Dynamics of Fungicide Sensitivity in *Venturia effusa* and Fungicide Efficacy under Field Conditions

J. R. Standish, T. B. Brenneman, and K. L. Stevenson,[†] Department of Plant Pathology, Coastal Plain Experiment Station, The University of Georgia, Tifton 31793

Abstract

Venturia effusa, which causes pecan scab, has developed resistance to fungicides that were once effective. Over 2 years, laboratory-based sensitivity of fentin hydroxide (TPTH) and tebuconazole in *V. effusa* and their efficacy under field conditions were compared. Leaf and nut scab were assessed on pecan trees receiving 10 applications of TPTH, tebuconazole, azoxystrobin, azoxystrobin plus tebuconazole, TPTH plus tebuconazole, or no fungicide (NTC) per year. Sensitivity of *V. effusa* on leaflets collected from treated and nontreated trees was assessed in June and September, respectively. The mean relative germination (RGe) on TPTH at 30 µg/ml was 10.9 and 40.9% in 2016 and 4.2 and 0.6% in 2017. Mean relative growth (RGr) on tebuconazole at 1 µg/ml

was 45.5 and 34.6% in 2016 and 69.3 and 56.3% in 2017. In both years, leaf and nut scab were significantly lower on trees treated with azoxystrobin, azoxystrobin + tebuconazole, or TPTH + tebuconazole when compared with NTC and tebuconazole-treated trees. Compared with the NTC, tebuconazole did not significantly reduce leaf scab in 2017 or nut scab in either year, indicating that an RGr value between 34.6 and 69.3% is likely to result in a control failure on tebuconazole-treated trees. Although better activity was expected, TPTH reduced scab with RGe values between 0.6 and 40.9%. These results are valuable for developing fungicide sensitivity thresholds to better predict fungicide performance.

Pecan scab, caused by *Venturia effusa* (G. Winter) Rossman & W. C. Allen (syn. *Fusicladium effusum*), is the most economically important disease affecting pecan (*Carya illinoensis* (Wangenh.) K. Koch) in the southeastern United States (Demaree 1924). This fungus causes small, olive-brown to black spots on infected leaves, fruit shucks, and twigs; young and actively growing tissues are most susceptible to infection and become resistant as they mature (Demaree 1924; Littrell and Bertrand 1981). Infections occurring on fruit during the early stages of development can cause premature fruit drop but those occurring after shell hardening are thought to be cosmetic (Bertrand 2002; Demaree 1924; Hunter 1983). The annual severity of scab is determined by cultivar susceptibility, the relative abundance of *V. effusa* inoculum, and the frequency of spring and summer rainfall (Latham 1982; Sparks et al. 2009). Rainfall events aid in the dispersal of conidia by wind and splash and the ensuing foliar moisture provides optimal conditions for conidial germination and infection (Gottwald 1982, 1985; Gottwald and Bertrand 1982; Latham 1982; Turechek and Stevenson 1998).

The most effective method of managing scab is through the use of resistant cultivars (Conner and Wells 2007). However, the scab pathogen exhibits a great amount of genetic and pathogenic diversity, which has allowed for its adaptation to cultivars with scab resistance (Bock et al. 2014, 2017b; Conner and Stevenson 2004; Demaree and Cole 1929; Sparks 1992). Thus, in the absence of durable host resistance, scab management is centered on preventive fungicide applications made throughout the growing season (Brock and Bertrand 2007a). In Georgia, the general guideline is to apply fungicides at 10- to 14-day intervals from bud break to pollination and at 14- to 21-day intervals from pollination to shell hardening, resulting in 10 or more applications per season (Brock and Bertrand 2007b).

However, the interval between sprays is often adjusted to once every 7 to 10 days during periods of frequent rainfall, which substantially increases the total number of applications per growing season (Brock et al. 2007; Latham 1995). Fungicides from different chemical groups are approved for use on pecan in the United States and include the methyl benzimidazole carbamates, demethylation inhibitors (DMIs), quinone outside inhibitors (QoIs), organotin compounds, phosphonates, dithiocarbamates, and guanidines (Fungicide Resistance Action Committee codes 1, 3, 11, 30, 33, M3, and U12, respectively) (Bock et al. 2017a). The inherent risk of resistance developing in *V. effusa* to the active ingredients within each of these fungicide groups ranges from low to medium to high and is magnified by the difficulty of providing uniform coverage in larger pecan trees (Bertrand and Brenneman 2001; Bock et al. 2013, 2015; FRAC 2017).

Practical fungicide resistance in *V. effusa* was first observed in the mid-1970s in association with benomyl control failures in Alabama and Georgia (Littrell 1976). Since that time, significant reductions in sensitivity have been reported in *V. effusa* isolates to the organotin fentin hydroxide (TPTH) in vitro and to the DMIs, both in vitro and as observed control failures in some locations (Reynolds et al. 1997; Seyran et al. 2010; Stevenson et al. 2004, 2015). Subsequently, a fungicide monitoring program was initiated in 2008 and further expanded in 2014 and 2015, allowing Georgia pecan producers to submit leaf samples showing typical scab symptoms for fungicide sensitivity testing (Seyran et al. 2010; Stevenson et al. 2015). Sensitivity was determined by collecting conidia directly from infected leaf material and testing either germination or microcolony growth on medium amended with dodine, TPTH, thiophanate-methyl, propiconazole, or tebuconazole, using a rapid in vitro method (Seyran et al. 2010). This assay utilized discriminatory concentrations of the aforementioned active ingredients to identify insensitivity when compared with a baseline population (Seyran et al. 2010). Studies utilizing similar in vitro laboratory assays provide an indication of relative sensitivity or insensitivity of an isolate or sample to a particular fungicide active ingredient and can be useful for detecting shifts in sensitivity over time. However, a decrease in sensitivity to a fungicide in vitro over time does not necessarily mean that practical resistance has developed or will develop if in vitro threshold levels have not yet been determined for individual fungicides. Understanding the relationship between fungicide efficacy in the orchard and sensitivity levels as measured in the laboratory is essential for development of fungicide recommendations and fungicide programs for effective scab management. The objectives of this research were to examine the

[†]Corresponding author: K. L. Stevenson; E-mail: ks@uga.edu

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within-season changes in sensitivity to TPTH and tebuconazole in *V. effusa* as influenced by fungicide treatment and to explore the relationship between fungicide sensitivity and efficacy in managing pecan scab under field conditions.

Materials and Methods

Field experiment and sample collection. Field experiments were conducted in a pecan orchard at the University of Georgia Ponder Farm located in Ty Ty, GA in 2016 and 2017. This orchard was originally established in 1988 with alternating rows of cultivars Desirable and Wichita planted in a square pattern with approximately 12.2 m between trees both within and across rows. Every other tree in all rows of both cultivars had been replanted in 2009, and these younger trees were used in this study. Each tree in the study was bordered on all sides by an older tree that was also sprayed for scab control with a program consisting primarily of Super Tin and Elast (dodine). Data were collected from Wichita, a cultivar known for being highly susceptible to scab. Fungicide treatments were applied using an air-blast sprayer in a volume of 950 liter/ha every 14 days for a total of 10 applications beginning at bud break (18 April 2016 and 8 April 2017). Although not a recommended use pattern, this strategy allowed for a direct evaluation of the efficacy of each treatment of interest. The experiment was arranged in a randomized complete block design with six treatments and five replications (blocks). The six treatments were Super Tin 4L (TPTH; United Phosphorus Inc., King of Prussia, PA), applied at a rate of 0.877 liter/ha; Orius 3.6F (tebuconazole; ADAMA Americas Inc., Aventura, FL), applied at a rate of 0.585 liter/ha; Abound (azoxystrobin; Syngenta Crop Protection, Greensboro, NC), applied at a rate of 0.877 liter/ha; Abound plus Orius 3.6F, applied as a tank mixture at rates of 0.877 and 0.585 liter/ha, respectively; Super Tin 4L plus Orius 3.6F, applied as a tank mixture at rates of 0.877 and 0.585 liter/ha, respectively; and a nontreated control. Treatments were applied to the same trees in both years of the study.

Disease assessments were made on a biweekly basis for 8 weeks after leaf scab symptoms were observed in each year and on a monthly basis for 2 to 3 months after nut scab symptoms were observed. Eight arbitrarily chosen terminals in the lower canopy of each tree were evaluated per tree for incidence of leaf scab and incidence and severity of nut scab. Leaf scab incidence was evaluated as the number of leaflets exhibiting symptoms of scab divided by total number of leaflets. Nut scab incidence and severity were measured as the number of nuts showing symptoms of scab divided by the total number of nuts and by estimating the percent diseased nut shuck area ranging from 0 to 100%, respectively.

Fungicide sensitivity. In each year, leaflets exhibiting scab symptoms were first sampled when disease incidence was high enough to obtain an adequate sample (typically postpollination) and again just prior to shuck-split. Approximately 50 leaflets were collected from each of the 30 trees in the study on 22 June and 15 September 2016 and 15 June and 8 September 2017. Leaflets were placed in plastic bags and refrigerated (4°C) until they were processed, typically within 5 days. To confirm sporulation and pathogen identity, leaf lesions were examined using a stereomicroscope to identify *V. effusa* conidia prior to testing.

Sensitivity to TPTH and tebuconazole was estimated using a slightly modified assay first described by Seyran et al. (2010). Technical-grade TPTH (98.7% a.i.; Chem Service, Inc., West Chester, PA) and tebuconazole (97.5% a.i.; Bayer CropScience, Research Triangle Park, NC) were each dissolved in acetone to reach concentrations of 30,000 and 1,000 µg/ml, respectively. A final concentration of TPTH at 30 µg/ml in media was obtained by adding the TPTH solution at 1 ml/liter to autoclaved 2% water agar (WA; 20 g of Bacto agar per liter of water) that had cooled to 50°C. Similarly, the final concentration of tebuconazole in media at 1 µg/ml was obtained by adding the tebuconazole solution at 1 ml/liter to autoclaved quarter-strength potato dextrose agar (qPDA; 9.75 g of PDA and 11.25 g of Bacto agar per liter of water), cooled to 50°C. A nonfungicide-amended control containing acetone at 1 ml/liter was included for both fungicides (Seyran et al. 2010). The prepared

medium was poured into disposable petri plates (15 by 100 mm in diameter) and refrigerated in the dark at 4°C for use within 3 days.

Each sample of leaflets per tree was divided into three groups and 15 individual lesions were selected from each group for a total of 45 lesions per sample. A 9-µl droplet of sterile deionized water solution amended with antibiotics and Tween 20 (chloramphenicol, streptomycin sulfate, and tetracycline, each at 50 µg/ml; and one drop Tween 20 per 100 ml, respectively) was placed onto each lesion and conidia were dislodged and collected. Conidia from each group of 15 lesions were combined in a 1.5-ml microcentrifuge tube and suspensions were mixed well with a vortex mixer (Seyran et al. 2010).

To determine sensitivity to TPTH, 23 µl of each of the three conidial suspensions per sample were placed onto two replicate plates of TPTH-amended and nonamended WA. Conidia were incubated in the dark at 25°C ± 2 for 48 h, and conidial germination was assessed for 50 arbitrarily selected conidia per replicate. A conidium was considered germinated if the length of the germ tube was at least four times the length of the conidium itself. Percent relative germination (RGe) was calculated as the proportion of germinated conidia on the fungicide-amended medium divided by the proportion of germinated conidia on nonamended medium, multiplied by 100. This value was generated for each of the three groups, which were then averaged to yield a mean RGe value for each sample (Seyran et al. 2010).

To determine sensitivity to tebuconazole, 23 µl of each of the three conidial suspensions per sample were placed onto two replicate plates of tebuconazole-amended and nonamended qPDA. Conidia were spread using a sterile glass rod and incubated in the dark at 25°C ± 2 for 72 h (Seyran et al. 2010). After incubation, plates were examined under a compound microscope (×100) and 10 single, well-separated colonies were arbitrarily selected and the maximum diameter of each colony was measured. The DMI fungicides are not expected to inhibit conidial germination but are expected to inhibit hyphal growth. To correct for the average combined length of a germinated spore and the germ tube, 15 µm was subtracted from all microcolony diameter measurements. Percent relative growth (RGr) was calculated as the corrected mean diameter of 10 microcolonies on the fungicide-amended medium divided by the corrected mean diameter of 10 microcolonies on the nonamended medium, multiplied by 100. This RGr value was calculated for each of the three groups, which were then averaged to yield a mean RGr value for each sample (Seyran et al. 2010).

Data analysis. Statistical analyses were performed separately for 2016 and 2017 datasets. A mixed-model analysis was conducted using PROC GLIMMIX in SAS (version 9.4; SAS Cary, NC) to examine the effects of fungicide programs on area under the disease progress curve, maximum leaf scab incidence, maximum nut scab incidence, and maximum nut scab severity. For each of these four response variables, fungicide treatment was treated as a fixed effect, with block as a random effect. The residuals for area under the disease progress curve, maximum leaf scab incidence, maximum nut scab incidence, and maximum nut scab severity met the assumptions of normally distributed data. A repeated-measures analysis of variance was performed in PROC GLIMMIX to identify whether sampling date, fungicide treatment, or sampling date–fungicide treatment significantly affected fungicide sensitivity of *V. effusa* on TPTH or tebuconazole amended medium. Tebuconazole sensitivity (RGr) residuals met the assumptions of normally distributed data. However, the residuals of TPTH sensitivity (RGe) were nonnormal and, as such, those data were analyzed using the lognormal distribution based on (RGe + 1) to avoid taking the logarithm of 0.

Results

Leaf scab symptoms were first observed in late May of both 2016 and 2017; however, scab pressure and epidemic development were vastly different between the 2 years (Fig. 1). In 2016, environmental conditions were conducive for early-season scab infections, with a cumulative rainfall between 1 March and 31 May of 362 mm over 18 events compared with 199 mm over 11 events in 2017 (Supplementary Table S1). This 2016 spring rainfall resulted in greater

amounts of leaf scab across all treatments (Table 1). Conversely, cumulative rainfall between 1 June and 31 August 2016 was 256 mm over 17 events compared with 379 mm over 28 events in 2017. The environment in 2017 was not conducive for early-season infection, which was reflected in the lower maximum leaf scab incidence and area under the disease progress curve values on the nontreated control trees (Table 1). The greater summer rainfall in 2017 led to first appearance of nut scab symptoms approximately 4 weeks earlier than in 2016 and maximum nut scab severity was greater on the nontreated control trees in 2017 than in 2016 (Fig. 1).

All fungicide treatments significantly reduced maximum leaf scab incidence and area under the disease progress curve when compared with the nontreated in 2016; however, Abound, Abound plus Orius 3.6F, and Super Tin 4L plus Orius 3.6F significantly reduced maximum leaf scab incidence and area under the disease progress curve when compared with the stand-alone Super Tin 4L and Orius 3.6F treatments (Table 1). In 2017, maximum leaf scab incidence and area under the disease progress curve were significantly lower in trees treated with Abound or Abound plus Orius 3.6F than in trees treated with all other fungicides. Trees that received the stand-alone Super Tin 4L or Super Tin 4L plus Orius 3.6F treatments had significantly reduced maximum leaf scab incidence and trees treated with these treatments or Orius 3.6F alone had significantly reduced area under the disease progress curve when compared with the nontreated trees. There were no significant effects of treatment on maximum nut scab incidence in either year but maximum nut scab severity was significantly reduced in both years on trees treated with Abound, Abound plus Orius 3.6F, Super Tin 4L, or Super Tin 4L plus Orius 3.6F when compared with the nontreated trees (Table 1).

For RGe values on TPTH-amended medium, the fungicide treatment by date of sampling interaction was not significant ($P = 0.3076$), nor was the effect of fungicide treatment alone ($P = 0.0626$); however, RGe values did differ significantly among sampling dates ($P < 0.0001$) and, therefore, data were pooled over treatment and compared by date. For all fungicide treatments combined, insensitivity to TPTH increased significantly between 22 June and 15 September 2016, decreased significantly between 15 September 2016 and 15 June 2017, and, again, decreased significantly between 15 June and 8 September 2017 (Table 2). Similarly, for RGr values on tebuconazole-amended medium,

the interaction between fungicide treatment and sampling date and fungicide treatment alone were not significant ($P = 0.1079$ and 0.8906 , respectively). As observed with TPTH RGe values, RGr values on tebuconazole differed significantly among sampling dates ($P < 0.0001$) and data were pooled over treatment and compared by date. Insensitivity to tebuconazole across all treatments decreased significantly between 22 June and 15 September 2016, increased significantly between 15 September 2016 and 15 June 2017, and decreased significantly from 15 June to 8 September 2017 (Table 2).

Discussion

Studies utilizing in vitro bioassays may provide an indication of relative sensitivity or insensitivity of an isolate or sample to a particular active ingredient and are useful for detecting shifts in sensitivity over time. However, a decrease in sensitivity to a fungicide in vitro does not necessarily indicate the occurrence of practical resistance. The goals of this study were to examine the within-season changes in sensitivity to TPTH and tebuconazole in *V. effusa* as influenced by fungicide treatment and to explore the relationship between fungicide sensitivity and efficacy in managing pecan scab under field conditions. As important members of the scab management arsenal, products containing these two active ingredients are heavily relied upon by pecan producers; therefore, monitoring and managing the development of resistance are very important to preserve their efficacy. The concentrations of TPTH and tebuconazole used in the sensitivity assays were initially selected based on a baseline study conducted by Seyran et al. (2010). For TPTH, the probability of an isolate sampled from the baseline population having an EC_{50} value greater than the discriminatory concentration of $30 \mu\text{g/ml}$ used herein was 0.00862 . As such, if RGe on this concentration was 0% , the population on the sampled tree could be considered sensitive and an RGe greater than 0% indicated that the population was less sensitive than the historic baseline population (Seyran et al. 2010). In the same study, for the DMI fungicide propiconazole, the probability of an isolate sampled from the baseline population having an EC_{50} value greater than the discriminatory concentration of $1.0 \mu\text{g/ml}$ was 1.49×10^{-7} (Seyran et al. 2010). Cross resistance among the DMI fungicides is common (Köller et al. 1997) and cross resistance between propiconazole and tebuconazole is known to occur in *V. effusa*; therefore, a discriminatory tebuconazole concentration of $1.0 \mu\text{g/ml}$ was appropriate (Stevenson et al. 2015). Thus, if RGr on this discriminatory concentration was 0% , the population on the sampled tree was considered sensitive to tebuconazole and an RGr greater than 0% indicated that the population was less sensitive than the historic baseline population (Seyran et al. 2010). Although a quantitative relationship between RGe or RGr values and efficacy in the field was not determined in this study, some important inferences were made, as described below.

The Super Tin 4L-treated trees had consistently less leaf and nut scab than the nontreated control trees in both years of this study (Table 1). In 2016, mean RGe values on TPTH-amended medium for samples collected from trees treated with Super Tin 4L were between 10.9 and 40.9% (Table 2), and maximum leaf scab incidence, area under the disease progress curve, and maximum nut scab severity on those trees were significantly lower compared with the nontreated control trees. However, these disease measurements were significantly greater on trees treated with Super Tin 4L than on those treated with Abound, Abound plus Orius 3.6F, or Super Tin 4L plus Orius 3.6F (Table 1). Mean RGe values in 2017 (4.2 and 0.6%) were considerably lower than in 2016; maximum leaf scab incidence, area under the disease progress curve, and maximum nut scab severity on Super Tin 4L-treated trees were significantly lower than on the nontreated control trees (Tables 1 and 2). Similarly, scab intensity in 2017 was significantly lower on trees treated with Abound (maximum leaf scab incidence and area under the disease progress curve), Abound plus Orius 3.6F (maximum leaf scab incidence, area under the disease progress curve, and maximum nut scab severity), or Super Tin 4L plus Orius 3.6F (area under the disease progress curve) than on trees treated with Super Tin 4L alone (Table 1).

Fig. 1. Effect of biweekly fungicide applications on disease progress of pecan scab epidemics in field experiments conducted in 2016 and 2017. Leaf scab incidence (indicated by the solid line) was assessed every 2 weeks after first appearance of symptoms, from 25 May to 20 July 2016 and 31 May to 28 July 2017; nut scab severity (indicated by the dashed line) was assessed monthly during fruit development, from 25 August to 15 September 2016 and 12 July to 8 September 2017.

In 2017, the efficacy of Super Tin 4L in relation to the other treatments was greater than in 2016. This could be due to the lower spring rainfall observed in 2017 that resulted in less leaf scab early in the year and the higher summer rainfall that occurred after leaves were no longer susceptible. Super Tin 4L is a protectant fungicide that is generally less effective at controlling leaf scab during the early part of the season, when rapid growth of susceptible leaf tissue occurs. Additionally, depending on the amount and timing of rainfall relative to fungicide applications, a considerable percentage of the fungicide may have been washed from the foliage during the early part of the 2016 growing season (Reynolds et al. 1994). Another possible explanation for greater relative efficacy of TPTH treatments in 2017 is the reduction in mean RGe values over time that resulted in a more TPTH-sensitive population in 2017. Fungicide treatment did not significantly affect sensitivity to TPTH but sampling date did (Table 2). On the whole, insensitivity to TPTH in this orchard increased significantly between June and September 2016, decreased significantly between September 2016 and June 2017, and, again, decreased significantly between June and September 2017 (Table 2). The fact that insensitivity to TPTH decreased significantly between growing seasons in the absence of fungicide exposure, as the pathogen overwintered, may provide evidence that insensitivity to TPTH is not stable and incurs a fitness cost to this pathogen. A similar phenomenon was observed in the sugar beet pathogen *Cercospora beticola*, in which insensitivity to TPTH was first identified in the United States in 1994 (Bugbee 1996). Results of a 10-year fungicide sensitivity monitoring program revealed that a decrease in the frequency of TPTH-insensitive isolates occurred over time when the annual number of TPTH applications was reduced from an average of 2.14 in 1998 to <1.0 from 2001 to 2008 (Secor et al. 2010). Additionally, isolates of *C. beticola* insensitive to the chemically related organotin fungicides fenitro acetate and fenitro chloride were found to be at a competitive disadvantage when sugar beet leaves were coinoculated with sensitive isolates (Giannopolitis and Chrysai-Tokousbalides 1980). Whether this is the case in *V. effusa* remains to be seen but future research is needed to further investigate the stability of TPTH insensitivity in this pathogen.

Table 1. Effects of biweekly fungicide applications on pecan scab epidemics^w

Year, treatment	Leaf scab (%)		Nut scab (%)	
	Max incd	AUDPC ^x	Max incd	Max sev
2016 ^y				
Super Tin 4L, 0.877 liter/ha	51.0 b	18.9 b	100.0 a	56.9 bc
Orius 3.6F, 0.585 liter/ha	43.4 b	18.3 b	100.0 a	77.4 ab
Abound, 8.77 liters/ha	29.8 c	11.0 c	96.5 a	26.0 d
Abound, 0.877 liter/ha + Orius 3.6F, 0.585 liter/ha	26.9 c	9.8 c	96.7 a	22.2 d
Super Tin 4L, 0.877 liter/ha + Orius 3.6F, 0.585 liter/ha	33.1 c	12.0 c	100.0 a	44.2 cd
Nontreated	61.7 a	25.7 a	100.0 a	86.1 a
2017 ^z				
Super Tin, 4L 0.877 liter/ha	28.8 bc	13.4 b	100.0 a	49.4 b
Orius 3.6F, 0.585 liter/ha	31.1 ab	13.4 b	100.0 a	84.3 a
Abound, 8.77 liters/ha	9.1 d	3.2 d	100.0 a	36.0 bc
Abound, 0.877 liter/ha + Orius 3.6F, 0.585 liter/ha	7.5 d	2.1 d	100.0 a	25.5 c
Super Tin 4L, 0.877 liter/ha + Orius 3.6F, 0.585 liter/ha	20.2 c	7.3 c	100.0 a	51.2 b
Nontreated	40.9 a	18.9 a	100.0 a	97.5 a

^w By year, means within columns followed by the same letter are not significantly different according to pairwise *t* tests of least squares means ($\alpha = 0.05$). Max incd = maximum incidence and Max sev = maximum severity.

^x Area under the disease progress curve (AUDPC) values calculated based on assessments of leaf scab incidence from 25 May to 20 July 2016 and 31 May to 28 July 2017.

^y Applications made in 2016: 18 and 28 April, 12 and 26 May, 9 and 23 June, 7 and 21 July, and 4 and 18 August.

^z Applications made in 2017: 8 and 21 April; 5 and 18 May; 1, 16, and 27 June; 13 and 27 July; and 10 August.

The efficacy of Orius 3.6F was greater relative to the other treatments in 2016 compared with 2017. In 2016, mean RGr values on tebuconazole-amended medium for samples retrieved from trees treated with Orius 3.6F were between 34.6 and 45.5% (Table 2). On those trees, significant reductions in maximum leaf scab incidence and area under the disease progress curve were observed when compared with the nontreated control trees whereas maximum nut scab severity was not significantly reduced (Table 1). Then, in 2017, mean RGr values were between 56.3 and 69.3% (Table 2); area under the disease progress curve was significantly reduced on trees treated with Orius 3.6F but maximum leaf scab incidence and maximum nut scab severity were not significantly different from the nontreated control trees (Table 1). Applications of Orius 3.6F alone did not provide significant control of leaf or nut scab, with the exception of significantly lower maximum leaf scab incidence compared with that on the nontreated trees in 2016. This was likely due to widespread insensitivity across this orchard (Table 2). Similar to TPTH, fungicide treatment did not significantly affect tebuconazole insensitivity but sampling date did (Table 2). Across all treatments, insensitivity to tebuconazole decreased significantly within the growing season in both years and increased significantly between years (Table 2). The fact that RGr values decreased within season could be an artifact of the sampling protocol. In both years, the early-season sensitivity measurement was taken toward the end of the leaf expansion phase, as leaves began to reach maturity and became less susceptible to new infection. It is likely that more disease cycles occurred on fruit shucks and late-season growth flushes after that time in both years, which could have been influenced by whether new infections were coming from the same tree (autoinfection) or different ones (alloinfection) (Mundt 2009). Based on the large size of individual pecan trees, it is likely that scab within the canopy is predominantly caused by autoinfections as conidia are washed down through the canopy from the foliage above during rain events; however, the dispersal of *V. effusa* inoculum between trees is not well understood. If there are relatively greater numbers of alloinfections compared with autoinfections, then our RGr values may reflect the sensitivity of incoming conidia that had not experienced the same selection pressure. Whether this is the case or not, Orius 3.6F still failed, for the most part, to significantly control scab in both years of this study, providing further evidence of practical resistance to tebuconazole in this orchard.

Previous research aimed at establishing the relationship between in vitro sensitivity and efficacy has produced mixed results. Köller et al. (1997) developed the first in vitro threshold for detection of DMI resistance when they found that isolates of *V. inaequalis* with RGr values greater than 80% on medium amended with fenarimol (0.05 µg/ml) or myclobutanil (0.1 µg/ml) were capable of causing practical field

Table 2. Effects of sampling date on insensitivity to fenitro hydroxide and tebuconazole in *Venturia effusa* from fungicide-treated pecan trees

Sampling date	Relative growth or germination (%) ^x	
	Fenitro hydroxide ^y	Tebuconazole ^z
22 June 2016	10.9 (1.7) b	45.5 (3.7) c
15 September 2016	40.9 (6.6) a	34.6 (4.6) d
15 June 2017	4.2 (0.8) c	69.3 (3.7) a
8 September 2017	0.6 (0.2) d	56.3 (4.0) b
Pr > <i>F</i>	<0.0001	<0.0001

^x Relative growth and germination values determined using a modified assay described by Seyran et al. (2010). Means within columns followed by the same letter are not significantly different according to pairwise *t* tests of least squares means ($\alpha = 0.05$).

^y Relative germination calculated as the proportion of germinated conidia on medium amended with fenitro hydroxide at 30 µg/ml divided by the proportion of germinated conidia on medium without fungicide, multiplied by 100; values represent the means of 30 samples collected from individual treated trees per time point, with standard errors in parentheses.

^z Relative growth calculated as the corrected mean diameter of 10 microcolonies on medium amended with tebuconazole at 1 µg/ml divided by the corrected mean diameter of 10 microcolonies on medium without fungicide, multiplied by 100; values represent the means of 30 samples collected from individual treated trees per time point, with standard errors in parentheses.

resistance. This was confirmed by conducting a greenhouse efficacy test revealing apple scab severity to be partially controlled by fenarimol or myclobutanil when leaves were inoculated with an isolate having an RGr of 88% compared with an isolate with an RGr of 42%. Miller et al. (2002) found that DMI-resistant isolates of *Sclerotinia homoeocarpa* with mean EC₅₀ values 5 to 10 times greater than the baseline isolates were not effectively controlled by DMI fungicides in the greenhouse. Also investigating *S. homoeocarpa*, Popko et al. (2012) determined that isolates from a native population exhibiting RGr values greater than 50% on medium amended with propiconazole (0.1 µg/ml) were capable of causing practical resistance. Conversely, Franke et al. (1998) found no significant correlation between in vitro sensitivity of *Sclerotium rolfii* to tebuconazole and control of southern stem rot of peanut with tebuconazole. This lack of correlation may have occurred because the isolates used in the study were sensitive enough to be adequately controlled by the labeled rate of tebuconazole. Similarly, Thomas et al. (2012) were unable to determine the relationship between fungicide sensitivity of *Stagonosporopsis citrulli* (syn. *Didymella bryoniae*) and efficacy for gummy stem blight (GSB) management because the initial population was highly resistant at the onset of the experiment. However, it was clear that, if the frequency of resistance to boscalid, azoxystrobin, or thiophanate-methyl in the initial population was greater than 90, 95, or 80%, respectively, then these fungicides were likely to be completely ineffective against GSB.

In both years of this study, Abound and Abound plus Orius 3.6F provided superior disease control when compared with all other fungicide treatments, except Super Tin 4L plus Orius 3.6F (Fig. 1; Table 1). Azoxystrobin is the active ingredient in Abound and belongs to the QoI fungicide group, known to have a high risk for resistance. The fact that disease control was not compromised after 10 consecutive applications in both 2016 and 2017 provides practical evidence of a reduced risk of resistance predicted by the presence of an intron in the *V. effusa* cytochrome *b* gene (Standish et al. 2016). These fungicides have been used on pecan since the late 1990s and practical resistance has never been reported; however, efforts to monitor QoI sensitivity have been hindered by the lack of a reliable screening method (Seyran et al. 2010; Standish et al. 2016).

An unexpected outcome of this study was that Super Tin 4L plus Orius 3.6F provided significantly greater leaf scab control in 2016 than that of either Super Tin 4L or Orius 3.6F alone, and significantly greater nut scab control than Orius 3.6F alone (Table 1). To a lesser extent, this trend was also observed in 2017, with the combination resulting in significantly lower leaf scab incidence and nut scab severity than Orius 3.6F alone, and a significantly lower area under the disease progress curve than Super Tin 4L or Orius 3.6F alone (Table 1). Fungicides in the DMI group such as Orius 3.6F are known to be stronger against leaf scab due to local systemic movement. However, this increased efficacy could be explained as preserving the activity of one or both fungicides because it follows an important resistance management strategy of tank-mixing an at-risk, systemic fungicide (Orius 3.6F) with a low-risk protectant (Super Tin 4L) (Köller and Wilcox 1999).

The results of this study provide insights into the relationship between efficacy of Super Tin 4L or Orius 3.6F and laboratory-based sensitivity of *V. effusa* to TPTH or tebuconazole, respectively. The active ingredients in these two fungicides are heavily relied upon by pecan producers for scab management; therefore, monitoring and managing the development of resistance to these two chemistries is of the utmost importance. The TPTH sensitivity and efficacy results reveal that RGe values between 0.6 and 40.9% could occur without causing a likely control failure, though efficacy was reduced when compared with other treatments. However, this study shows that an RGr value between 34.6 and 69.3% is likely to result in a scab control failure for trees treated with Orius 3.6F at the rate of 0.585 liter/ha. In such cases, growers are advised to keep resistance management practices in mind and utilize tank mixtures to preserve fungicide activity as a means of better managing pecan scab epidemics.

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