Timing and Efficacy of Fungicide Applications for Diplodia Ear Rot Management in Corn

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ABSTRACT


Diplodia ear rot, a corn (Zea mays L.) disease caused by the fungus Stenocarpella maydis (Berk.) B. Sutton, has been a persistent ear rot across the United States. Management options are currently limited. Field trials conducted under inoculated and non-inoculated conditions were established at two locations in Indiana from 2011 to 2013 to test the fungicides azoxystrobin plus propiconazole and prothioconazole against Diplodia ear rot. Fungicides were applied at three individual growth stages during each year. Fungicides did not consistently reduce Diplodia ear rot compared to non-fungicide-treated controls in any year. Applications also did not consistently increase yield at any timing under inoculated and non-inoculated plots compared with the non-fungicide-treated control. Fungicides were tested in an in vitro assay to determine the effective fungicide concentration at which 50% of mycelial growth or conidial germination of S. maydis was inhibited (EC50). Propiconazole and prothioconazole EC50 values indicated efficacy in reducing fungal growth under controlled conditions; however, current fungicide application methods and plant barriers to fungicide contact with the pathogen may prevent these products from effectively reducing Diplodia ear rot in a field setting.

INTRODUCTION

In recent years, Diplodia ear rot, caused by the fungus Stenocarpella maydis (Berk.) B. Sutton, has become a persistent ear rot disease of corn (Zea mays L.) in the United States Corn Belt. Yield losses vary annually; however, combined yield loss of Diplodia ear rot in 2012 and 2013 including 22 states in the United States and Canada was estimated to be 1.36 million metric tons (Mueller and Wise 2012; Mueller and Wise 2013). Early foliar symptoms of the disease begin with leaves exhibiting discoloration and having a bleached and dry appearance (Rossouw et al. 2009). Infection generally occurs during the silking growth stage, typically beginning at the bottom of the ear and continuing to the ear tip (Bensch 1995; Koehler 1959). Disease severity may increase when humid conditions occur during reproductive growth stages (Van Rensburg and Ferreira 1997). At plant maturity, ears will show external symptoms and signs, including a white fungal mat on the ear, and husk discoloration.

Stenocarpella maydis needs to remain on the surface of corn debris to survive and can remain viable for almost one year (Casa et al. 2003). Each year, infected corn debris is the primary source of inoculum (Flett et al. 1992), especially if no tillage or crop rotation is implemented in a field. Disease development and severity is determined by the proximity between host and inoculum source (Ullstrup 1964).

Currently, general management guidelines include planting less susceptible hybrids, crop rotation, and tillage (Flett et al. 1992; Flett et al. 1998; Ullstrup 1964). However, commercial resistance that can withstand high disease pressure is currently unavailable (Dorrance et al. 1998; Flett and McLaren 1994). While tillage practices and crop rotation with non-host crops can reduce inoculum, these are not feasible management strategies for all farmers, since many farmers use conservation tillage practices to reduce erosion and improve soil properties (Olson and Sander 1988). Additionally, some farmers in the Corn Belt prefer to remain in continuous corn production since other crops are not always as economically competitive with corn (USDA 2012). These production practices can result in increased Diplodia ear rot when environmental conditions are favorable for disease development (Rossouw et al. 2009).

Until 2007, the use of foliar fungicides in corn hybrid production was rare (Bradley and Ames 2009). Farmers did not consider using fungicides due to their high cost and the low market price of corn, but now that corn demand has increased, farmers are more interested in using foliar fungicides against a variety of diseases, including ear rots, and as a way to potentially increase yields even in the absence of disease (Bradley and Ames 2009; Wise and Mueller 2011). Foliar fungicide applications occur over a wide range of growth stages in corn, beginning at between four and six visible leaf collars (V4-V6) and may extend through the early dough stage of grain fill (R3) (Abendroth et al. 2011). If plants are under stress during these growth stages, flowering may be inhibited and/or yield might be reduced (Westgate et al. 2004). Traditionally, plant pathologists have recommended fungicide applications at the end of the vegetative growth stage, when the tassel is fully emerged, and at the beginning of the reproductive stage, when silks are visible outside the husks (VT-R1) (Mueller et al. 2013). This timing has primarily targeted foliar disease control, and has been confirmed by previous studies on northern corn leaf blight caused by Exserohilum turcicum and gray leaf spot caused by Cercospora zeae-maydis (Bowen and Pedersen 1988; Munkvold et al. 2001; Ward et al. 1997).
Despite the increased use of fungicides in corn and the prevalence of Diplodia ear rot in corn production, there is limited and inconsistent research on fungicide efficacy for management of this disease. Kleinschmidt and White (2002) assessed several fungicides for control of Diplodia ear rot in two studies. Their results indicated that treatments containing azoxystrobin from the Quinone outside inhibitor (QoI) group [Fungicide Resistance Action Committee (FRAC) group 11], reduced disease severity compared with the non-treated control in one experiment. The most recent work, by Lee et al. in 2008, indicated that fungicides did not significantly affect Diplodia ear rot under field conditions.

In 2010, a commercial fungicide containing two different active ingredients—propiconazole from the demethylation-inhibiting (DMI) fungicide group (FRAC group 3); and azoxystrobin, a QoI fungicide (FRAC group 11)—was labeled as a fungicide able to suppress Diplodia ear rot (Syngenta Crop Protection, Greensboro, NC). Currently, there is limited information on efficacy and timing to determine if this or other fungicides should be recommended for Diplodia ear rot management in commercial corn production.

The objectives of this study were to: (i) assess the efficacy of fungicide applications against Diplodia ear rot under inoculated and non-inoculated field conditions and observe impact of fungicide programs on corn yield; (ii) establish the plant growth stage where fungicides will be most effective at reducing disease development; (iii) determine the effect of fungicides on in vitro mycelial growth or conidial germination of S. maydis.

SITE DESCRIPTION AND FIELD EXPERIMENT DESIGN

Research trials were established at the Agronomy Center for Research and Education (ACRE) in Tippecanoe County, IN, from 2011 to 2013. In 2012 and 2013, the experiment was established at an additional location at the Southwest Purdue Agricultural Center (SWPAC), in Knox County. In both locations, experiments in 2011 and 2012 were planted following corn. In 2013 experiments followed soybeans. In all years and locations, trials were conducted under standard agricultural practices for the area. One corn hybrid, Pioneer 34F97 (114-day maturity), was planted with a John Deere 1700 six-row planter at a seeding density of 84,016 viable seed/ha in all years and locations of the study. The hybrid was selected based on its score of 5 for Gibberella and Fusarium ear rot, and 6 for Diplodia ear rot on a rating scale of 1 to 9, was chosen for its ability to suppress Diplodia ear rot (Syngenta Crop Protection, Greensboro, NC). Currently, there is limited information on efficacy and timing to determine if this or other fungicides should be recommended for Diplodia ear rot management in commercial corn production.

The objectives of this study were to: (i) assess the efficacy of fungicide applications against Diplodia ear rot under inoculated and non-inoculated field conditions and observe impact of fungicide programs on corn yield; (ii) establish the plant growth stage where fungicides will be most effective at reducing disease development; (iii) determine the effect of fungicides on in vitro mycelial growth or conidial germination of S. maydis.

CULTURE MAINTENANCE AND INOCULUM PREPARATION

Cultures used to generate inoculum each year were collected from ears naturally infected with S. maydis at ACRE and at SWPAC. Isolates were collected from infected corn kernels and prepared as described in Romero and Wise (2015). Each year, field inoculum was prepared from an isolate mix of ten isolates, which was obtained using four circular pieces (17 mm diameter) from each plate, and then placed in a blender with 100 ml of ddH2O and mixed for 1 min at 22,000 rpm. Due to variability in isolate growth, the resulting suspension may have contained different concentrations of each isolate. After mixing, 5 ml of the resulting suspension was plated onto natural oatmeal agar (NOA). Isolates were placed in an incubation chamber at 28°C with 12-h light and dark cycles. Sterilized sorghum seed was used as a carrier for inoculum. To prepare inoculum, 5 lbs of white sorghum seed was added to clear autoclave bags (60.96 × 76.2 cm), to which 2 liters of ddH2O was added. Sorghum was soaked for 12 h, then autoclaved for 1 h, allowed to cool, and autoclaved for an additional hour. Each sterilized sorghum seed bag was inoculated with a single previously cultured isolate.

Inoculated bags of sorghum were incubated at 28°C until fully colonized (approximately two weeks). During the incubation period, bags were mixed by hand to distribute fungal growth. After seed was fully colonized, sorghum seeds were dried for five days at 28°C as a single layer of seeds spread across newspaper. To prevent clumping, sorghum seeds were separated by hand, and stored at 4°C until plants were ready to be inoculated.

FIELD INOCULATIONS

Experimental plots were inoculated using a previously established protocol, which consisted of placing 5 g of sterilized sorghum seeds colonized with S. maydis in the whorl of each plant per row at the V7 growth stage (7 visible leaf collars) to ensure inoculum presence when ears emerge from the plant (Romero 2012). To create a conducive environment for inoculum to bind to the plant, 5 ml of water/plant was sprayed into the whorl just prior to inoculation using a 15.14-liter BioLogic backpack sprayer equipped with adjustable brass cone nozzle (Mossy Oak, West Point, MS). Plots that did not receive inoculum served as non-inoculated controls.

FUNGICIDE APPLICATION AND TIMING

The two fungicides tested were azoxystrobin plus propiconazole (Quilt Xcel; Syngenta Crop Protection, Greensboro, NC), which was labeled in 2010 as a fungicide able to suppress Diplodia ear rot, and prothioconazole (Proline 480 SC; Bayer CropScience, Research Triangle Park, NC). Azoxystrobin plus propiconazole was the only fungicide tested in 2011. Prothioconazole was included in 2012 and 2013 due to its Environmental Protection Agency (EPA) 2ee special use label in several U.S. states and Canada for other ear rot pathogens, including Fusarium verticillioides and Gibberella zeae. Activity on Diplodia ear rot was unknown prior to this trial. Azoxystrobin plus propiconazole was applied at the labeled rate of 735 ml/ha and prothioconazole was applied at the rate of 280 ml/ha at all locations and timings. The nonionic surfactant (NIS) (Preference; Winfield Solutions LLC, St. Paul, MN) was included at a rate of 0.125% v/v in all fungicide applications in each location and year.

Fungicides were applied in a single application at one of three growth stages—V6 (six visible leaf collars), VT-R1 (tasselling-
silkig), and R3 (early dough) — through CO₂—pressurized backpack sprayers (TeeJet Technologies, Wheaton, IL) using a handheld boom fitted with four TJ-8001VS nozzles spaced 45 cm apart, which delivered 140 liter/ha at 275 kPa. Non-fungicide-treated controls were included in each experiment. The early fungicide application at V6 was intended to be a preventative application, while later applications targeted disease suppression.

**DATA COLLECTION AND STATISTICAL ANALYSIS**

Forty ears from each treatment (10 per plot) were hand-harvested at maturity from the center two rows of each plot. Percent disease severity was rated on these ears based on the estimation of ear exhibiting symptoms or signs of Diplodia ear rot (Romero 2012). The inner two rows of each experimental plot were harvested with a Kinaird 8-XP (Kinaird Equipment Mfg., Haven, KS) small-plot research combine. The total seed weight and seed moisture were determined, and converted to yield in kilograms per hectare. Yield data were calculated with corrections for moisture content (standardized to 15% moisture).

The normal distribution and homogeneity of variances for the disease severity data were verified using PROC UNIVARIATE in SAS software version 9.2. Disease severity values were arcsine transformed (arc sine-square-root transformed). Analysis of variance were conducted for each year and location for disease severity and yield using a general linear model in SAS. Mean separations were based on Fisher’s least significant difference value (LSD) at P ≤ 0.05.

**IN VITRO SENSITIVITY OF STENOCARPELLA MAYDIS**

In vitro assays were conducted using ten isolates of *S. maydis*, of which the majority originated from non-inoculated corn plants taken from ACRE during the 2010 to 2013 field seasons. Single-spore isolates of *S. maydis* were obtained from infected corn kernels as previously described. Stock solutions of technical grade chemicals for azoxystrobin (96% a.i.; Syngenta Crop Protection, Greensboro, NC), propiconazole (95% a.i.; Syngenta Crop Protection, Greensboro, NC), and prothioconazole (97.70% a.i; Bayer, CropScience, Research Triangle Park, NC) were prepared in acetone.

Sensitivity of each of the ten *S. maydis* isolates to propiconazole and prothioconazole were tested using an in vitro mycelial growth assay, since these fungicides primarily inhibit mycelial growth. Mycelial plugs cut from the margin of 4-day-old cultures of each isolate were transferred to two replicate plates of PDA amended with each fungicide at concentrations of 0, 0.001, 0.01, 0.1, and 1 µg a.i./ml. The 0 µg/ml concentration served as a control and was amended with acetone. Plugs were inverted so that the sporulating fungal growth was in contact with the fungicide-amended media and placed in the center of a 100 × 15-mm petri dish. Plates were arranged in a completely random design (CRD) and incubated in the dark for 4 days at room temperature. The diameter of each colony was measured (cm) in two perpendicular directions after 4 days. These two colony diameter measurements were averaged to obtain the mean radial growth for each isolate. The percent radial growth for each isolate was obtained and converted to percentage inhibition: 100 – [mean radial growth on fungicide-amended medium / (mean radial growth on non-amended medium control)] × 100. From this, EC₅₀ values were determined (effective fungicide concentration at which 50% fungal growth is inhibited) from each isolate was estimated by linear interpolation method (Pasche et al. 2004). Fungicide sensitivity of the same ten *S. maydis* isolates to azoxystrobin was determined by assessing conidial germination on PDA amended with fungicide at concentrations of 0, 0.01, 0.1, 1, and 10 µg a.i./ml plus 100 µg/ml salicylhydroxamic acid (SHAM; 99% Sigma-Aldrich, St. Louis, MO). A conidial germination assay was selected because QoI fungicides primarily target spore germination. SHAM was dissolved in methanol and added to all fungicide-amended media to reduce the effects of the alternative oxidative pathways that allow some fungi to overcome QoI-fungicidal effects in vitro (Olaya and Köller 1999; Ziogas et al. 1997). A spore suspension was prepared by adding 5 ml of ddH₂O to a 15-day-old culture of *S. maydis* growing on NOA. The spore suspension was adjusted to 1.7 × 10⁷ conidia/ml. A conidial suspension of 50 µl was pipetted onto each of two replicate petri plates (60 × 15 mm) and spread with a petri disposable L-shaped spreader (USA scientific, Ocala, FL). Plates were allowed to dry for 30 min and were incubated 24 h under dark conditions at room temperature. A conidium was considered to be germinated if the germ tube was visually determined to be at least as long as the conidium. The experiment was repeated three times. Data from each in vitro fungicide experiment were analyzed independently using an F-test to determine if variances were homogeneous (F ≤ 0.05) among experiments. EC₅₀ values were determined for each fungicide active ingredient and analyzed using the general linear models procedure in SAS. Means for each treatment were compared using Fisher’s least significant difference value (LSD) at P ≤ 0.05.

**EFFECT OF FUNGICIDE APPLICATIONS ON DIPLODIA EAR ROT AND YIELD**

Monthly air temperature and precipitation data for June, July, August, and September for each year are presented in Table 1 (Scheeringsa 2014). Except for fungicide trials in 2011, where only one location was evaluated, the location main effect for 2012 and 2013 was highly significant, therefore data from locations were not combined and fungicide trials were analyzed separately by year and location. No significant interaction of fungicide by application timing was observed for disease severity at any location or year. Foliar disease pressure in each experiment was noted, but not severe enough to impact yield.

**Field trials 2011. ACRE.** Azoxystrobin plus propiconazole did not reduce disease severity at any application timing in inoculated or non-inoculated plots (Fig. 1A). Fungicide applied at VT/R1 in non-inoculated plots increased yield when compared to yield in inoculated plots and when fungicide was applied at the V6 growth

![Table 1](https://climate.agr.purdue.edu/climate/index.asp)

<table>
<thead>
<tr>
<th></th>
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<th></th>
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<td>76.2</td>
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<td>Temperature</td>
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<td>21.5</td>
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<td>Temperature</td>
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<td>24.2</td>
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<td>21.0</td>
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<td>Precipitation</td>
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<td>44.2</td>
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<td>24.6</td>
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<td>SWPAC 2012</td>
<td>Precipitation</td>
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<td>7.2</td>
<td>12.7</td>
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* Location: Agronomy Center for Research and Education (ACRE) in Tippecanoe County, IN; and Southwest Purdue Agricultural Center (SWPAC), in Knox County, IN.

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**Summary of air temperatures (°C) and precipitation (mm) for June, July, August, and September, from 2011 to 2013 at Indiana locations.a**

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a Weather data were obtained from the Indiana State Climate Office. [https://climate.agr.purdue.edu/climate/index.asp](https://climate.agr.purdue.edu/climate/index.asp)
stage in non-inoculated plots. However, this application did not increase yield compared to the non-inoculated, non-fungicide-treated control (Fig. 1B).

Field trials 2012. ACRE. The main effects of fungicide and timing were significant for disease severity and yield (Table 2). Azoxystrobin plus propiconazole applied at any plant growth stage did not reduce disease severity or increase yield compared to the non-inoculated or inoculated, non-fungicide-treated controls (Fig. 2A, B). Likewise, prothioconazole applications did not reduce disease severity or increase yield compared to the non-inoculated and inoculated, non-fungicide-treated controls (Fig. 2C, D).

FIGURE 1
Effect of application timing of azoxystrobin plus propiconazole on (A) Diplodia ear rot disease severity caused by *Stenocarpella maydis* and (B) yield under inoculated and non-inoculated conditions at the Agronomy Center for Research and Education (ACRE) in 2011. Values with the same letter are not significantly different within each treatment based on Fisher's Least Significant Difference (LSD) test (P = 0.05).

TABLE 2
Detailed statistical analysis from general lineal model analysis of data from field experiments established across Indiana from 2011 to 2013 to evaluate the effect of azoxystrobin + prothioconazole and prothioconazole on Diplodia ear rot disease severity and yield under inoculated and non-inoculated conditions at three single application timings.

<table>
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<tr>
<th>Experiment</th>
<th>Year</th>
<th>Fungicide</th>
<th>Source</th>
<th>df</th>
<th>Disease severity F value</th>
<th>Disease severity p</th>
<th>Yield F value</th>
<th>Yield p</th>
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a Field experiments conducted across Indiana from 2011 to 2013 at the Agronomy Center for Research and Education (ACRE) in Tippecanoe County, IN, and at the Southwest Purdue Agricultural Center (SWPAC), in Knox County, IN.
b Timing: for all trials treatment consisted of a single fungicide application under inoculated and non-inoculated conditions at a specific growth stage—V6 (six visible leaf collars), VT-R1 (tasselling-silking), and R3 (early dough). A non-treated control was included in each inoculation treatment. Fungicides: azoxystrobin + propiconazole and prothioconazole.
c Degrees of freedom.
d F statistic and P values based on general linear model analysis of arcsine-transformed disease severity, and yield.
e No significant effect on disease severity or yield respectively was observed when fungicides were analyzed together.
f Due to drought conditions, the effect of treatment on yield could not be assessed for the trial, and yield data could not be used to test yield response for fungicide treatment.
The main effects of fungicide and timing were significant for disease severity (Table 2). Due to drought conditions at SWPAC, yield could not be measured in 2012. Azoxystrobin plus propiconazole did not reduce disease severity compared to the non-inoculated and inoculated, non-fungicide-treated controls (Fig. 3A). Prothioconazole applied at V6 in inoculated plots reduced disease severity compared with the inoculated, non-fungicide-treated control. Applications of prothioconazole at VT/R1 and R3 in non-inoculated and inoculated plots did not reduce disease severity (Fig. 3B).

**Field trials 2013. ACRE.** The main effects of fungicide and timing were significant only for disease severity. No significant effect was observed on yield (Table 2). Azoxystrobin plus propiconazole applied at VT/R1 and R3 in non-inoculated plots reduced disease severity compared to fungicide applications at V6. However, disease severity was not significantly different from the non-inoculated, non-fungicide-treated control (Fig. 4A). Azoxystrobin plus propiconazole did not reduce disease severity at any plant growth stage in inoculated plots. Prothioconazole applied at R3 in non-inoculated plots reduced disease severity compared with the non-inoculated, non-fungicide-treated control (Fig. 4B), whereas fungicide applied at V6 and VT/R1 did not. Prothioconazole application did not reduce disease severity in the inoculated plots at any plant growth stage compared with the inoculated, non-fungicide-treated control.

**SWPAC.** The main effects of fungicide and timing were significant for yield at SWPAC (Table 2), but only the main effect of timing was significant for disease severity. Therefore, disease severity data for azoxystrobin plus propiconazole, and prothioconazole were analyzed together (Table 2). Fungicides applied at V6 and VT/R1 in inoculated plots reduced disease severity compared with the inoculated, non-fungicide-treated control, whereas fungicides applied at R3 did not. Fungicides did not reduce disease severity at any plant growth stage in non-inoculated treatments (Fig. 5A). Azoxystrobin plus propiconazole and prothioconazole did not increase yield at any plant growth stage compared to the non-inoculated and inoculated, non-fungicide-treated controls (Figs. 5B and 5C).

**FIGURE 2**
Effect of application timing of azoxystrobin plus propiconazole on (A) Diplodia ear rot disease severity caused by *Stenocarpella maydis* and (B) yield, and effect of application timing of prothioconazole on (C) disease severity and (D) yield under inoculated and non-inoculated conditions at the Agronomy Center for Research and Education (ACRE) in 2012. Values with the same letter are not significantly different within each treatment based on Fisher’s Least Significant Difference (LSD) test ($P = 0.05$).

a IN=Inoculation (Treatment that received an artificial inoculation of *Stenocarpella maydis*).
FIGURE 3
Effect of (A) azoxystrobin plus propiconazole and (B) prothioconazole application timing on Diplodia ear rot disease severity caused by *Stenocarpella maydis* under inoculated and non-inoculated conditions at the Southwest Purdue Agricultural Center (SWPAC) in 2012. Values with the same letter are not significantly different within each treatment based on Fisher’s Least Significant Difference (LSD) test ($P = 0.05$).

FIGURE 4
Effect of (A) azoxystrobin plus propiconazole and (B) prothioconazole on Diplodia ear rot disease severity caused by *Stenocarpella maydis* and effect of fungicide application timing of (C) yield under inoculated and non-inoculated conditions at the Agronomy Center for Research and Education (ACRE) in 2013. Values with the same letter are not significantly different within each treatment based on Fisher’s Least Significant Difference (LSD) test ($P = 0.05$).
Experimental variances were homogeneous for azoxystrobin ($P = 0.1439$), propiconazole ($P = 0.8428$), and prothioconazole ($P = 0.1580$). Therefore, data were combined by fungicide for further analysis. *Stenocarpella maydis* sensitivity to propiconazole and prothioconazole ranged from 0.09 to 0.19 $\mu$g/ml and from 0.11 to 0.23 $\mu$g/ml, with mean values of 0.12 and 0.16 $\mu$g/ml, respectively. *Stenocarpella maydis* sensitivity to azoxystrobin ranged from 2.00 to 2.85 $\mu$g/ml, with a mean of 2.27 $\mu$g/ml. Analysis of *S. maydis* isolate EC$_{50}$ values for each fungicide by year revealed that fungal sensitivity to each fungicide did not vary significantly over time (Table 3).

## CONCLUSIONS AND DISEASE MANAGEMENT CONSIDERATIONS

The use of multiple fungicide application timings in our study was intended to identify a growth stage within the window of common application timings at which fungicides might suppress Diplodia ear rot under inoculated and non-inoculated conditions. We hypothesized that fungicide application at the R1 growth stage would be most likely to suppress *S. maydis* infection since it occurs at the silking growth stage of corn (Chambers 1988; Koehler 1959), and foliar fungicides typically offer 14 to 21 days of protection to the plant (Mueller et al. 2013). However, across the three years of this study, the R1 fungicide application only

### TABLE 3

<table>
<thead>
<tr>
<th>Years of isolate collection</th>
<th>Range of EC$_{50}$ value</th>
<th>EC$_{50}$ means$^a$</th>
<th>Range of EC$_{50}$ value</th>
<th>EC$_{50}$ means$^a$</th>
<th>Range of EC$_{50}$ value</th>
<th>EC$_{50}$ means$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>2.12–2.19</td>
<td>2.16a</td>
<td>0.09–0.15</td>
<td>0.11a</td>
<td>0.12–0.16</td>
<td>0.15a</td>
</tr>
<tr>
<td>2011</td>
<td>2.06–2.41</td>
<td>2.19a</td>
<td>0.09–0.15</td>
<td>0.11a</td>
<td>0.11–0.19</td>
<td>0.14a</td>
</tr>
<tr>
<td>2012</td>
<td>2.00–2.85</td>
<td>2.46a</td>
<td>0.10–0.19</td>
<td>0.13a</td>
<td>0.14–0.23</td>
<td>0.18a</td>
</tr>
<tr>
<td>Total</td>
<td>Overall mean = 2.27 $\mu$g/ml</td>
<td></td>
<td>Overall mean = 0.12 $\mu$g/ml</td>
<td></td>
<td>Overall mean = 0.16 $\mu$g/ml</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Means followed by same letters are not significantly different based on Fisher’s protected least significant difference at the $P = 0.05$ level.
significantly reduced disease severity once in inoculated plots compared to disease severity in the inoculated, non-fungicide-treated control. An application of prothioconazole in 2012, and azoxystrobin plus propiconazole in 2013 applied at V6 each reduced disease severity compared to the respective inoculated, non-fungicide-treated controls in each trial. Although these reductions were detectable, the level of disease severity at these timings did not differ significantly from disease severity levels observed with other fungicide application timings within the same trial, and we were not able to establish a plant growth stage at which fungicides would be most effective at reducing Diplodia ear rot.

Despite the consistent lack of fungicide efficacy observed in individual application timings under field conditions, the in vitro assay results indicate that the fungicides tested are capable of inhibiting growth under controlled conditions. These in vitro results corroborated Kleinschmidt and White (2002) who previously observed that azoxystrobin reduced Diplodia ear rot under field conditions. However, we observed limited fungicide efficacy for Diplodia ear rot under field conditions, which is similar to Lee et al. (2008), where disease was not significantly reduced by fungicide applications. These inconsistencies in efficacy could be attributed to the fungal infection process and plant morphology. *Stenocarpella maydis* infection occurs between the ear shank and the stalk, and from there, the fungus grows to the ear tip (Bensch 1995). This is an ideal spot for infection since the shank retains water and pollen residue which is a nutrient reservoir (Ullstrup 1953). This area is well protected by natural plant barriers (i.e., upper leaves, husks, and ear position) (Du Costa 2009). Since foliar fungicides are commonly applied over the top of the corn plant with ground or aerial equipment (Mueller et al. 2013), it is possible that these barriers limit the amount of fungicide able to penetrate the plant and contact the fungus.

Additionally, theDMI and QoI fungicides used in this study are locally systemic fungicides that can have upward mobility into the xylem (Watschake et al. 2013), but differences exist with the extent of protection after the uptake event, and the systemic movement into new leaves (Bartlett et al. 2002). These differences may also limit their activity against *S. maydis* in the plant, since the fungicide must penetrate the thick ear husk and shank and move through this tissue to contact the fungus. Finally, we hypothesized that the high humidity present at silking stage (20 to 30%) within the husk (Aldrich et al. 1975) that favors *S. maydis* infection might be an additional factor of the reduced fungicide longevity and activity within the plant. High temperatures, strong winds, dew or rain during and after application compromise the effectiveness of fungicides (Mueller et al. 2013). Therefore, it appears that although these fungicide active ingredients may have efficacy against *S. maydis* in vitro, the current application methods may not allow fungicide penetration or movement through the husk and shank, and fungicide that is available in husk tissue may break down before infection occurs.

Diplodia ear rot severity varied across locations and years, with low disease levels observed in 2013. Across the trial, rainfall was limited in all years in July and August, but in 2011 and 2012, increased rainfall in September likely helped to increase Diplodia ear rot (Table 1). Low September rainfall may have resulted in the lower disease severity observed in 2013 in both inoculated and non-inoculated trials. This influence of late-season rain on Diplodia ear rot development has been previously reported, and it was observed that Diplodia ear rot severity was more severe when drought conditions during reproductive growth stages were followed by late season rains (Van Rensburg and Ferreira 1997).

In our study, each of the fungicides significantly affected yield; however, results were inconsistent among years and application timing. Our results indicate that yield improvement cannot be attributed with Diplodia ear rot control, and no other significant foliar disease pressure existed in these trials (data not shown). Therefore, it is possible that environmental or physiological plant effects impacted yield. Several studies report that fungicides induce physiological changes in plants that potentially contribute to yield gains (Bryson et al. 2000; Glaab and Kaiser 1999; Ruske et al. 2003), and in recent years, farmers have become interested in using fungicides not only for disease control but also for increasing yield.

Wise and Mueller (2011) examined the impact of QoI fungicides on corn yield from trials conducted from 2000 to 2010, and results indicate that QoI fungicides can have a positive impact on yield even in the absence of disease, although consistent yield increases are observed when high disease pressure occurs in trials. However, based on the inconsistent results of fungicide on yield observed in this study, it is difficult to recommended the use of foliar fungicides for the sole purpose of yield improvement in corn.

Currently, no hybrid is known to have durable resistance to Diplodia ear rot under high disease pressure (Dorrance et al. 1998; Flett and McLaren 1994), and *S. maydis* may become a more common pathogen due to continuous corn production and reduced tillage practices (Flett et al. 1992; Flett et al. 1998; Flett et al. 2001; Rheeder et al. 1990; Ullstrup 1964). Although farmers have increased adoption of fungicides to manage other corn diseases, our research indicates that the fungicides tested in this experiment at three different plant growth stages did not consistently reduce Diplodia ear rot or improve yield under low and high disease pressure, and across multiple environments in Indiana. The absence of consistent yield improvement with fungicide application does not likely justify the use of these fungicides even when environmental and field conditions favor Diplodia ear rot, and producers should focus on other management methods, such as crop rotation and tillage, to reduce severity of this disease.

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**LITERATURE CITED**


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