The Role of Phosphites in Scab Management and Residues in Pecan Kernels

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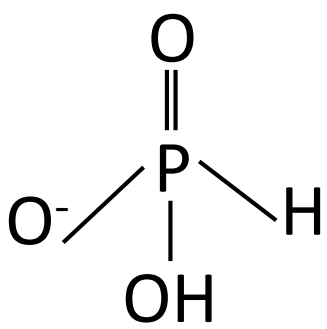
University of Georgia, Tifton, GA

What is phosphite?

- Phosphites ($H_2PO_3^-$) are salts of phosphorous acid [$HPO(OH)_2$]
- Not a phosphate (HPO_4^{2-}), which are important in plant nutrition
- The phosphite ion ($H_2PO_3^-$) is readily absorbed in plants
- Phosphite travels systemically in both the xylem and phloem
- Formulated with a cation (most often an alkali metal, Na, K, Al, NH_4) and is sold both in fungicide and nutritional packages for use in agriculture



Phosphite ion $H_2PO_3^-$

Aliette

Viathon

Reliant®

KPHITE®

Helena ProPhyt®
A Systemic Fungicide Containing Potassium Phosphite

FUNGI-PHITE®

Phostrol®

AGRI-FOS

Claims of nutritional benefits of phosphites should be approached with caution

- No evidence for **direct** benefit of phosphites to crop health (no studies performed on pecan to date)
- But data demonstrating no or adverse effects exists from other crops:
  - If phosphate is adequate, additional phosphate or phosphite has no effect
  - If plants are phosphate deficient, additional phosphate benefits dry matter accumulation, but phosphite retards it

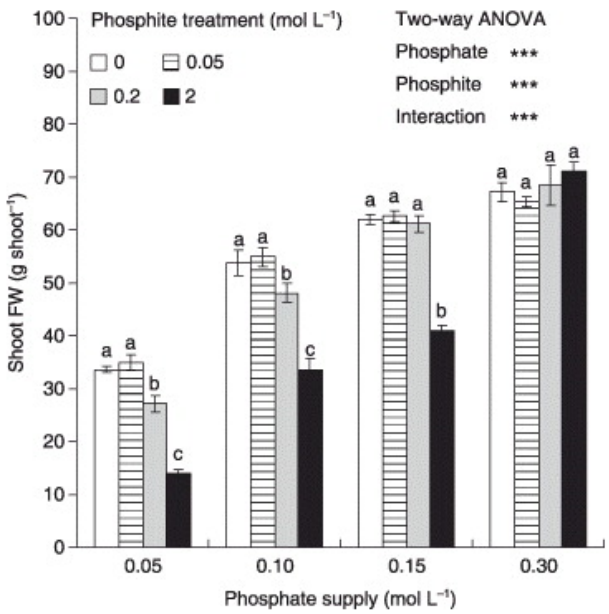
Table 4:
Influence of different P fertilization treatments on the dry matter yield of maize plants [g DM per plant] (FV4, Braunschweig 2004)

Soil P fertilization	Foliar P fertilization		
	Without	Phosphate[1)]	Phosphite[2)]
	Plant dry matter [g]		
P deficiency	35 ab	45 b	29 a
P sufficiency	45 a	45 a	42 a

[1)] one-time 15 mg P as 0.2 % w/v KH_2PO_4 solution
[2)] one-time 15 mg P as 0.2 % w/v KH_2PO_3 solution
All values are means of five replicates. Means followed by dissimilar letters in rows are significantly different (Duncan's test, P = 0.05).

Schroetter, S, Angeles-Wedler, D, Kreuzig, R and Schnug, E. 2006. Effects of phosphite on phosphorus supply and growth of corn (Zea mays). *Landbauforschu Volk., 3/4*, 56: 87–99.

Effects of phosphite treatments on shoot fresh weight of hydroponic lettuce under different phosphate supplies



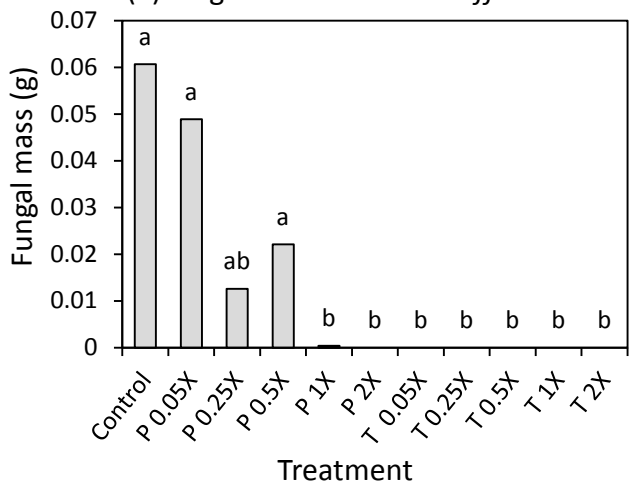
- The phosphate levels 0.05, 0.1, 0.15 and 0.3 mmol L−1 were phosphate supplies for 50, 80, 90 and 100% of maximum plant growth, respectively.
- Error bars are standard error (n = 3).
- ***P < 0.001.
- Different letters indicate significant differences between means within the same phosphate supply by least significant difference tests (P < 0.05).
- Thao and Yamakawa, 2010

H. T. B. Thao and T. Yamakawa. 2010. Phosphite (phosphorous acid): Fungicide, fertilizer or bio-stimulator? Soil Science and Plant Nutrition Vol. 55: 228-234.

Mode of action

- 1 - Direct toxicity to fungus: demonstrated that it is directly toxic to many pathogens at the rates applied in the field (but does not explain the full level of control witnessed in field situations)
- 2 - Phosphites have been demonstrated to enhance the plants natural defense through a mechanism called systemically acquired resistance (SAR)
  - SAR are broad-spectrum plant defense responses that can be induced biologically by challenging a plant with an attenuated pathogen or chemical

The effect of phosphite (P) and triphenyltin hydroxide (T) on growth of *Venturia effusa 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. Means comparisons are based on Tukey's test: numbers with different letter are significantly different (*P*=0.05).

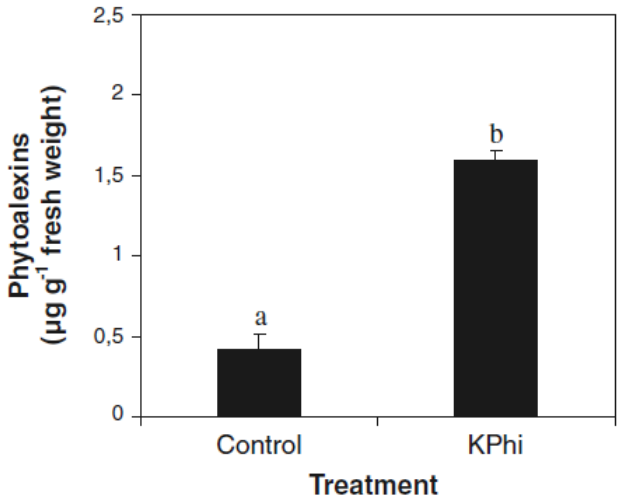


Fig. 2 Phytoalexins accumulation after *P. infestans* infection in tuber slices obtained from KPhi-treated or control plants. Phytoalexins were measured seven days after inoculation with *P. infestans*

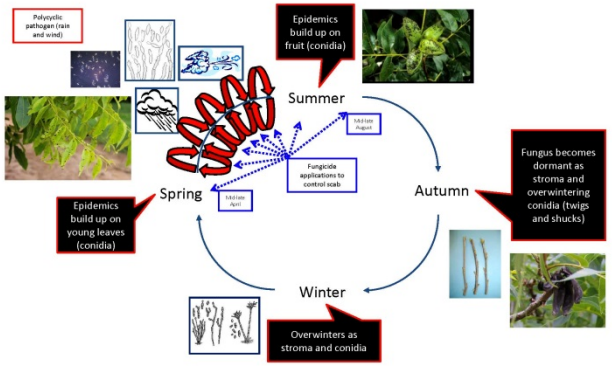
Bock, C.H., Brenneman, T.B., Hotchkiss, M.W. and Wood, B.W. Evaluation of a phosphite fungicide to control pecan scab in the southeastern USA. Crop Protection 36: 58-64. 2012.

Lobato, M.C. , Machinandiarena, M.F. , Tambascio, C. , Dosio, G.A.A. , Caldiz, D.O., Daleo, G.R. , *et al. 2011.* Effect of foliar applications of phosphite on post-harvest potato tubers. Eur J Plant Pathol 30: 155–163

Control of pecan scab with phosphites

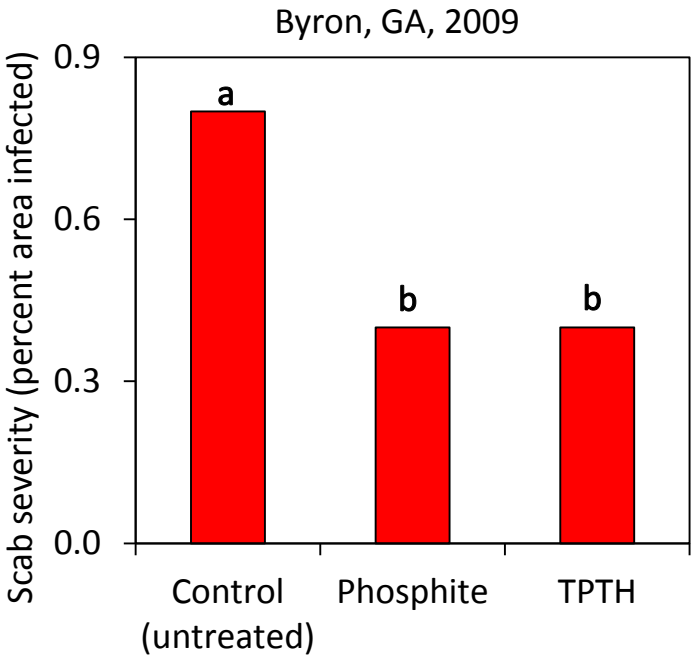
- Pecan scab, caused by *Venturia effusa* is the most important disease of pecan
- Several fungicides are used to manage the disease, including organotins, DMIs, MBCs, QoIs and Ziram (unfortunately resistance to some of these is a reality)
- Recently, phosphites were demonstrated to provide good control of pecan scab

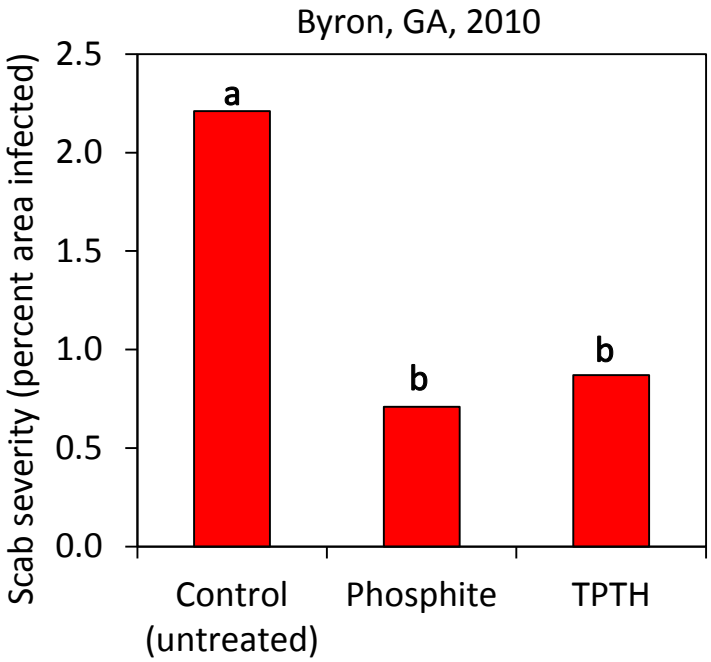
- Lifecycle of *Venturia effusa*, cause of pecan scab



Phosphites are effective fungicides against pecan scab on foliage

- In multiple experiments across the Southeast, phosphites have been shown to effectively reduce scab on foliage when compared to other industry standard fungicides





- Fungicide treatments: Potassium phosphite (Prophyt, 36 fl oz/100 gallons) or triphnyl tin hydroxide (TPTH) (Super Tin 4L, 12 fl oz/100 gallons). Applications made biweekly. Prepollination - end of July
- Scab severity assessed visually on foliage . Data was analyzed using General linear modeling with Tukey's means separation (P = 0.05)

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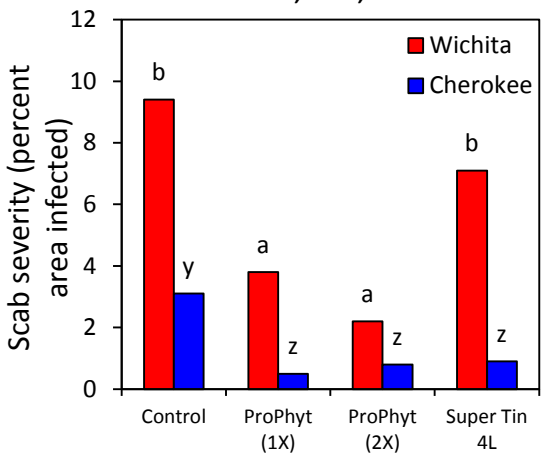
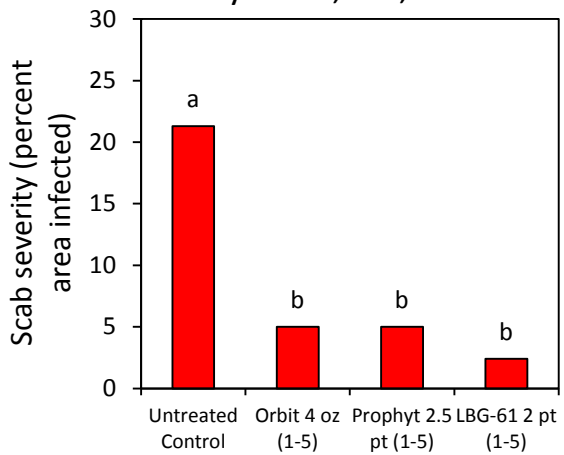
Phosphites are effective fungicides against pecan scab on foliage

- Further studies demonstrating efficacy on foliage:

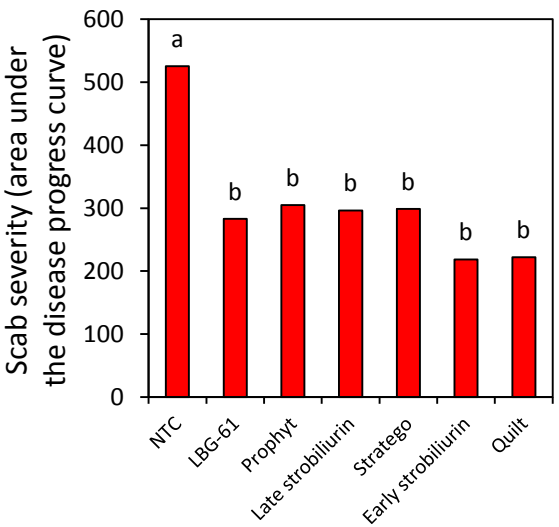
Tifton, GA, 2010



Raymond, MS, 2010



Stillwater, OK, 2010



- Fungicide treatments (6 applications): Potassium phosphite (Prophyt, 36 fl oz/100 gallons) or triphnyl tin hydroxide (TPTH) (Super Tin 4L, 12 fl oz/100 gallons). Applications made biweekly. Prepollination - end of July
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- Fungicide treatments (5 applications): Orbit 4 oz (1-5), Prophyt 2.5 pt (1-5), LBG-61 2 pt (1-5)
- Scab severity assessed visually. Data was analyzed using ANOVA with means separation an LSD test (P = 0.05).

Ingram, D.M. 2011 Evaluation of products for control of scab in pecans, 2010. Plant Disease Management Reports 5:STF007

- Fungicide treatments (6 applications):

NTC = Non-treated control.
LBG-61: 1 = Quilt 1.66SC 27.5 oz, 2-5 = LBG-61 F 32.0 oz , 6 = Enable 2F 8.0 oz
Prophyt: 1 = Quilt 1.66SC 27.5 oz, 2-5 = ProPhyt F 40.0 oz, 6 = ProPhyt F 40.0 oz + Elast 400F 25.0 oz
Late strobiliurin: 1 = Folicur 3.6F 8.0 oz, 2, 4 = Enable 2F 8.0 oz, 3, 6 = Topsin 4.5FL 20.0 oz, 5 = Headline 2.09EC 7.0 oz
Stratego: 1,3,5 = Stratego 2.08EC 10.0 oz, 2,4,6 = Topsin 4.5FL 20.0 oz
Early strobiliurin: 1, 5 = Headline 2.09 EC 7.0 oz, 2, 4 = Topsin 4.5FL 20.0 oz, 3 = Abound 2.08EC 12.3 oz , 6 = Enable 2F 8.0 oz
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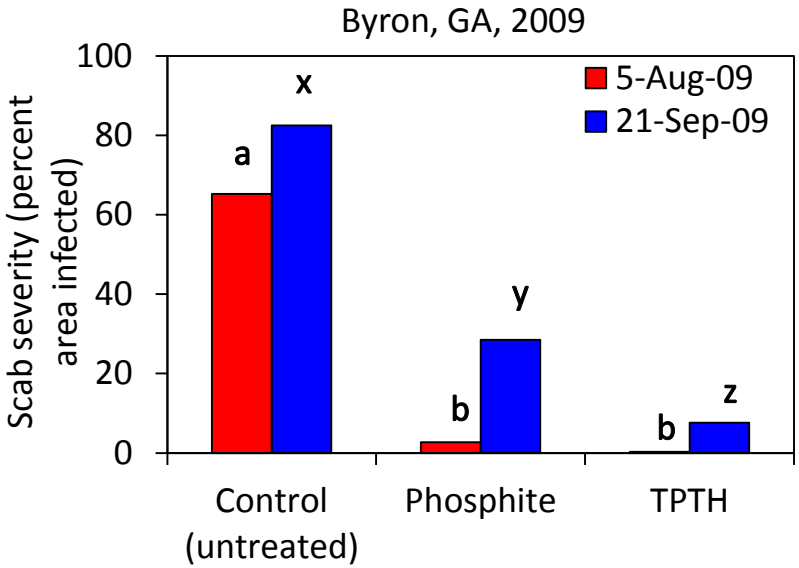
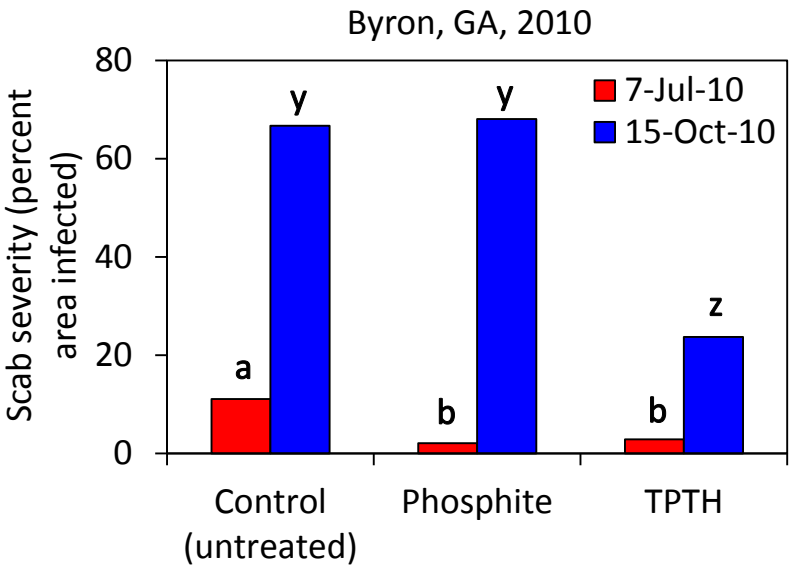
- Scab severity assessed visually. Data was analyzed using ANOVA with means separation using Fishers protected LSD test (P = 0.05).

Smith, D.L., Payne, A.F. and Moore, J.W. 2011 Evaluation of conventional fungicde programs and new fungicde formulations for control of pecan scab in Oklahoma, 2010. Plant Disease Management Reports 5:STF007.

Phosphites are effective fungicides against pecan scab on developing fruit

- In multiple experiments across the Southeast, phosphites have been shown to effectively reduce scab on fruit when compared to other industry standard fungicides





- Phosphite and TPTH applied to Apache, Desirable and Wichita
- Randomized complete block design. Non-treated control, potassium phosphite (Prophyt, 36 fl oz/100 gallons) or TPTH (Super Tin 4L, 12 fl oz/100 gallons). Applications made biweekly. Prepollination - end of July
- Scab severity assessed visually twice on fruit
- Data was analyzed using General linear modeling with Tukey's means separation (P = 0.05)

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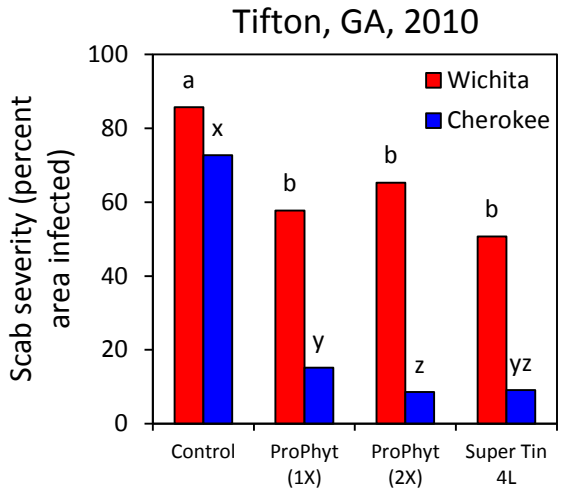
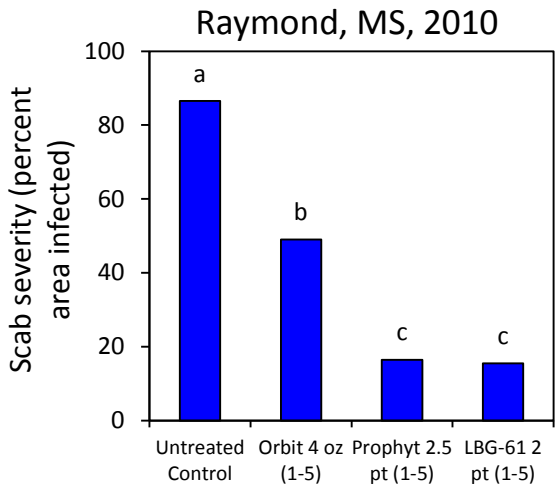
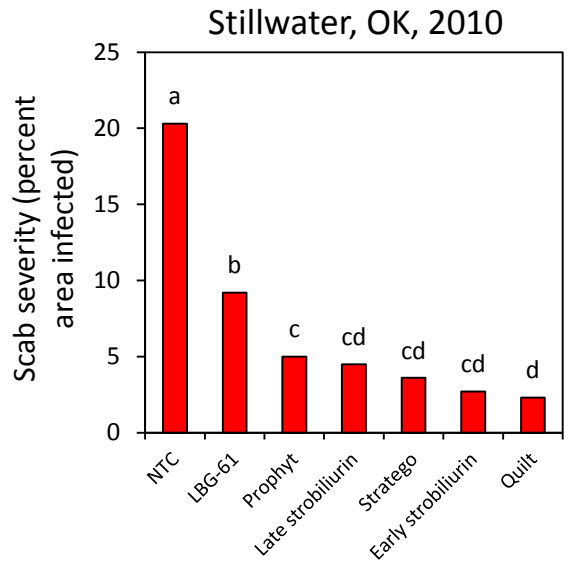
- Further studies demonstrating efficacy on fruit:







- Fungicide treatments (6 applications): Potassium phosphite (Prophyt, 36 fl oz/100 gallons) or triphnyl tin hydroxide (TPTH) (Super Tin 4L, 12 fl oz/100 gallons). Applications made biweekly.
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How are phosphites used in the pecan industry?

- Phosphites are applied as both single application treatments and tank mixes
- There is much information we do not have, but current usage recommendations are:

- Prepollintation applications (every 10-14 days from bud-break to nut-set)

phosphorous acid Phostrol ProPhyt FungiPhite Reliant	33	 2-5 pt 2-3 pt 2-3 pt 4 pt	4 H/ --	For best control apply in 100 gpa by ground. DO NOT apply in consecutive applications. The phosphite (phosphorous acid-based) fungicides listed are EPA approved and considered to be very safe products. However, there is currently an unresolved issue regarding potential residues of these products in tree nuts exported to the EU. This affects only nuts exported to the EU, but growers who know their crop is going to that market may want to consider not using phosphite fungicides until this issue is resolved. Check labels for potential limitations on maximum number of applications or amount of active ingredient allowed per season.
phosphorous acid + tebuconazole Viathon	33 + 3	2-2.5 pt	12 H/ 0 D	The phosphite (phosphorous acid-based) fungicides listed are EPA approved and considered to be very safe products. However, there is currently an unresolved issue regarding potential residues of these products in tree nuts exported to the EU. This affects only nuts exported to the EU, but growers who know their crop is going to that market may want to consider not using phosphite fungicides until this issue is resolved. Check labels for potential limitations on maximum number of applications or amount of active ingredient allowed per season.

- Postpollintation applications (every 10-21 days from nut-set to shell hardening)

phosphorous acid Phostrol ProPhyt Viathon FungiPhite Reliant	33	 2-5 pt 2-3 pt 2 pt 2-3 pt 4 pt	4 H/ --	For best control, apply in 100 gpa by ground. DO NOT apply in consecutive applications. The phosphite (phosphorous acid-based) fungicides listed are EPA approved and considered to be very safe products. However, there is currently an unresolved issue regarding potential residues of these products in tree nuts exported to the EU. This affects only nuts exported to the EU, but growers who know their crop is going to that market may want to consider not using phosphite fungicides until this issue is resolved. Check labels for potential limitations on maximum number of applications or amount of active ingredient allowed per season. Use the highest labeled rate; tank-mix with other fungicides on highly susceptible cultivars
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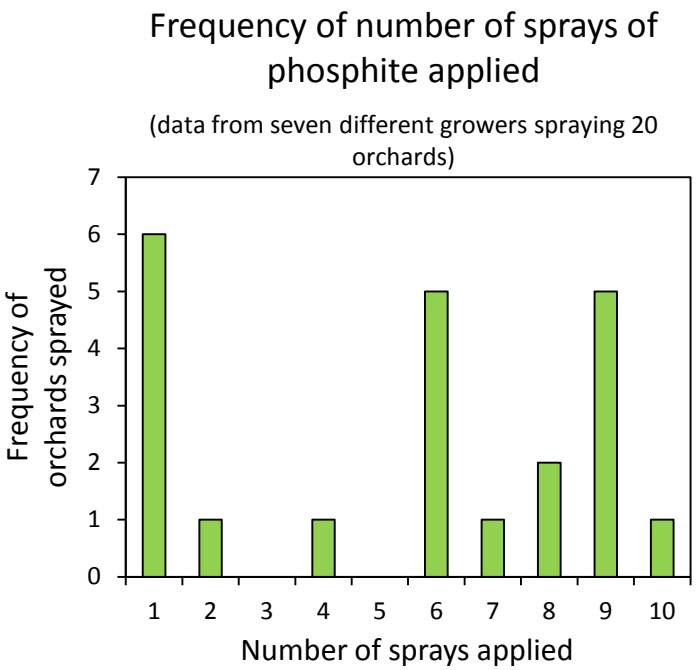
Wells, M. L. 2016 Commercial Pecan Spray Guide. University of Georgia Extension. UGA Extension Bulletin 841.

How are phosphites used in the pecan industry?

- Most labels state that there is no limit to the number of applications applied per season, although some labels do specify minimum volumes and also minimum time periods before reapplying the product
- Some growers do not use phosphites
- But many growers apply from one to as many as 10 sprays of phosphite per season (either as single sprays, tank mixes, or a combination – often at ~1Q rate)



Orchard	Total sprays	Number that contain phosphite	% of sprays with phosphite
1	12	4	25%
2	12	7	58%
3	14	6	43%
4	14	0	0%
5	13	1	8%
6	13	0	0%
7	24	8	33%
8	16	10	63%
9	15	9	60%

Advantages/disadvantages of phosphites

- They have low toxicity. The EPA has four toxicity ratings. Phosphite salts fell into categories III and IV, depending on toxicity test (oral, dermal, inhalation, ocular). (https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-076416_1-Oct-98.pdf)
  - Toxicity category III is slightly toxic and slightly irritating
  - Toxicity category IV is practically non-toxic and not an irritant
- Apparently not harmful to the environment (used in Australia to control a disease of Eucalyptus forests 'Jarrah')
- They are effective fungicides/oomyticides
- Cases of field resistance to phosphites are rare
  - *Bremia lactucae* (lettuce downy mildew) in CA and *Phytophthora cinnamomi* (Jarrah dieback) in Australia, both oomycetes
  - Risk factors for resistance include repeated use of phosphite-containing fungicides/fertilizers
- They might cause phosphite-induced phosphorous deficiency
- They have potential to be phytotoxic (also reports of delayed shuck-split)
- Phosphite application can result in residues in harvested crop products (such as pecan nutmeats)

Brown S, Koike ST, Ochoa OE, Laemmlen F, Michelmore RW, 2004. Insensitivity to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen, Bremia lactucae. Plant Disease 88, 502–8.

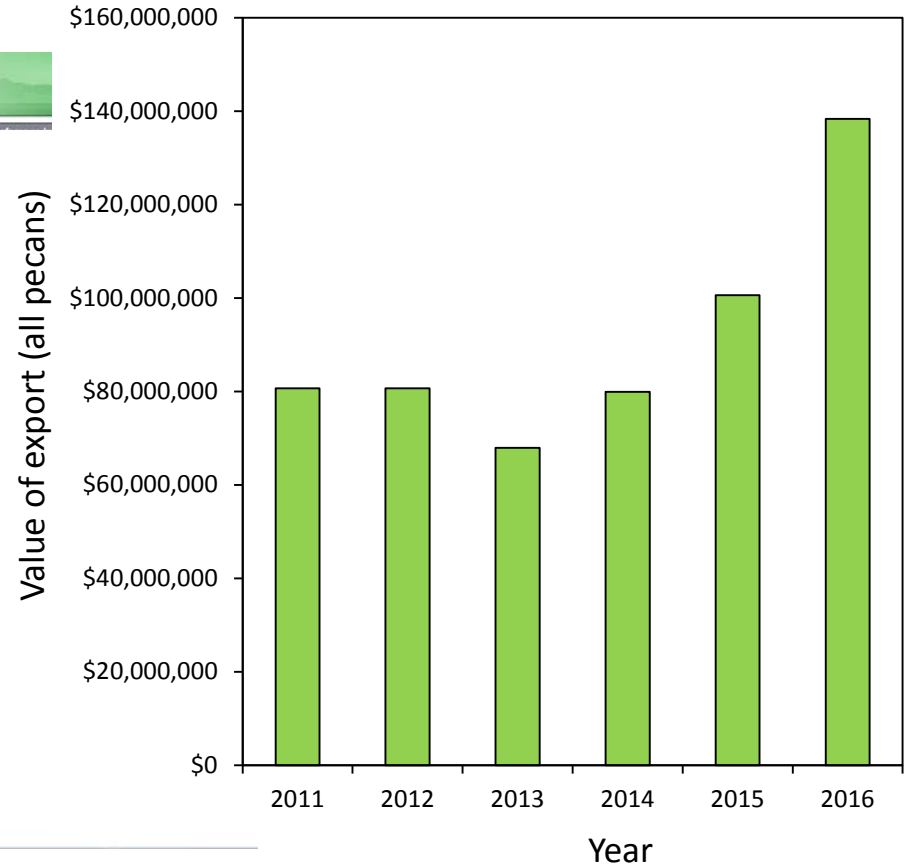
Dobrowolski, M. P., Shearer, B. L., Colquhoun, I. J., O'Brien, P. A. and Hardy, G. E. StJ. (2008), Selection for decreased sensitivity to phosphite in Phytophthora cinnamomi with prolonged use of fungicide. Plant Pathology, 57: 928–936.

Residues, MRLs, EU rulings and other nut crops in relation to pecan

- In 2013 the EU changed the designation of phosphites as both fertilizer and pesticide to only pesticide, thus defaulting phosphites to a 2 ppm MRL
- They provided a temporary MRL of 75 ppm to nut and other crops to 31 December 2015 to allow time for producer industries to respond
- In September 2015, the US tree nut industry submitted a package based on IR-4 phosphite residue testing (*which did not include pecan data*) for the EU to determine a final import tolerance to replace the temporary MRL
- The temporary MRL was extended on other nut cops (*which did not include pecan*) until 1 March 2019 (announced January 2016). The permanent MRL for nut crops is to be decided before that date
- Pecan is likely to be included in the permanent MRL, but in the meantime the level for pecan has defaulted to 2 ppm
- Thus it has become expedient to obtain a temporary MRL for pecan to allow exports to the EU (valued at US $138,314,000)
- We need to obtain some residue data for pecan to achieve this goal

Exports of pecans to the EU

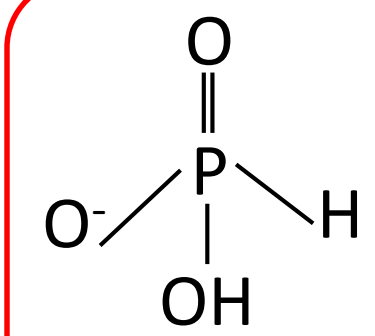
- Valuable export market
- Increasing in size as a market for pecans



https://apps.fas.usda.gov/Gats/default.aspx

Method used to test for residues of phosphite by the EU

- Quick method for the analysis of numerous highly polar pesticides (QuPPe-method)
- Liquid Chromatography coupled with Mass Spectroscopy
- A 68-page methods description is available (http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf)



Phosphite ion $H_2PO_3^-$

EURL-SRM
EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method)

- Version 9.2 (October 2016, Document History, see page 65)

Authors: M. Anastassiades; D. I. Kolberg; A. Benkenstein; E. Eichhorn; S. Zechmann; D. Mack; C. Wildgrube; I. Sigalov; D. Dörk; A. Barth

Note: Changes from V8 to V9 are highlighted in yellow, changes from V9 to 9.1 are highlighted in turquoise and changes from V9.1 to 9.2 are highlighted in green

Content

- Accurate testing for phosphites is not trivial
- Specialized equipment is required, it is expensive and only a few labs have it
- Highly trained and skilled personnel are needed to operate the equipment
- Commercially, each sample costs ~US$ 250
- There are other less expensive methods, but they could be questioned by the EU due to potential inaccuracies
- The following data is based on tests performed by a commercial laboratory using LC-MS (Eurofins)
- Collected preliminary samples in the fall of 2015 in Tifton and Byron, GA

Residues detected in samples of pecan

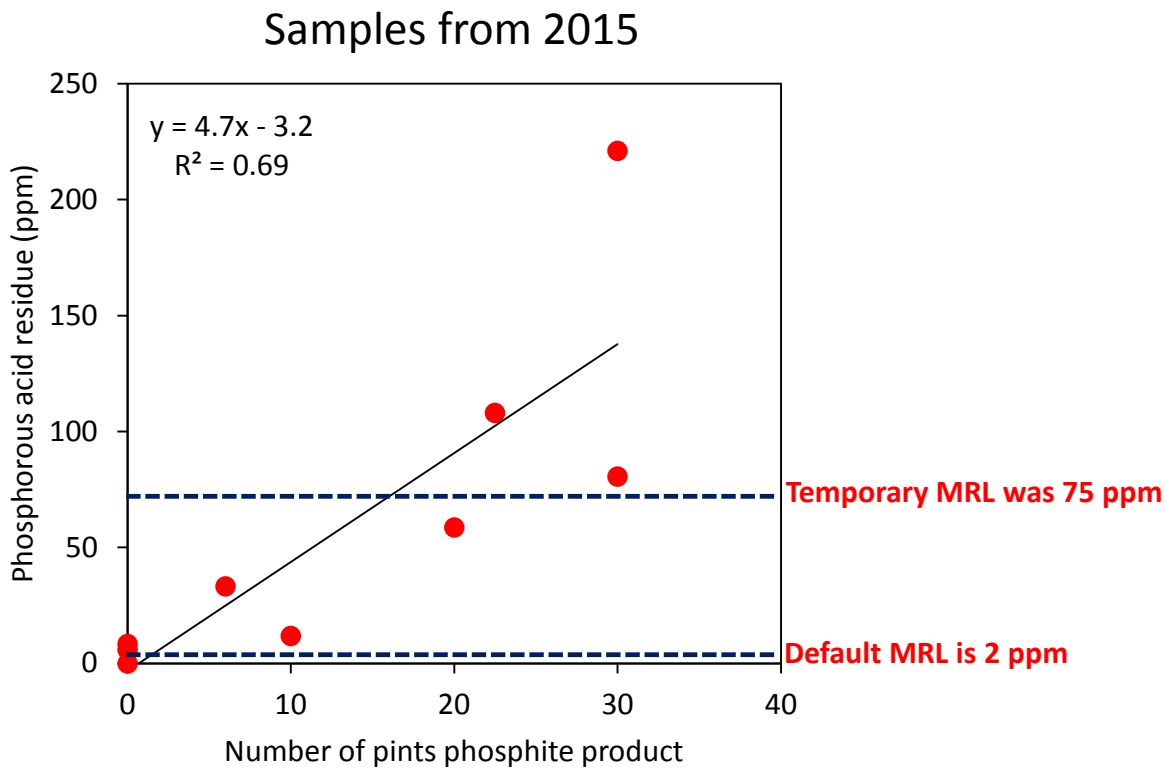
Samples from 2015

Test	Location	Product	Exposure – rate and number of applications	Total exposure	EUROFIN Total detected (mg/kg)	UC – Davis Total detected (mg/kg)
1	Tifton 1		0 pts, 0 x	0 pt	Not detected	-
	Byron 1		0 pts, 0 x	0 pt	7.9	-
	Albany 1	Phostrol	2 pts, 3 x	6 pt	44.5	-
	Byron 1	Prophyt	2 pts, 5 x	10 pt	15.8	-
	Tifton 1	Prophyt	4 pts, 5 x	20 pt	78.6	-
	Tifton 1	Rampart	6 pts, 5 x	30 pt	296.0	-
2	Byron 1		0 pts, 0 x	0 pt	-[a]	5.7
	Byron 1	Prophyt	4.5 pts, 5 x	22.5 pt	-	108.0
	Byron 1	Prophyt	6 pts, 5 x	30 pt	-	80.6

[a] Not tested

- Most programs had >2 ppm
- Some had >75 ppm
- Even some pecan crops receiving no phosphite had detectable residue

Relationship between total number of pints of phosphite applied and phosphite residue



- A linear relationship was observed between total number of pints of phosphite applied and phosphite residue
- Based on preliminary data

Status of knowledge and recent sampling

- Status:
  - Phosphite residues are readily detectable in pecan (at levels >75 ppm)
  - There is no directly transferrable information from other crops, and we don't know if the "nut-crop group" MRL will be realistic for pecans
  - Even when a nut group MRL is granted, use of phosphites will need to be managed to ensure residues <MRL (spray frequency, timing etc)
  - Phosphites are a vital chemistry for the industry
  - The pecan industry is currently pursuing a temporary MRL, which is being spearheaded by Dr. Randy Hudson and Dr. Tim Brenneman
- Sampling:
  - In 2016, samples (~300) were obtained from growers orchards to provide base-line data on residue levels (Drs. Rohla (Noble Foundation, Ardmore), Brenneman (University of Georgia, Tifton), and Bock (USDA-ARS-SEFTNRL, Byron))
  - The samples will be processed and phosphite levels determined using the appropriate methods. The Noble Foundation is instrumental in this task
  - Field experiments (2016 and onwards) are being designed to investigate effects of different usage patterns on residues as a basis for recommendations
  - Data will be compiled and interpreted by the researchers, and provided to the industry for approaching the EU, and possibly IR-4 for further tests if required

Preliminary results of phosphite residue testing in relation to number and timing of sprays - 2016

- Experiment at ARS-SEFTNRL, Byron, GA, in 2016, cv. Pawnee
- Applied Prophyt (3 pt/100 G/acre) following two different protocols

i) 1 to 5 applications additively (1, 2, 3, 4 or 5 sprays)

- No spray
- 15-Jul (1 spray)
- 15, 28-Jul (2 sprays)
- 15, 28-Jul, 15-Aug (3 sprays)
- 15, 28-Jul, 15, 30-Aug (4 sprays)
- 15, 28-Jul, 15, 30-Aug, 9-Sep (5 sprays)

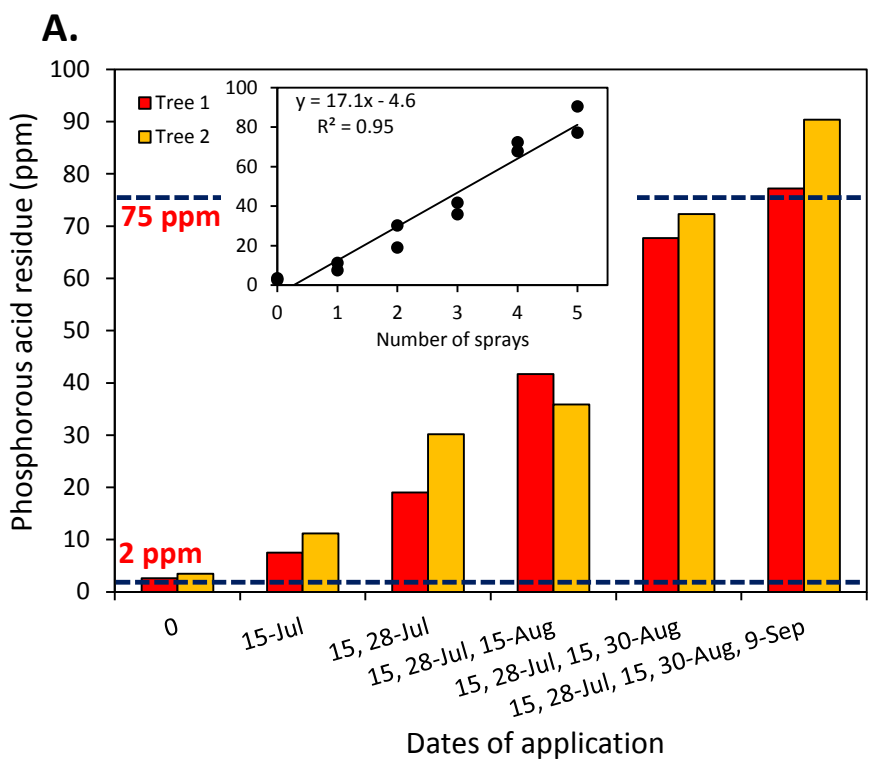
ii) 1 spray applied at each of 5 spray dates (only 1 application, but on different dates)

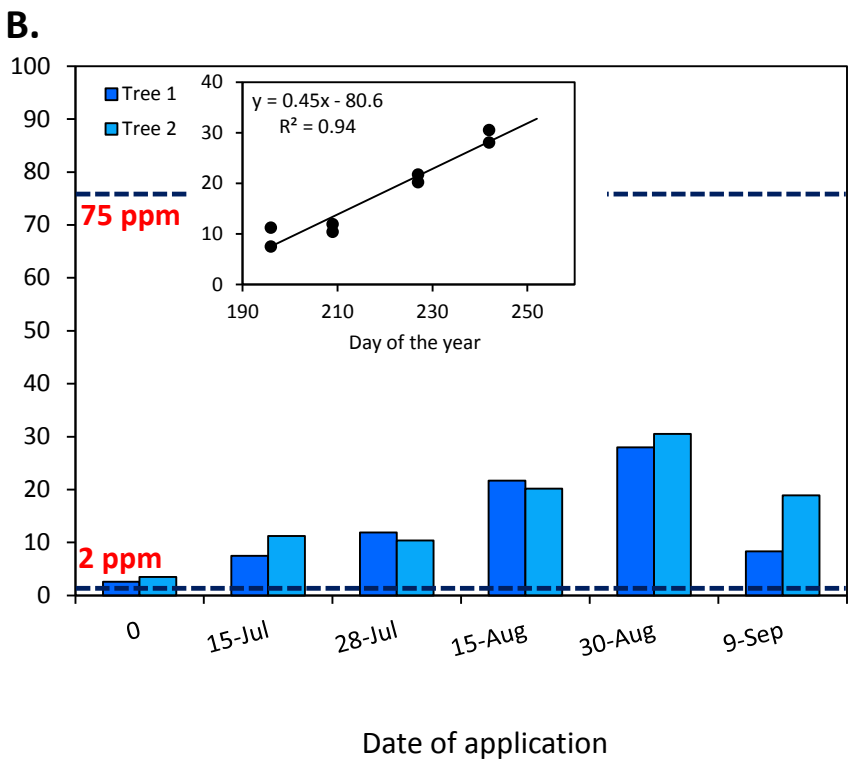
- No spray
- 15-Jul (1 spray)
- 28-Jul (1 spray)
- 15-Aug (1 spray)
- 30-Aug (1 spray)
- 9-Sep (1 spray)

- Only two trees of each treatment – so based on limited data

Preliminary results of phosphite residues in relation to number and timing of sprays

- Profound effects of number of sprays and spray timing on the residue levels of phosphite
  - **A. There was an almost additive increase in phosphite residue with each spray**
  - **B. Later sprays applications resulted in greater residue**
- Even the control exceeded the EU MRL of 2 ppm





Phosphites are a valuable chemistry to pecan production

- Particularly efficacious against scab on foliage and also fruit
- Needed as a tool for managing resistance to fungicides in the scab pathogen
- Reducing impact of other diseases in addition to scab (e.g. anthracnose, *Phytophthora*). Phosphites are broad spectrum fungicides – they can control several different diseases
- Phosphites are a safe and environmentally friendly fungicide compared to many others. Particularly important as the world becomes less tolerant of less safe or environmentally harmful pesticides
- These needs require that we establish acceptable use procedures for phosphites in pecan where nuts are exported to the EU
- And we need data to ensure realistic MRLs are granted by the EU for pecan

Conclusions

- Any application of phosphite will exceed 2 ppm. Just a few sprays can result in >75 ppm
- As long as the 2 ppm ruling is in place, growers choosing to export to the EU should avoid using phosphites
- Even if a nut group MRL is granted, use of phosphites will need to be managed in a manner to ensure EU-bound nuts have residues that do not exceed this value
- Phosphite spray timing and frequency used by growers will be critical, and dependent on the final MRL ratified by the EU
- We need to determine:
  - How spray frequency/concentration/timing affects phosphite residue in nuts
  - Whether there is season to season carry over of phosphites in pecan trees (could become cumulative over years)
  - Cultivar/environment effects

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- We also appreciate manufacturers of various phophite products for providing material for testing purposes

Thank you!