 a	49 a	35 a	40 a	31 a	23 a	10 a	2 a	1842 a

<sup>†</sup>Calendar = fungicide was applied on a 14-day interval on the following dates: 1, 15, and 29 June, 13 and 27 July, and 10 and 24 Aug, 1993.

<sup>‡</sup>Brown patch (BP) Model = fungicide was applied on the following dates: 8 June, 2 and 15 July, and 2 and 17 Aug, 1993.

<sup>§</sup>Area under disease progress curve (AUDPC) data were collected between 9 June and 27 Sept, 1993.

<sup>¶</sup>Means followed by same letter in column are not significantly different according to Fisher's protected least significant difference test at  $P \leq 0.05$ .