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Evaluation of a phosphite fungicide to control pecan scab in the southeastern USA

Clive H. Bock [a,*], Tim B. Brenneman [b], Michael W. Hotchkiss [a], Bruce W. Wood [a]

[a] SEFTNRL-USDA-ARS, 21 Dunbar Road, Byron, GA 31008, USA
[b] Department of Plant Pathology, Coastal Plain Experiment Station, The University of Georgia, Tifton, GA 31793, USA

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ABSTRACT

The efficacy of phosphite, a potential elicitor of systemically acquired resistance (SAR) was compared to the protectant fungicide triphenyltin hydroxide (TPTH) to control pecan scab caused by *Fusicladium effusum*. Efficacy was evaluated in four field experiments over a two-year period involving biweekly foliar applications of both fungicides to trees of five susceptible cultivars of pecan (*Carya illinoinensis*) and assessment of disease severity on foliage and fruit. Both phosphite and TPTH reduced scab severity on foliage equally well compared to the non-treated control, with the exception of one of the TPTH treatments in 2010. Both phosphite and TPTH provided equally good control of disease early in fruit development (Jul/Aug). However, by the final assessment (Sep/Oct), scab severity on phosphite-treated trees was most often greater than those receiving TPTH and in 2010, severity was equivalent to the non-treated control. Despite a suggested lack of late-season protection with phosphite, there was no difference in fruit volume between phosphite and TPTH-treated plots in 2009, and no difference in nut volume in 2010, although there were treatment differences in kernel weight and fruit weight in 2010. Phosphite-treated trees showed some symptoms of phytotoxicity. Regression analysis demonstrated the effect of scab on yield loss and confirmed the value of scab control on susceptible cultivars. *In-vitro* tests showed that phosphite is toxic to scab at rates applied in the field, thus implying direct fungitoxicity. Results indicate that phosphite provides useful control of pecan scab on both foliage and fruit early in the growing season, but might not provide prolonged late-season protection compared to an industry standard (i.e., TPTH).

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1. Intoduction

Pecan scab (caused by *Fusicladium effusum*) is widespread on pecan [*Carya illinoinensis* (*Wangeth.*) Koch] cultivated in the southeastern U.S. (Goff et al., 1996), and elsewhere in the world where pecan is cultivated as an exotic in humid environments (Kobayashi, 1984; Mantz et al., 2008). Scab is the most economically important pecan disease (Gottwald and Bertrand, 1983, 1988; Sanderlin, 1995; Stevenson and Bertrand, 2001). The pathogen produces conidia that are wind- and splash-dispersed (Gottwald and Bertrand, 1982), and require prolonged surface wetness for infection. Thus, epidemics are most severe in high-rainfall years (Gottwald, 1985; Turechek and Stevenson, 1998; Sparks et al., 2009).

Many pecan cultivars are susceptible to scab, with relatively resistant cultivars becoming susceptible after a few years of cultivation (Goff et al., 1996). Consequently, growers rely heavily on fungicides to control scab in commercial orchards (Demaree, 1925; Cole and Large, 1939; Brenneman et al., 1999; Seyran et al., 2010). The presence of fungicide resistance in *F. effusum* to different chemical classes of fungicides makes a compelling case for careful management of fungicides to prolong efficacy (Isakeit, 2010; Littrell and Bertrand, 1981; Stevenson, 1998; Brenneman et al., 1999; Stevenson et al., 2004; Seyran et al., 2010), and for testing new fungicides as alternatives to conventional options. The need to minimize use of synthetic fungicides for environmental and health reasons also justifies exploration of disease management alternatives (Percival et al., 2009; Percival and Haynes, 2008; Schnabel and Parisi, 1997; Gozzo, 2003; Agostini et al., 2003). One such approach is stimulation of systemically acquired resistance (SAR), in which the *in planta* defense system is stimulated to prevent infection. Phosphites are thought to elicit a SAR effect in some hosts species (Guest and Grant, 1991; Kessmann et al., 1994; Sticher et al., 1997; Becot et al., 2000; Jackson et al., 2000; Gozzo, 2003; Percival et al., 2009; Miller et al., 2006) and can also exhibit direct fungitoxicity against certain pathogens (Fenn and Coffey, 1984; Wilkinson et al., 2001). Phosphite is especially efficacious against oomycetes, but can also protect against certain other fungal pathogens (Jackson et al., 2000; Miller et al., 2006; Kessmann et al., 1994; Gozzo, 2003). Although useful as fungicides, variation in phosphite sensitivity is reported for certain pathogens, especially within the oomycetes (Wilkinson et al., 2001;

* Corresponding author. Tel.: +1 478 956 6409.
E-mail addresses: clive.bock@usda.ars.gov, clive.bock@ars.usda.gov (C.H. Bock).

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Fenn and Coffey, 1984; Brown et al., 2004); suggesting a potential for fungal pathogen resistance to phosphites.

Phosphites exhibit useful efficacy against apple scab (*Venturia inaequalis*) and pear scab (*Venturia pirina*); although control is less than with conventional fungicides (Percival et al., 2009). In apple, Rosenberger and Cox (2009) found weekly foliar phosphite application somewhat effective for control of apple scab, but trunk applications were ineffective. Because phosphite is potentially useful for controlling *V. inaequalis* on apple and *V. pirina* on pear, and because a recent phylogenetic study indicates that *F. effusum* is related to *V. inaequalis* (Seyran et al., 2010), it is possible that phosphites might be beneficial for control of pecan scab. An early report on one years' field data suggested that phosphites possess at least a degree of activity against pecan scab (Sanderlin, 2010) thus, comprehensive field-testing of phosphites was initiated to determine whether they provide control of pecan scab on susceptible cultivars.

First, the present study investigates whether phosphites can effectively reduce severity of pecan scab in pecan orchards. Second, the degree of scab control achieved with phosphite is compared to an industry fungicide standard, triphenyltin hydroxide (TPTH). Third, the study assesses influence of phosphite on yield traits, and fourth, the relative *in-vitro* fungitoxicity of phospite and the protectant, TPTH, to *F. effusum* is compared.

2. Materials and methods

2.1. Orchard location and layout

Test orchards were located at the USDA-ARS research farm in Peach County, GA (latitude $+32^{\circ}$ 39′ 54″ N, longitude $+83^{\circ}$ 44′ 31″ W), with an elevation of ~156 m and a freeze-free growing period of ~240 d, and an annual precipitation of ~118 cm. The site has Faceville sandy loam soils [FoA; fine, Kaolintic, thermic Typic Kandiudult soil]. The efficacy of phosphite compared to TPTH and a non-treated control was investigated in four field experiments – one in 2009 and three in 2010. In 2009 and in one 2010 experiment the orchard used was a mixed planting of eight different cultivars of pecan planted as bare-root transplants in 1998 at 4.05 × 9.1 m spacing, with trees thinned in 2006 to 9.1 × 9.1 m spacing. The orientation of the rows in this multiple-cultivar orchard was north to south. The final orchard configuration consisted of single consecutive series of two to three trees of each of the eight cultivars assigned randomly in each of eight blocks assigned randomly in the orchard. Orchard trees were approximately 6–10 m tall. In 2009, three cultivars were included (Wichita, Desirable and Apache), and in 2010 four were included (Wichita, Desirable, Apache and Cheyenne). A third and fourth experiment were established in 2010 on two small, separate single-cultivar orchards of cvs (Wichita and Cherokee) planted adjacent to each other. Cv. Wichita was planted in 1975 and thinned to 18.2 × 12.1 m in 1998, and cv. Cherokee was planted in 1984, and thinned to 18.2 × 12.1 m in 2007. Trees in both orchards were reproductively mature and approximately 15–20 m tall. All orchards received standard farm practice fertilizer and weed control (Hudson et al., 2011), and sub-surface drip irrigation as required, but received no insect or disease control other than that specified below. Scab susceptibility varied among cultivars with Wichita being extremely susceptible, and cvs. Cheyenne, Cherokee and Apache as a group being susceptible, and cv. Desirable being slightly less susceptible to scab.

2.2. Treatments and experiment design

Timing of fungicide application based on calendar date is a common practice for managing scab-susceptible pecan (Gottwald and Bertrand, 1988; Brenneman et al., 1999), with sprays starting mid-April and applied approximately every two weeks for 16 weeks until early-mid-August, at shell-hardening (Gottwald and Bertrand, 1988; Brock and Brenneman, 2011). This approach was followed, although weather conditions (rainfall and wind) affected exact timing and frequency of applications in both seasons. In the mixed-cultivar experiment in 2009, the two fungicide treatments were ProPhyt (potassium phosphite, 54.5% a.i., at the standard rate of 2.64 L ProPhyt 1000 L^{-1} ha^{-1}, Helena Chemical Company, Collierville, TN) applied eight times (between April 15th, and July 31st, 2009), and Super Tin 4L, (TPTH, 40.0% active ingredient, at the standard rate of 0.90 L 1000 L^{-1} ha^{-1}, United Phosphorous, Inc., King of Prussia, PA) applied following the same spray schedule. In the mixed-cultivar experiment in 2010, there were four fungicide treatments; on all plots ProPhyt and Super Tin 4L were applied to trees as described for 2009, but with a total of six applications between April 21st and July 16th, 2010. Half the fungicide-treated trees received two additional applications on August 5th and 27th, 2010. The six-spray regime was termed the part-season (PS) treatment, and the eight-spray regime the full-season (FS) treatment. The two single-cultivar experiments in 2010 (cvs. Wichita and Cherokee) received the following treatments: i) non-treated control, ii) a standard rate of ProPhyt (described above), iii) a high rate of phosphite (ProPhyt at 4.70 L per 1000 L ha^{-1}), and iv) a standard rate of Super Tin 4L (described above) with all treatments applied nine times between April 22nd and September 1st, 2010. All fungicide treatments were applied to provide full coverage of individual trees using a hand-held spray gun (Ford's and Gantt, Macon, GA) either from the back of a pick-up truck or from a hydraulic boom lift (JLG Industries Inc., McConnelsburg, PA).

In the mixed-cultivar experiments in 2009 and 2010 a fully randomized factorial design (cultivar × treatment) was imposed on the structure of the existing orchard planting. Treatments were assigned randomly to trees of each cultivar, with three replicate trees of each of the three cultivars receiving one of the three treatments in 2009 ($n = 27$), and two replicate trees of each of the four cultivars receiving one of the five treatment in 2010 ($n = 40$), where a treatment is a single fungicide treatment × cultivar combination. In the single-cultivar orchards in 2010 (cvs. Wichita and Cherokee) the design was completely randomized with each one of the four treatments assigned randomly to two replicate trees of each cultivar ($n = 8$).

2.3. Disease severity and yield parameters

Disease severity was assessed on leaves and fruit. In 2009, scab severity (% area diseased) was assessed visually on August 5th (four leaves sampled at random from each of five terminals in the lower and mid-third of each tree), with severity estimated for each leaf. Fruit samples were assessed visually for scab severity (% area infected) on August 5th and September 21st (five individual fruit clusters [2–5 fruit each] collected at random from the lower and mid-third of each), and mean percent area diseased per fruit was estimated. In the multiple-cultivar experiment in 2010, foliage was assessed visually for scab severity on June 11th by evaluating each leaflet on 10 compound leaves per tree. Fruit assessments were completed on July 7th by assessing 15 fruit per tree and on October 15th by assessing 10 fruit per tree. For each fruit, the four valve-sides were assessed individually for percent area diseased. In the single-cultivar experiments in 2010, foliage was assessed visually for scab severity on June 16th as described for the 2010 multi-cultivar experiment. Visual fruit assessments were made on June 30th by assessing 15 fruit per tree and on September 23rd by assessing 10 fruit per tree. In all 2010 experiments all leaflet/leaf/fruit samples were taken randomly from the entire canopy of each tree, and the individual leaflet and individual fruit disease severity estimates were used as the raw data in the subsequent analyses.

In late July of 2010, ProPhyt treatment-specific symptoms of foliar damage were noticed. The symptoms were manifested at leaflet tips as a necrotic zone extending to a maximum of half-way down affected leaflets, often with all leaflets on a leaf and most leaves on the terminals affected. The whole canopy of each tree was assessed for severity of phytotoxicity using a 0–3 scale on 16 August 2010, where 0 = no symptoms, 0.1 = a few leaflets with mild tip burn, 1 = several leaflets on most terminals with tip burn, 2 = numerous leaves on terminals with tip burn, 3 = most leaflets with severe burn, in some cases extending over >25% of the leaflet.

Yield parameters were also measured. In 2009 fruit volume (cm^3, fruit defined here as the inner nut and the surrounding fleshy pericarp, or shuck) was measured using samples collected for disease assessment on September 21st, as described above. Individual fruit volume was measured by water displacement. In the mixed-cultivar experiment in 2010, the sample of ten fruit per tree collected and assessed for disease on October 15th were used to obtain yield data. Fruit weight, nut weight (nut defined here as the inner nut minus the surrounding fleshy pericarp, or shuck) and volume (by displacement), and kernel weight were used as indicators of yield. In the single-cultivar experiments in 2010 yield was measured using fruit weight (g). In all 2010 experiments, individual fruit yield parameters were used as the raw data in the subsequent analyses.

2.4. In-vitro toxicity of phosphite and TPTH to F. effusum

A suspension of conidia (1×10^6 ml^{-1}) in sterile distilled water was prepared from cultures of a single-spore isolate of *F. effusum* isolated from cv. Desirable in Byron, GA. A 0.5-ml aliquot of the conidial suspension was used to seed 50 ml Potato Dextrose Broth (PDB, 24 g L^{-1}) in 125 ml Erlenmeyer flasks (final concentration 1×10^4 conidia ml^{-1}). The PDB was amended with ProPhyt or Super Tin 4L at different concentrations (0 = non-treated control, 0.05X, 0.25X, 0.5X, 1X, and 2X) compared to the recommended rate of the fungicide, where 1X = the recommended rate of 2.64 L (54.5% a.i.) 1000 L^{-1} (1.44 ppm) for ProPhyt and 0.90 L (40.0% a.i.) 1000 L^{-1} (0.36 ppm) for Super Tin 4L. The non-treated control received 0.5 ml sterile distilled water. Each treatment was replicated three times. Cultures were placed in an orbital shaker for three weeks at 27°C, when the *F. effusum*-PDB culture was filtered through previously dried (70°C for 48 h), weighed, No. 1 filter paper (Whatman International, Maidstone, England). The filter papers were rinsed through with sterile distilled water and re-dried, and weighed again. The experiment was repeated once.

2.5. Data analysis

General linear modeling (GLM) was used to analyze disease severity and yield from the mixed-cultivar experiments in 2009 and 2010 with main effects and interactions of fungicide treatment and cultivar (in 2009 canopy position was nested within cultivar, but was not significant). Tukey's test was used for multiple comparisons among means ($P = 0.05$) of fungicide treatments and cultivar for scab severity and yield variables described. The data from the single-cultivar experiments in 2010 (cvs. Wichita and Cherokee) were analyzed using GLM, but with the main effect of fungicide treatment only, and Tukey's test was used for the comparisons among means. Linear regression analysis ($y = a + bx$) was used to explore the relationship between disease severity on fruit at harvest and yield on treated and non-treated trees in the mixed- and single-cultivar experiments in 2009 and 2010 (data for each cultivar was analyzed individually). As described above, GLM was used to analyze the effect of phosphite and TPTH *in-vitro* on mass of *F. effusum* in liquid culture, using Tukey's test for multiple comparisons of treatment means. SAS V9.2 (SAS Systems, Cary, NC) was used for all data analyses.

3. Results

3.1. Severity of scab

In the mixed-cultivar experiments in 2009 and 2010 there was a significant effect of cultivar, fungicide treatment and a cultivar*-treatment interaction (Tables 1 and 2). In 2009 both ProPhyt and Super Tin 4L reduced foliar disease severity compared to the control, but scab severity on the fruit of phosphite-treated trees on September 21st was greater than that on Super Tin 4L-treated trees, but significantly less than the control. The significant cultivar*treatment interaction indicated the two more susceptible cvs., Wichita and Apache not only had more severe disease compared to cv. Desirable, but also showed a greater response to fungicides. ProPhyt and TPTH gave similarly good control of scab on foliage and young fruit on all three cultivars, but TPTH gave superior control of scab on fruit on cvs. Wichita and Desirable later in the season (September 21st).

In 2010, scab severity was again low on foliage. Both the PS and FS ProPhyt treatments had similar, and less severe foliar scab severity compared to the control. The Super Tin 4L treatment was inconsistent, with the FS Super Tin 4L-treated trees having similar, or more severe disease compared to the non-treated control, whereas the PS Super Tin 4L-treated plots had less severe disease on foliage compared to the non-treated control. On July 7th, immature fruit receiving PS or FS ProPhyt treatments had less disease compared to the non-treated control. Both Super Tin 4L treatments also had less severe disease compared to the non-treated control. By October 15th, disease had increased on fruit on both ProPhyt treatments and were no different to the non-treated control. In contrast, both PS and FS Super Tin 4L treatments had significantly less disease compared to the non-treated control. Cvs. Wichita and Apache were the most

Table 1
The efficacy of phosphite (ProPhyt) and triphenyltin hydroxide (Super Tin 4L) for controlling scab on pecan foliage and fruit in 2009 in a mixed-cultivar orchard in Byron, Georgia, southeastern USA.

Cultivar	Fungicide[a]	% Leaf area[b]	% Fruit area		Fruit vol (cm^2)
		Aug 5th	Aug 5th	Sep 21st	
Apache	–	1.3b[c]	25.9b	34.6a	28.5b
Desirable	–	0.2a	12.8a	32.4a	29.6b
Wichita	–	2.3c	29.5b	51.6b	23.4a
–	Control	2.2b[d]	65.2b	82.5c	18.7a
–	ProPhyt	0.7a	2.7a	28.5b	31.2b
–	Super Tin 4L	0.9a	0.3a	7.6a	31.5b
Apache	Control	1.9ab	74.5c	85.7d	21.8b
	ProPhyt	1.1ab	2.9a	8.7a	34.6d
	Super Tin 4L	1.0ab	0.3a	9.4a	29.2cd
Desirable	Control	0.4ab	36.7b	63.9c	25.9bc
	ProPhyt	0.1a	1.5a	26.0b	28.6cd
	Super Tin 4L	0.0a	0.1a	7.2a	34.2d
Wichita	Control	4.4c	84.5c	98.0d	8.5a
	ProPhyt	0.9ab	3.6a	50.8c	30.5cd
	Super Tin 4L	1.6ab	0.4a	6.1a	31.3cd
Effect[d]:	Cultivar	<0.0001	<0.0001	<0.0001	<0.0001
	Treatment	0.0001	<0.0001	<0.0001	<0.0001
	Cultivar*Treatment	0.003	<0.0001	<0.0001	<0.0001

[a] Rate for Super Tin 4L = 0.90 L 1000 L^{-1} ha^{-1}, and for ProPhyt = 2.64 L 1000 L^{-1} ha^{-1}. Fungicides were applied eight times at about 2-week intervals between April 15th and July 31st.
[b] Disease severity was assessed visually for the percent area of the leaf or fruit surface infected, with treatment means based on three replicate trees receiving each treatment combination.
[c] Mean comparisons are based on Tukey's test. Numbers with different letter are significantly different ($P = 0.05$).
[d] Treatment effect P-values indicate the probability that the F-value for the treatment effects for the null hypothesis are not significant.

Table 2
The efficacy of phosphite (ProPhyt) and triphenyltin hydroxide (Super Tin 4L) for controlling scab on pecan foliage and fruit in 2010 in a mixed-cultivar orchard in Byron, Georgia, southeastern USA.

Cultivar	Fungicide[a]	% Leaflet area[b]	% Fruit area		Fruit weight (g, fresh)	Nut vol (cm^2)	Kernel wt (g)
		Jun 11th	Jul 7th	Oct 15th			
Apache	–	1.0c[c]	6.2c	66.0c	25.4b	8.3a	3.22a
Cheyenne	–	0.6b	3.9b	19.6a	23.1b	8.4a	3.17a
Desirable	–	0.1a	0.6a	23.9a	26.0c	11.6b	4.31b
Wichita	–	0.9c	6.6c	53.9b	21.1a	8.2a	3.18a
–	Control	0.8b	11.0c	59.3b	20.2a	8.2a	2.81a
–	ProPhyt (PS)	0.4a	2.1a	47.5b	22.9ab	9.0b	3.47b
–	ProPhyt (FS)	0.4a	4.8b	63.4b	24.2b	9.3b	3.22b
–	Super Tin 4L (PS)	0.4a	2.9a	25.6a	27.2c	9.7b	4.14c
–	Super Tin 4L (FS)	1.3c	1.4a	24.7a	25.0bc	9.4b	3.67b
Apache	Control	1.5c	14.9cd	93.7e	20.0ab	6.9a	2.51ab
	ProPhyt (PS)	0.3ab	2.4a	46.3bcd	24.6b	7.8ab	3.04ab
	ProPhyt (FS)	0.1a	6.5ab	74.8e	27.3bc	8.8b	3.30bc
	Super Tin 4L (PS)	2.4d	2.7a	52.5cde	32.0c	9.4b	4.31c
	Super Tin 4L (FS)	0.7bc	4.5ab	62.7de	22.9b	8.5b	2.97ab
Cheyenne	Control	0.2a	10.9bc	50.7bcd	19.7ab	7.5ab	2.42ab
	ProPhyt (PS)	1.1c	1.4a	9.9a	21.6ab	8.6b	3.26b
	ProPhyt (FS)	1.1c	3.5a	36.4abcd	26.1bc	7.6ab	3.28bc
	Super Tin 4L (PS)	0.1a		1.8a	28.0bc	9.3b	3.95bc
	Super Tin 4L (FS)	0.4ab	0.02a	14.5a	22.5ab	8.5b	3.03ab
Desirable	Control	0.1a	1.5a	15.4ab	24.1b	11.4c	3.99bc
	ProPhyt (PS)	0.0a	0.3a	50.7cde	24.4b	11.4c	4.12bc
	ProPhyt (FS)	0.0a	1.6a	63.5de	26.2bc	13.6d	3.91bc
	Super Tin 4L (PS)	0.1a	0.1a	7.7a	25.0b	11.1c	4.52c
	Super Tin 4L (FS)	0.1a	0.04a	2.2a	30.4c	11.5c	4.82c
Wichita	Control	1.2c	16.8d	73.1de	16.8a	6.5a	2.12a
	ProPhyt (PS)	0.4ab	4.0a	83.0e	21.2ab	8.2ab	3.46bc
	ProPhyt (FS)	0.3ab	5.4ab	65.5de	19.3ab	8.6b	2.79ab
	Super Tin 4L(PS)	2.3d	1.4a	28.7abc	24.2b	8.7b	3.69bc
	Super Tin 4L (FS)	0.2a	5.7ab	23.2abc	23.6b	8.8b	3.77bc
Effect[d]:	Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Treatment (Trt)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cultivar*Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0003

[a] Rate for Super Tin 4L = 0.90 L 1000 L^{-1} ha^{-1}, and for ProPhyt = 2.64 L 1000 L^{-1} ha^{-1}. PS (part-season) treated-trees received six applications at about 2-week intervals between April 21st and July 16th. The FS (full-season) treated trees received two additional sprays on August 5th and 27th.
[b] Disease severity was assessed visually for the percent area of the leaflet or fruit surface infected, with treatment means based on two replicate trees for each treatment. Yield variable means based on 10 fruit from two replicate trees for each treatment.
[c] Mean comparisons are based on Tukey's test. Numbers with different letter are significantly different ($P = 0.05$).
[d] Treatment effect *P*-values indicate the probability that the *F*-value for the treatment effects for the null hypothesis are not significant.

severely diseased on both foliage and fruit. As in 2009, there was a significant cultivar*treatment interaction, with the two more susceptible cultivars (cvs. Apache and Wichita) most often having the most severe disease, and generally having the greatest response to fungicide treatments compared to the less susceptible cvs. Desirable and Cheyenne, although individual treatments were not always consistent. On the final assessment date, October 15th disease severity was relatively high on all cultivars and treatments, and cv. Desirable ProPhyt-treated trees had more severe scab symptoms compared to the untreated control.

The data from the two single-cultivar experiments (Table 3) showed that on foliage both the standard and high rates of ProPhyt reduced scab compared to the non-treated control, although the high rate of ProPhyt did not give better scab control than the standard rate, or Super Tin 4L treatment; and Super Tin 4L treatments were not always better than the non-treated control. Similarly on the fruit, both rates of ProPhyt and Super Tin 4L reduced scab severity compared to the non-treated control, except on cv. Wichita on September 23rd, when the high rate of ProPhyt did not result in better control. On cv. Wichita, disease severity on fruit of trees treated with Super Tin 4L was not different from either ProPhyt treatment, but on cv. Cherokee Super Tin 4L was superior early in the season compared to the low rate of ProPhyt.

Phytotoxicity symptoms were observed on trees of all cultivars sprayed with ProPhyt in all three experiments in 2010. In the multi-cultivar experiment, trees receiving the PS ProPhyt treatment had

Table 3
The efficacy of ProPhyt (phosphite) and Super Tin 4L (triphenyltin hydroxide) for controlling scab on pecan foliage and fruit on cvs. Wichita (A) and Cherokee (B) in 2010 in two orchards in Byron, Georgia, southeastern USA.

Treatment[a]	% Leaflet area[b]	% Area fruit		Nut length (cm)[b]	Nut weight (g, fresh)
	Jun 16th	Jun 30th	Sep 23rd		
A. Wichita					
Control	9.4b[c]	25.1b	85.7b	5.3a[c]	11.8a
ProPhyt (1X)	3.8a	8.9a	57.7a	6.2b	20.0b
ProPhyt (2X)	2.2a	4.6a	65.3ab	6.0b	17.8b
Super Tin 4L	7.1b	8.2a	50.7a	6.5b	21.7b
P-value[d]	<0.0001	<0.0001	0.0023	<0.0001	<0.0001
B. Cherokee					
Control	3.1b	20.9c	72.7b	4.4a	10.6a
ProPhyt (1X)	0.5a	5.7b	15.2a	4.8b	14.6b
ProPhyt (2X)	0.8a	1.2ab	8.6a	5.1c	19.9c
Super Tin 4L	0.9a	0.9a	9.1a	5.1bc	17.4bc
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Rates for Super Tin 4L = 0.90 L 1000 L^{-1} ha^{-1}, and for ProPhyt 1X = 2.64 and 2X = 4.70 L 1000 L^{-1} ha^{-1}. Nine sprays were applied at about 2-week intervals between April 22nd and September 1st.
[b] Disease severity was assessed visually for the percent area of the leaflet or fruit surface infected on 10–20 (depending on sample date) leaves or fruit per tree. Yield and quality variables variable means based on 10 fruit from two replicate trees for each treatment, with treatment means based on two replicate trees for each treatment.
[c] Mean comparisons are based on Tukey's test. Numbers with different letter are significantly different ($P = 0.05$).
[d] Treatment effect *P*-values indicate the probability that the *F*-value for the treatment effects for the null hypothesis are not significant.

a mean damage severity of 1.06 compared to 1.56 for trees receiving the FS treatment. In the single-cultivar experiments, cvs. Wichita and Cherokee had injury severities of 0.55 and 0.1, and 1.5 and 1.0, respectively, at the standard and high rates of ProPhyt, respectively. Although phytotoxicity symptoms were observed on ProPhyt-treated trees, they were not assessed in 2009. No symptoms were observed on the Super Tin 4L-treated trees or the non-treated control trees. The leaflet tip death did not result in noticeable leaflet or leaf drop.

3.2. Fruit yield variables

In the mixed-cultivar experiment in 2009 fruit volume was significantly greater in both ProPhyt and Super Tin 4L-treated trees compared to the control, and there was no significant difference between ProPhyt and Super Tin 4L-treated trees (Table 1). There were significant differences among cultivars in fruit volume. The significant cultivar*treatment interaction showed that the two more susceptible cultivars (cvs. Apache and Wichita) had the greatest yield response to the fungicides compared to cv. Desirable. Use of both ProPhyt and TPTH gave similarly good yield response with scab-susceptible cvs. Wichita and Apache, but not with cv. Desirable.

In the mixed-cultivar experiment in 2010 (Table 2), fruit weight was greatest for all ProPhyt and Super Tin 4L-treated trees compared to control, except the PS ProPhyt-treated trees. The Super Tin 4L-treated trees receiving the PS treatment had the highest fruit weight, whereas the FS Super Tin 4L-treated trees produced fruit that were not significantly larger than the ProPhyt-treated trees. Nut volume was not significantly different among the treatments, but all treatments had larger nuts compared to the non-treated control. Kernel weight was least for the non-treated control, and only kernels from trees receiving the PS Super Tin 4L-treatment were significantly heavier than kernels on trees receiving the other fungicide treatments. As in 2009, there were significant differences among cultivars in yield parameters. There were significant cultivar*treatment interaction for all yield parameters. All cultivars appeared to have a positive response to at least one of the treatments but the interactions were not consistent with treatment although cvs. Wichita

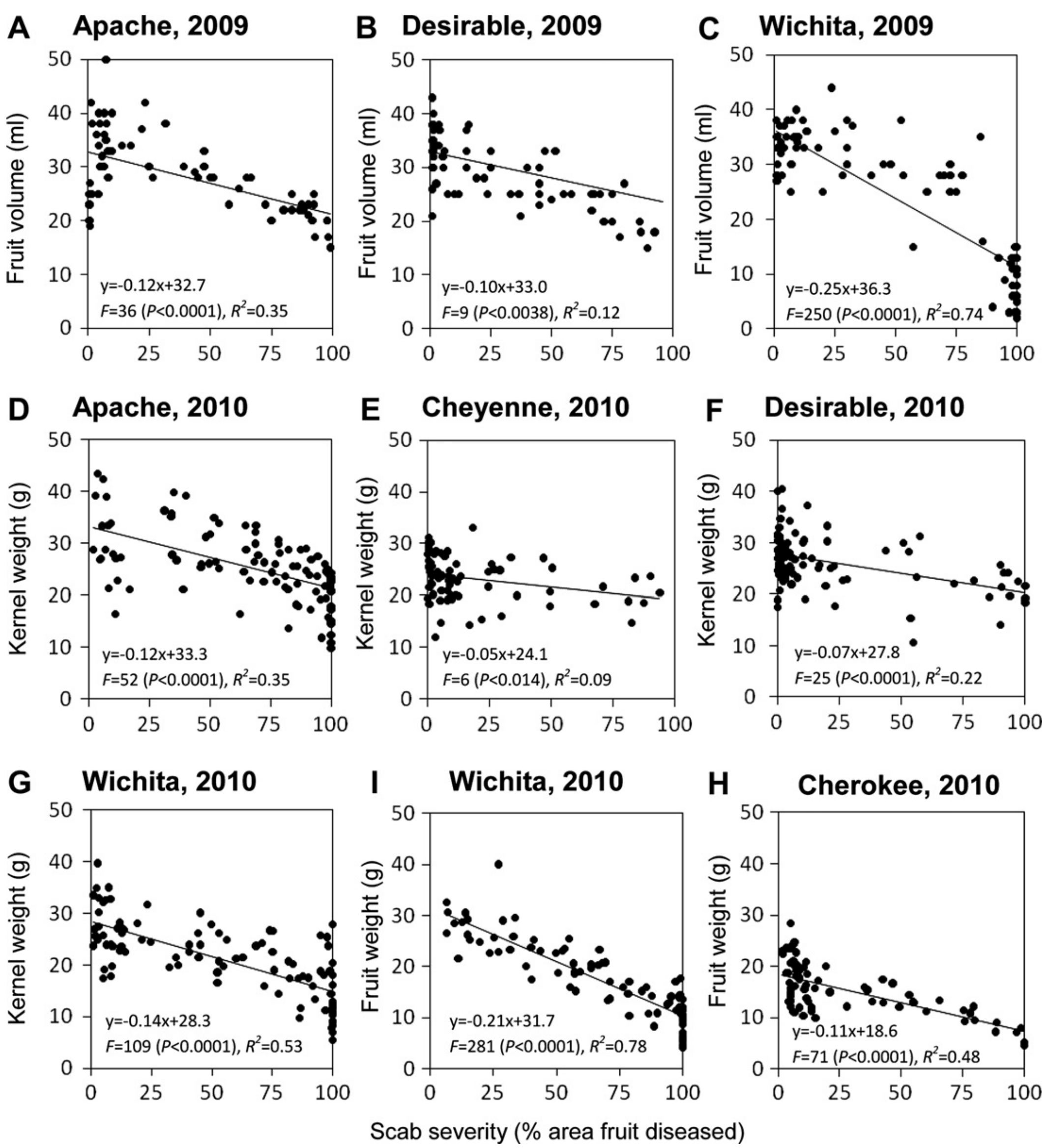

Fig. 1. Linear regression analysis showing the relationship between direct and indirect indicators of yield and pecan scab severity for combined treatments (ProPhyt- (phosphite) treated, Super Tin 4L- (triphenyltin hydroxide) treated and non-treated control trees) from four experiments in 2009 and 2010 at Byron, Georgia, USA. A–C (fruit volume, ml), and D–G, fruit weight, g) in multi-cultivar experiments in 2009 and 2010, respectively, and H and I, fruit weight, g) in single-cultivar experiments in 2010.

and Apache more often showed a significant effect between at least some of the fungicide treatments and the non-treated control.

The data from the two single-cultivar experiments (Table 3) on cvs Wichita and Cherokee showed both nut length and nut weight were greater on trees receiving either the standard or high rate of ProPhyt and the Super Tin 4L treatment compared to the non-treated control. The high rate of ProPhyt did not consistently improve these measures of yield compared to the standard ProPhyt rate. Super Tin 4L treatment resulted in a greater nut length and weight on cv. Cherokee (but not on cv. Wichita) compared to the standard rate of ProPhyt, but not compared to the high rate of ProPhyt.

There was a significant negative linear relationship between disease severity on fruit and yield for all cultivars in all experiments (Fig. 1A–H), demonstrating that reduction in disease by applying ProPhyt or Super Tin 4L improved yield. Cv. Wichita, the most susceptible cultivar, consistently had the highest coefficient of determination (R^2 = 0.53–0.78). Cvs. Apache and Cherokee, also susceptible, had lower coefficients of determination (R^2 = 0.35 and 0.48, respectively). Cvs. Cheyenne and Desirable, both less susceptible, had the lowest coefficients of determination (R^2 = 0.09–0.22).

3.3. In-vitro toxicity of phosphite and TPTH to F. effusum

There were significant differences between the two experiments (Fig. 2). Nonetheless, in both experiments *F. effusum* did not grow in the Super Tin 4L treatments. ProPhyt amended media showed that phosphite was toxic to growth of *F. effusum*, but did not significantly reduce mycelia mass at ≤0.25X. However, at ≥1.0X it had a toxic effect and fungal growth was almost completely inhibited.

4. Discussion

Phosphite effectively reduced severity of scab on foliage and developing fruit early in the season on pecan, with the level of control afforded by the phosphite application being consistently similar to, or better than that by TPTH. Severity of disease increased on fruit from August onwards and in some experiments TPTH-treated trees had less severe disease on fruit at harvest, compared to phosphite-treated trees, although this was not entirely consistent. In a previous study on apple scab (Percival et al., 2009), phosphite provided a useful degree of scab control when used throughout the season compared to synthetic fungicides, but disease suppression was not as good as a conventional fungicide. The effect of phosphite on pecan scab appears to be comparable, providing useful protection against the pathogen (also observed by Sanderlin (2010)), but perhaps not as efficacious as TPTH for providing control of scab on fruit late in the season. All five cultivars used in this study were very susceptible or moderately susceptible to *F. effusum*, and there were differences in the severity of disease that developed relating to their reported resistance to scab (Goff et al., 1996).

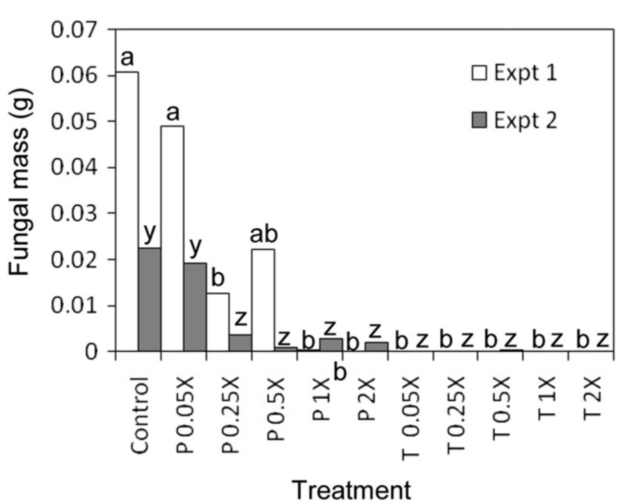

Fig. 2. The effect of phosphite and triphenyltin hydroxide (TPTH) on growth of *Fusicladium effusum in-vitro*. The fungus was grown in potato dextrose broth amended with ProPhyt (a.i. phosphite) or Super Tin 4L (a.i. TPTH) at different concentrations: 0, 0.05X, 0.25X, 0.5X, 1X, 2X as the factor of the recommended rate of the fungicide where 1X = 2.64 L 1000 L^{-1} for ProPhyt and 0.90 L 1000 L^{-1} for Super Tin 4L. General linear modeling analysis indicated significant difference between experiments (P = 0.0002), thus data is presented individually for each experiment. Means comparisons are based on Tukey's test and numbers with different letter are significantly different ($P = 0.05$). P = phosphite, T = TPTH.

The observation that scab severity tended to increase more on mature fruit of phosphite-treated trees compared to TPTH-treated trees could indicate that a late-season application of phosphite in mid-September is needed. A maximum number of phosphite applications provided best control of apple scab on apple fruit compared to reduced spray programs (Percival et al., 2009), which were applied at key times previously identified for apple scab control (Bevan and Knight, 2001). The single-cultivar experiments on cvs. Wichita and Cherokee did not indicate that a higher rate of phosphite was advantageous for controlling scab. The greater phytotoxicity and the economic implications of increasing the application rate suggest that this approach is less feasible than extending the spray schedule. Phytotoxicity of phosphite has been reported previously on other crops (Walker, 1989; Seymour et al., 1994), and the phosphite-induced damage to foliage observed in 2010 on all phosphite-treated trees suggests that phosphites should be applied to pecan with caution. The phytotoxicity appears to be a consequence of a salt-effect, with concentration of phosphite greatly increasing as the pooled solution at leaflet tips and lower margins evaporated – it is not known whether this will occur using application methods other than a spray gun.

The reason phosphite tended not to provide more prolonged protection of maturing fruit is unknown, but might be due to a relatively transient putative elicitation of SAR in pecan, or differential expression of SAR in maturing fruit later in the season as the fruit valves senesce. In some situations with other host-pathogen systems SAR can last for a year or more and provide protection against more than one disease (Percival, 2001), but the length of any effect has not been established with phosphite-treated pecan for scab. Phosphite also appears to be directly toxic to scab at rates applied in the field, which could directly impact field populations of the pathogen. Previous studies have demonstrated the direct toxicity of phosphite to some plant pathogens (Fenn and Coffey, 1984; Wilkinson et al., 2001). The proportion of the reduction in disease in the field that is due to direct toxicity vs. SAR mechanisms has not been established. Furthermore, the longevity of phosphite efficacy on the pecan leaf or fruit surface is unknown, but if it is more rapidly dissipated compared to TPTH - which is removed by increasing rainfall (Reynolds et al., 1994), the direct effect of phosphite would rapidly be lost.

Measured yield parameters indicated that although later-season scab was often more serious on phosphite-treated trees compared to TPTH-treated trees, this did not always translate into a difference in yield. The regression analysis for all cultivars showed the productivity response associated with reduced disease. The severity of pecan scab prior to shell-hardening (mid-August) is known to have a greater effect on yield than disease later in the season (Gottwald and Bertrand, 1983; Stevenson and Bertrand, 2001), and disease at shell-hardening is most often the best predictor of components of yield at harvest. Thus, disease developing later in the season, after mid-August, is less important to yield; which can explain why relatively severe disease on phosphite-treated plots in September or October had relatively little impact on yield compared to the non-treated control, which showed more severe disease earlier in the season.

Certain plant pathogens have developed phosphite resistance, or exhibit a range of sensitivity (Brown et al., 2004; Wilkinson et al., 2001). Although observed among oomycetes, there is a risk that other pathogen, like *F. effusum*, could also develop resistance. Thus, as with all fungicides, phosphite should be used in conjunction with alternative chemistries, and in the event they become used in pecan scab control, a resistance screening program developed and maintained to monitor *F. effusum* population changes (Isakeit, 2010; Brenneman et al., 1999; Stevenson et al., 2004; Seyran et al., 2010). The degree of phosphite efficacy demonstrated herein indicates that the chemical can play an especially useful role in the early-season management of pecan scab on foliage and fruit, while growers might consider using alternative fungicides on maturing fruit later in the season.

It has been noted that presumed SAR agents are generally less effective for controlling disease compared to conventional fungicides (Agostini et al., 2003; Percival et al., 2009). The control of scab on pecan afforded by phosphites in this study demonstrated they were as effective as an industry standard protecting foliage, and also efficacious protecting fruit, at least early in the season. Unlike apple and certain other crops, non-yield limiting infections and blemishes on pecan fruit do not preclude sale of nuts. Additional work is needed to confirm whether phosphite spray timing, frequency, or usage in conjunction with alternative chemistry might provide improved control. Nonetheless, the present work shows that phosphite can contribute to management of pecan scab on scab-susceptible trees in orchards, particularly early in the season on the foliage and on young, developing fruit.

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