

Application Pressure and Carrier Volume Affects the Concentration of Azoxystrobin on Peanut Foliage and in Soil

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ABSTRACT

Azoxystrobin can be used to manage pod rot of peanuts, and applications are typically made at mid-season or later, when it can be difficult to move the fungicide through the foliage and down into the soil. Spanish peanuts were sprayed with azoxystrobin in 2013 at carrier volumes ranging from 252 to 1038 L ha⁻¹ and pressure ranging from 138 to 552 kPa; and in 2014 virginia peanuts were sprayed with carrier volumes ranging from 131 to 524 L ha⁻¹ and pressure ranging from 138 to 414 kPa. Square root transformed azoxystrobin concentration (\sqrt{A} in ppm) on foliage at carrier volumes < 300 L ha⁻¹ was higher in 2 of 3 experiments (\sqrt{A} averaged 2.4 times higher) with spanish peanuts and 1 of 2 experiments (\sqrt{A} averaged 1.3 times higher) on virginia peanuts, than at volumes \geq 524 L ha⁻¹. A linear model could be used to describe the negative relationship between carrier volume and (\sqrt{A}) on foliage for all three applications on spanish peanuts. Azoxystrobin concentrations in soil were 3 times higher for carrier volumes < 300 L ha⁻¹ in 2 of 3 experiments on spanish peanuts and were not affected by carrier volume on virginia peanuts, than carrier volumes \geq 524 L ha⁻¹. However, application pressure of 138 kPa resulted in higher concentrations of azoxystrobin (1.7 times higher) in the soil in 1 of 2 trials than using an application pressure of 515 kPa on virginia peanuts. Foliar concentrations of azoxystrobin on virginia peanuts declined over time and irrigation events. However, soil concentrations of azoxystrobin generally increased between 0 and the first irrigation event for the higher carrier volume (524 L ha⁻¹), but did not change between the first and second irrigation event. Attempts to force azoxystrobin through the peanut canopy with high pressure (414 to 552 kPa), high carrier volume (\geq 524 L ha⁻¹) or combinations of high pressure and high carrier volumes were unsuccessful at increasing azoxystrobin concentrations in soil compared to more traditional carrier volume and pressure.

Key Words: Fungicide application, pod rot control, *Pythium* spp.

Pod rot of peanuts can be caused by a number of organisms, including *Rhizoctonia solani* and *Pythium* spp. (Frank, 1968; Hollowell *et al.*, 1998; Wheeler *et al.*, 2005). *Pythium* spp. isolated from peanut pods included *P. myriotylum*, *P. ultimum*, and *P. irregulare* in Texas (Wheeler *et al.*, 2005); whereas, *P. irregulare*, *P. spinosum*, *P. dissotocum*, and *P. vexans* were associated with the disease in North Carolina (Hollowell *et al.*, 1998).

Azoxystrobin (methyl (E)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl]-3-methoxyacrylate) has activity against both *R. solani* and *Pythium* spp. (Grichar *et al.*, 2000; Mihajlović *et al.*, 2013; Windels and Brantner, 2005) and is labelled for *Pythium* pod rot suppression/control and peg and pod rot control of *R. solani* and *Sclerotium rolfsii* in peanut in the US (Anonymous, 2013). Uptake of azoxystrobin into leaves is a gradual process with 1 to 3% of the applied material absorbed into a grape leaf within 24 hr of foliar application (Bartlett *et al.*, 2002). Strobilurins (like azoxystrobin) move across the leaf surface and into the waxy cuticle of the leaf (locally systemic) and may even move into the cuticle on the underside of the leaf (translaminar activity) (Balba, 2007). Some azoxystrobin may also move into the xylem and be transported upwards (Bartlett *et al.*, 2002). However, the plant does not transport much if any fungicide down to the roots. Therefore, a foliar application of azoxystrobin to peanuts for pod rot control must reach the pod zone by another mechanism.

There is information available about the effects of application parameters like nozzle type, carrier volume, and pressure on distribution of fungicides on foliage (Egel and Harmon, 2001; Tompkins *et al.*, 1983; Zhu *et al.*, 2004), however there is almost no information on the deposition of fungicides through the plant canopy into the soil. Spray penetration into lower peanut canopies was better with air induction nozzles than with flat fan nozzles (Zhu *et al.*, 2004). Increasing carrier volume from 190 L ha⁻¹ to 375 L ha⁻¹ increased spray coverage on the bottom leaves of snap beans from 29 to 43% (Tompkins *et al.*, 1983). Increasing carrier volume

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from 100 to 200 or 300 L ha⁻¹ resulted in better control of *Ascochyta* blight of chickpea (*Cicer arietinum* L.) and resulted in subsequent yield increases (Armstrong-Cho *et al.*, 2007). However, increasing carrier volume is not always associated with higher concentration of fungicide in the lower canopy. Using different application methodology to increase carrier volume from 120 to 17,800 L ha⁻¹ resulted in a higher concentration of chlorothalonil distributed throughout the plant (top, middle, and bottom) canopy associated with the lowest carrier volume (Brenneman *et al.*, 1990). While it is possible that the fungicide was redistributed into the soil with the higher carrier volumes, soil concentration was not measured in Brenneman *et al.* (1990).

When sprayer pressure was increased from 345 kPa to 690 kPa, bottom leaf coverage increased only from 37 to 40% in snap beans (Tompkins *et al.*, 1983). Application pressure of 207 to 620 kPa did not result in improved disease control for *Alternaria* blight of muskmelon, though in one situation, application of chlorothalonil at 827 kPa did result in better disease control than with 207 and 414 kPa (Egel and Harmon, 2001). Applications at two pressures (500 kPa and 1400 kPa) in a pear orchard resulted in a similar amount of deposits overall on the soil, but there was a higher concentration of deposits in close proximity to the tree with the lower pressure, and a higher concentration on the ground a farther distance from the tree with higher pressure (Di Prinzio *et al.*, 2010).

Deposition to soil increased dramatically when sugar beets and potatoes were treated earlier in the season (Jensen and Spliid, 2003). They developed a model that predicted the percentage of the application that reached the soil was proportional to the percent cover of the crop. At the time that first pod rot applications are made (60 d after planting), peanut foliage is already covering the ground over the central area near the stem where the pods are forming. Zhu *et al.* (2004) reported that average peanut height and width increased by 100% between 45 and 75 d after planting. However, average height and width increased only 13%, respectively between 75 and 104 d after planting.

Irrigation after application has been used to move fungicides from the foliage to the soil for peanut soil-borne disease management. *Sclerotium rolfsii* colonized approximately 30% of pods after an application with azoxystrobin immediately followed with irrigation versus around 50% pod colonization when irrigation was delayed 96 hours (Woodward *et al.*, 2012). Azoxystrobin application at night (when leaves are folded resulting in a sparser canopy), combined with a post application irrigation improved disease control of

S. rolfsii, relative to a day application with no irrigation (Augusto and Brenneman, 2011).

The objective of this project was to examine the influence of the application parameters pressure and carrier volume, on the concentration of azoxystrobin on peanut foliage and in soil, with an emphasis on identifying applications parameters that improve soil deposition. There is a critical gap in our understanding of how much fungicide concentration from foliar applications actually reaches the soil depth where pegging occurs. Since irrigation or rainfall can impact results of different trials, an effort was made to measure how soon these events occurred after an application. Irrigation or rainfall timing or amount was not a specific treatment for this project.

Materials and Methods

Spray Coverage

For tests conducted in 2013 (test 1), Teejet 8010 flat-fan nozzles were calibrated to compare three application volumes (252, 505, and 1038 L/ha⁻¹) at a pressure of 138 kPa (Table 1). There are no carrier volume recommendations on the label for azoxystrobin (Anonymous, 2013), however, these volumes represent a range that likely exceeds what a producer would be willing to use. The same nozzles were also calibrated to compare four different application pressures (138, 276, 414, and 552 kPa) at a similar carrier volume of approximately 281 L ha⁻¹ (actual range was from 252 to 299 L/ha, Table 1). A CO₂ pressurized application system with a two-row spray boom (nozzles were 101 cm apart centered on the beds) was placed on the quick hitch of a tractor at a height of 56 cm above the canopy, and driven at various speeds to establish the desired combination of application volume at a given pressure. The fungicide azoxystrobin (Abound FL, Syngenta Crop Protection LLC, Greenboro, NC) was banded (51 cm) over the rows (peanuts were planted in a single row/bed) at the same rate (0.45 kg a.i. ha⁻¹ = 0.225 kg a.i. banded ha⁻¹) for all treatments. Peanut foliage at the time of applications was continuous down the row and wider than the banded applications across the row.

In 2014, the application volumes of 131 and 524 L ha⁻¹ were compared at two pressure rates of 138 and 414 kPa in test 2 and carrier volumes of 131, 262, and 524 L ha⁻¹ were compared at 138 and 414 kPa in test 3. Teejet 8002VS flat-fan nozzles and Teejet 8015E flat-fan nozzles were used to calibrate the appropriate rates (Table 1). The same CO₂ pressured system was used and the same rate of

Table 1. Application details for three tests where the effect of carrier volume and pressure on application of azoxystrobin was examined.

Year	Test	Volume L ha ⁻¹	Pressure kPa	Nozzle ^a
2013	1	252	138	TJ8010
2013	1	290	276	TJ8010
2013	1	281	414	TJ8010
2013	1	299	552	TJ8010
2013	1	505	138	TJ8010
2013	1	1038	138	TJ8010
2014	2 and 3	131	138	TJ8002VS
2014	2 and 3	131	414	TJ8002VS
2014	3	262	138	TJ8015E
2014	3	262	414	TJ8002VS
2014	2 and 3	524	138	TJ8015E
2014	2 and 3	524	414	TJ8015E

^aAbbreviations; TeeJet, TJ

azoxystrobin was applied in both years. The 2013 spray parameters were designed to explore the range of carrier volume and pressure that might be used in commercial applications, but did not permit testing for interactions between carrier volume and pressure. The 2014 application parameters still maintained a range of parameter values that showed differences in 2013, but allowed for carrier volume x pressure interactions to be tested.

Test Sites

In 2013, the test design consisted of six treatments (Table 1), that were applied at three different times during the season (24 July, 7 Aug, and 16 Aug, each plot was only treated one time during the season), in a randomized complete block design with four replications per application time. A spanish-type cultivar (Tamnut OL06) was planted in early May on single beds. The soil series was a Patricia fine sand with a texture of 80% sand, 6% silt, and 14% clay, with 0.3% organic matter and a pH of 8.2. Plots were four rows wide, where only the middle two rows were treated, and were 10.7 m in length. Soil moisture was monitored with a Watermark soil moisture (capacitance-type) sensor buried to a depth of 10 cm, and attached to a WatchDog 1200 series data logger (Spectrum Technologies, Inc., Aurora, IL). Volumetric soil moisture starting at the time of application was used to determine the approximate length of time between application and the first rain or irrigation event. All three application dates were followed within a few hours with an irrigation (24 July and 16 Aug) or rain event (7 Aug). This measurement method does not indicate the amount or intensity of water that impacts the plants. Hourly soil moisture can be seen in Fig. 1A. For the 24 July application, which was completed around 17:30 hr, there was an increase in soil moisture from 3.2 to 12.6% at

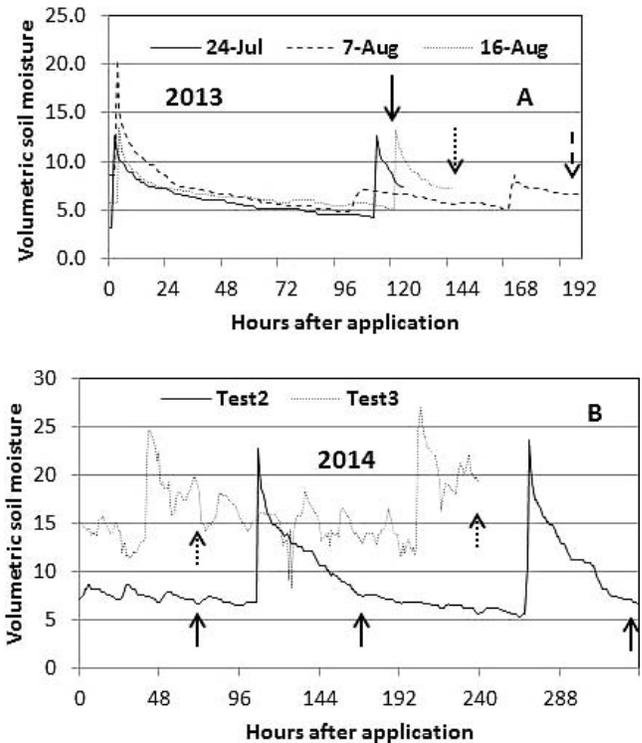


Fig. 1. Volumetric soil moisture at a depth of 10 cm from the time of fungicide application until the last sampling date. (A) There were three application dates in 2013 and one sampling time for each application date. An arrow indicates sampling time and is in the same pattern as the line representing the soil moisture for that application time; (B) There were two application times in 2014. Arrows indicate the sampling time associated with each test. There were three sampling times for test 2 and two sampling times for test 3.

18:00 hr, and then a second irrigation event on 29 July at 5:00 hr when soil moisture changed from 4.3% to 12.6% in one hr. For the 7 Aug application, which was completed around 16:00 hr, there was an increase in soil moisture at 17:00 hr (from 8.6 to 20.5% due to rain), then on 14 Aug at 10:00 there was an increase from 5.2 to 8.6% in two hr. For the 16 Aug application which finished around 15:00 hr, there was an increase in soil moisture at 17:00 hr from 5.8 to 13.6% in one hr, and then on 21 Aug at 10:00 hr there was an increase of soil moisture from 5.1 to 13.2% in one hr.

In 2014, test 2 consisted of four treatments (two carrier volumes x two pressures; Table 1) applied on 24 July, and test 3 consisted of six treatments (three carrier volumes by two pressures, Table 1) applied on 28 July, each with four replications in a randomized complete block design. The two tests were located at different parts of the field. The field was planted with a virginia-type cultivar (Florida Fancy) in mid-May on a single bed. The soil series was an Amarillo fine sandy loam, and the texture was 68% sand, 10% silt, and 22% clay with 0.6% organic matter and a pH of 7.5. Plots were four

rows wide with only the middle two rows treated and were 6.1 m in length.

Soil moisture was monitored for tests 2 and 3 similarly as in 2013. Average daily soil moisture can be seen in Fig. 1B. Test 2 was irrigated initially five d after application and test 3 was irrigated two d after application. Soil moisture in test 2 went from 7.1% at 0 hr to 22.7% moisture at 107 hr. Soil moisture in test 3 went from 15.1% at 0 hr to 24.6% at 40 hr after application. A second irrigation occurred with test 2 at 11 d after the original application and with test 3 at 8 d after the original application (Fig. 1B).

Sampling After Application

In 2013, plant and soil samples were taken when two irrigation or rain events had occurred after the fungicides were applied. Sampling was done 5, 8, and 6 d after the applications on 24 July, 7 Aug, and 16 Aug, respectively (Fig. 1A). An area in the middle of the plot consisting of 50 cm of row length was selected. Plants in this area were trimmed so that only the center 30 cm (width) remained, and then all the leaflets from top to bottom were removed, placed in a bag, mixed, and then placed in a cooler with ice, until they could be frozen at -20 C. The soil in this area to a depth of 10 cm was removed (4 cores in 50 cm of row), mixed, and a subsample of 20 g was placed in a plastic bag, and then placed in a cooler with ice until they could be frozen at -20 C. Samples were shipped overnight with ice packs to Omic USA Inc. (Portland, OR) to be analysed for azoxystrobin concentration using the European Standard EN 15662 (2008). In this procedure, the pesticide is initially extracted with a mixture of acetonitrile and water. The acetonitrile containing the pesticide is separated from water and cleaned using dispersive solid phase extraction. The cleaned extract is analysed using gas chromatography tandem mass spectrometer (GC-MS/MS) and/or ultra-performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS). The pesticide detected in the sample is confirmed using multiple MSMS transitions. The quantification is done using a matrix matched calibration standard. The minimum detectable level for this assay was 0.02 ppm.

In 2014, sampling foliage was similar to 2013, but soil samples were taken at 0 to 8 cm, and 8-16 cm depths with a 2.5-cm diameter sampling probe (four cores/middle 50 cm of plot). In 2014, plots were sampled multiple times, with test 2 at 4, 8, and 15 d after fungicide application, representing sampling after 0, 1, and 2 irrigation events (no rainfall occurred, Figure 1B). Test 3 was sampled at 3 and 7 d after application which represented samples taken after 1 and 2 irrigation events. The expansion in sampling in 2014 both over time and

at two depths was done in response to questions that were raised over the results in 2013, primarily where had the fungicide gone with the higher carrier volumes.

Statistical Analysis

In 2013, the effect of application parameter combinations and date that the plots were sprayed were evaluated using analysis of variance with the PROC MIXED procedure of SAS (version 9.3, SAS Institute, Cary, NC). Significant treatment effects ($P < 0.10$) were separated using the least-squared mean function and pair-wise differences (PDIFF) at $P < 0.10$ in 2013. A square root transformation (\sqrt{A}) was used to normalize variance of azoxystrobin concentration on leaves, but was unnecessary with soil azoxystrobin concentrations.

Regression analysis (PROC REG, SAS) was conducted on subsets of the data for each application date, to examine the linear relationship between carrier volume and azoxystrobin concentration, and the linear and quadratic relationship between pressure and azoxystrobin concentration. A model was accepted if all parameters were significant at $P < 0.10$. The pattern of residuals was also examined to determine model suitability.

In 2014, the plots for each spray date were in two separate areas (not randomized within the same area as was done in 2013), so each spray date and sampling time was analysed separately for effect of carrier volume, pressure, and their interaction, with the PROC MIXED procedure. The dependent variables measured in 2014 were transformed azoxystrobin concentration in the foliage, soil from 0 to 8 cm depth, and soil from 8 to 16 cm depth. Significant interactions ($P < 0.10$) and main effects ($P < 0.10$) were separated using the least-squared mean function and pair-wise differences (PDIFF) at $P < 0.10$.

The individual treatment combinations were compared across each sampling date (sampling dates after 0, 1, and 2 irrigations for test 2 and sampling dates after 1 and 2 irrigations for test 3) with a t-test at $P=0.10$. The purpose was to determine if the concentration of azoxystrobin changed with each subsequent irrigation event/time. Finally, the impact that initial irrigation timing had on azoxystrobin concentration was compared with a t-test ($P \leq 0.10$) at each treatment combination (i.e. trt 1 was compared across test 2 and test 3 for differences after 1 and 2 irrigation events; trt 2 was compared across test 2 and 3 after 1 and 2 irrigation events, etc.).

There were a total of five trials conducted (three on spanish peanuts and two on virginia peanuts) that examined the impact of application parameters

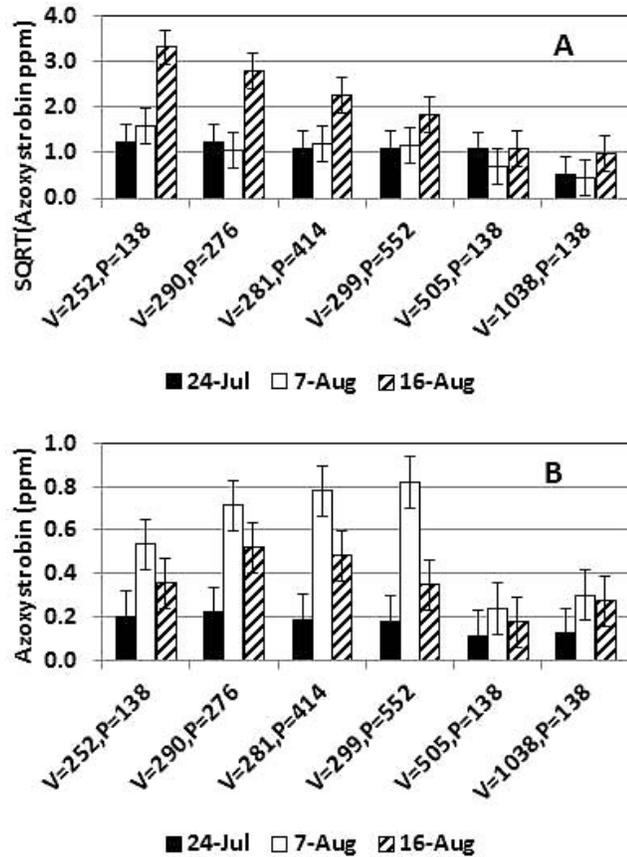


Fig. 2. Square root transformed (SQRT) azoxystrobin concentration on foliage (A) and azoxystrobin concentration in soil (B) after different application dates in 2013. Application parameters that were compared are carrier volume (V in $L ha^{-1}$) and pressure (P in kPa). Standard error bars for A) 0.388 and B) 0.117.

(carrier volume and pressure) on azoxystrobin concentration on foliage and in the soil. The focus of this work was on controllable application parameters, i.e. carrier volume and pressure. While irrigation/rainfall and plant size effects were not tested, they were measured and used in discussing results that may have differed between experiments.

Results and Discussion

Foliage in 2013

The plant size (height [SD] x width [SD]) at the time of application was 45.1 [SD=7.5] x 64.0 [SD=5.8] cm, 49.4 [SD=4.2] x 78.5 [SD=6.9] cm, and 53.6 [SD=8.1] x 73.6 [SD=9.8] cm, for the first, second, and third application times, respectively. There was a significant interaction ($P = 0.034$) between application parameters (six treatments) and date of application with respect to azoxystrobin concentration, therefore each application date will be presented separately. Carrier volume did not affect foliar \sqrt{A} for the 24 July application (Fig. 2A). For the 7 Aug application, \sqrt{A} in ppm was higher

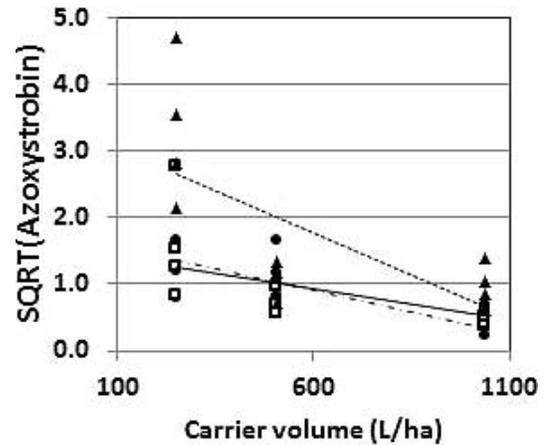


Fig. 3. The relationship between foliar transformed (square root) azoxystrobin concentration (A) in ppm and carrier volume (V), applied at three different times during the season (24 July; 7 Aug; 16 Aug). The equations fitted for each application time were:

$$24 \text{ July } \sqrt{A} = 1.48 - 0.00091(V), R^2 = 0.49$$

$$7 \text{ Aug } \sqrt{A} = 1.70 - 0.0013(V), R^2 = 0.43$$

$$16 \text{ Aug } \sqrt{A} = 3.31 - 0.0025(V), R^2 = 0.46.$$

for the combination of $252 L ha^{-1} + 138 kPa$ ($\sqrt{A} = 1.60$) than for the carrier volumes of $505 L ha^{-1} + 138 kPa$ ($\sqrt{A} = 0.69$) and $1038 L ha^{-1} + 138 kPa$ ($\sqrt{A} = 0.45$). After the 16 Aug application, azoxystrobin concentration was highest ($\sqrt{A} > 2.78$) for those treatments with carrier volumes of $< 300 L ha^{-1}$ and/or $< 414 kPa$ (Fig. 2A). The combination of $281 L ha^{-1}$ and $414 kPa$ had a higher \sqrt{A} (2.25) than treatments with $> 300 L ha^{-1}$ and $138 kPa$, which averaged \sqrt{A} between 0.97 and 1.09 ppm.

The \sqrt{A} on foliage could be adequately fitted with equations based only on carrier volume (Fig. 3), and there was no improvement in R^2 value by combining both carrier volume and pressure in the model. The models fitted were:

$$24 \text{ July: } \sqrt{A} = 1.5 - 0.00091(V), SE = 0.00030, P = 0.012, R^2 = 0.49$$

$$7 \text{ Aug: } \sqrt{A} = 1.7 - 0.0013(V), SE = 0.00048, P = 0.020, R^2 = 0.43$$

$$16 \text{ Aug: } \sqrt{A} = 3.3 - 0.0025(V), SE = 0.00086, P = 0.015, R^2 = 0.46,$$

V was the carrier volume in $L ha^{-1}$. The slope value of the 16 Aug application was significantly different than that of the 24 July application ($P < 0.10$). Foliar \sqrt{A} decreased as carrier volume increased.

Soil in 2013

There was a significant interaction ($P=0.053$) between application parameter combinations and date of application with respect to azoxystrobin concentration in the soil (Fig. 2B). There were no differences between treatments for the 24 July application. After

Table 2. Probability of spray volume (V), pressure (P) or their interaction (VxP) being significant with analysis of variance (PROC MIXED, SAS) in 2014.

Test	Sampling time	Foliage			Soil		
		V	P	VxP	V	P	VxP
2	0	NS ^a	0.097	NS	NS	NS	NS
2	1	0.003 ^b	NS	NS	0.076	NS	NS
2	2	0.005 ^c	0.002 ^d	NS	NS	0.006 ^f	NS
3	1	NS	NS	NS	NS	NS	NS
3	2	NS	0.006 ^e	NS	NS	NS	NS

^aNS indicates parameter was not significant at $P \leq 0.10$.

^bAzoxystrobin foliage averaged 37.3 and 23.1 ppm for 131 and 524 L ha⁻¹, respectively.

^cAzoxystrobin foliage averaged 14.1 and 7.7 ppm for 131 and 524 L ha⁻¹, respectively.

^dAzoxystrobin foliage averaged 14.5 and 7.3 ppm for 138 and 414 kPa, respectively.

^eAzoxystrobin foliage averaged 8.0 and 4.6 ppm for 138 and 414 kPa, respectively.

^fAzoxystrobin soil averaged 0.15 and 0.09 ppm for 138 and 414 kPa, respectively.

the 7 Aug application, azoxystrobin concentration was lower (0.24 to 0.3 ppm) when carrier volume was > 300 L ha⁻¹ than for all treatments with a carrier volume < 300 L ha⁻¹ (concentration ranged from 0.54 to 0.82 ppm, Fig. 2B). When carrier volume was between 252 and 299 L ha⁻¹, but pressure differed, there was a higher concentration of azoxystrobin for 414 and 552 kPa (0.78 and 0.82 ppm, respectively) than with 138 kPa (0.54 ppm, Fig. 2B). Following the 16 Aug application, azoxystrobin concentration was higher (0.52 ppm) for the combination of 290 L ha⁻¹ and 276 kPa than for carrier volumes > 300 L ha⁻¹ (0.18 and 0.27 ppm for 505 and 1038 L/ha, respectively, Fig. 2B).

The concentration of azoxystrobin in the soil across all treatments averaged 0.17, 0.56, and 0.36 ppm for application dates of 24 July, 7 Aug, and 16 Aug, respectively, which are all significantly different ($P < 0.001$) from each other. Application date had more impact on the treatments where carrier volume was < 300 L ha⁻¹, than for the applications with 505 and 1038 L ha⁻¹ (Fig. 2B). The treatments with higher carrier volumes also had the lowest concentration of azoxystrobin overall (summed across foliage and soil). These results caused us to question where the fungicide actual went for the higher carrier volume treatments? Changes were made then in 2014 sampling to look at fungicide concentration over more time periods after application and to a greater depth in the soil.

2014

Volume and Pressure Effects on Foliar Azoxystrobin.

Plant size at the time of application for test 2 was 24.3 cm [SD=3.0] (height) x 71.6 cm [SD=3.7] (width), and for test 3 was 25.6 cm [SD=3.9] (height) x 68.4 cm [SD=6.9] (width). There were no significant interactions at any sampling times between carrier volume and pressure for azoxystrobin concentration in the foliage or soil (Table 2). In test 2 (107 hr until the first

irrigation), there was a higher concentration of \sqrt{A} on the foliage associated with 131 L ha⁻¹ carrier volume compared with 524 L ha⁻¹ (6.1 versus 4.7 at Irr=1 and 3.7 versus 2.7 at Irr=2, Fig. 4A). There was also a higher concentration of \sqrt{A} on the foliage associated with pressure at 138 kPa (3.7) than with 414 kPa (2.6) after the second irrigation (Fig. 4B). In test 3 (40 hr until first irrigation), there was no significant effect of carrier volume on foliar concentration of \sqrt{A} (Fig. 4C). The lower pressure (138 kPa) was associated with a higher concentration of \sqrt{A} after the second irrigation (2.8) than with 414 kPa (2.1) (Fig. 4D).

Volume and Pressure Effects on Soil Azoxystrobin.

There was little to no azoxystrobin detected at the 8 to 16 cm soil depth, so those results will not be discussed further, other than it is unlikely that azoxystrobin was being leached below the 0-8 cm depth. Soil (0 to 8 cm) concentration of azoxystrobin was affected by carrier volume only in test 2, and only after the first irrigation. The 131 L ha⁻¹ carrier volume was associated with lower azoxystrobin concentration (0.076 ppm) than the 524 L ha⁻¹ carrier volume (0.126 ppm), and only after the first irrigation (Fig. 5A). Lower pressure (138 kPa) was associated with higher concentration of azoxystrobin after the second irrigation, compared to 414 kPa (Fig. 5B). In test 3, neither carrier volume nor pressure had any effect on azoxystrobin concentration in soil (Fig. 5C,D). Overall, soil concentration of azoxystrobin was low in both tests, averaging < 0.16 ppm for all treatments after at least one irrigation event.

Changes in Azoxystrobin Concentration Over Irrigation Events.

There was a decline in \sqrt{A} over subsequent irrigation events on the foliage for all treatments (Fig. 4). However in the soil, azoxystrobin concentration either did not change over subsequent irrigation events or increased (Fig. 5). Soil concentration increased between 0 and 2 irrigation events for both carrier volumes in test 2 (Fig. 5A), and for

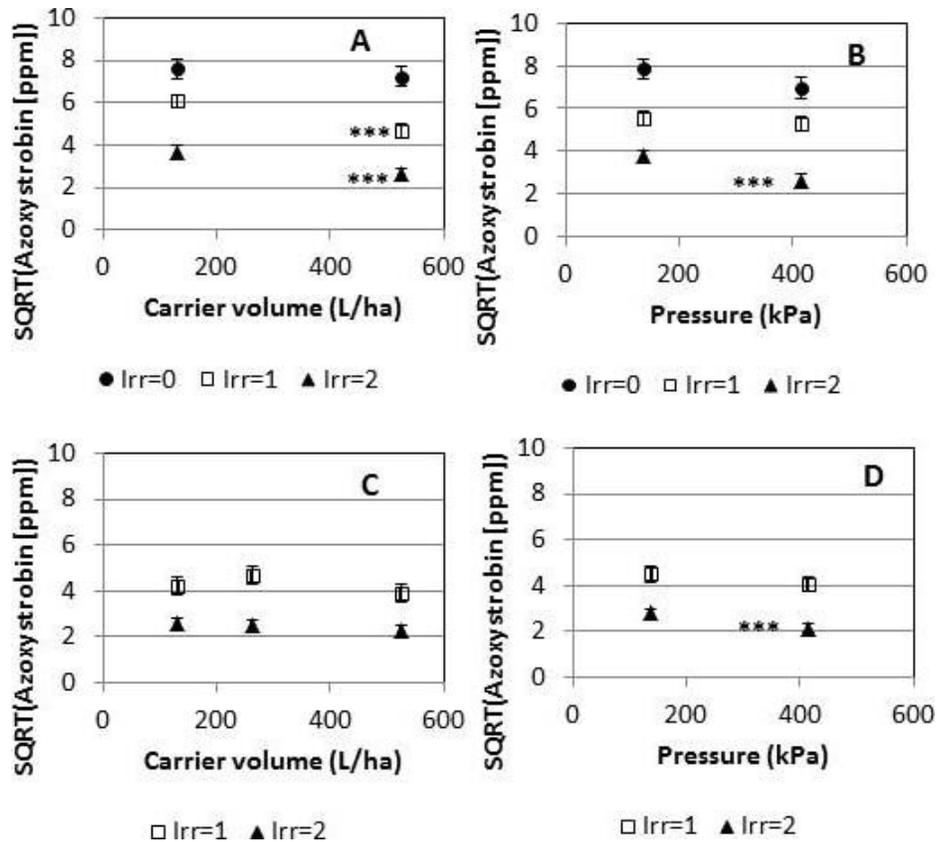


Fig. 4. Effect of carrier volume and application pressure on foliar azoxystrobin (square root transformed = SQRT) concentrations. A and B) First irrigation occurred approximately 107 hr after application; C and D) First application occurred approximately 40 hr after application. There were no significant ($P > 0.10$) interactions between carrier volume and pressure in either trial. Error bars represent the standard error within an irrigation event. Samples were taken after application, but before the first irrigation event (Irr=0), after the first irrigation, but before the second irrigation event (Irr=1), and after the second irrigation event (Irr=2). ***F-test for treatment was significant at $P < 0.01$.

pressure at 138 kPa (Fig. 5B). However, there were few differences in concentration between the first and second irrigation events, except at 138 kPa, where soil concentration increased with the additional irrigation (Fig. 5B). In test 3, which did not include sampling before the first irrigation, there were no differences in azoxystrobin concentration between the first and second irrigations (Fig. 5 C,D).

Changes in Azoxystrobin Concentration between Tests 2 and 3 Where First Irrigation was Either 40 or 107 Hours After Application. Irrigation at 40 hr after application resulted in a reduction in azoxystrobin concentration on the foliage compared to 107 hr of 36 to 64% for a carrier volume of 131 L ha⁻¹, depending on pressure combinations and sampling time (Fig. 4A,C; Table 3). The combination of 524 L ha⁻¹ and 138 kPa also resulted in marginally higher azoxystrobin concentration on foliage after the first irrigation (Table 3). In this case there was a 49% reduction in foliar azoxystrobin concentration associated with the 40-hr irrigation test.

Timing of the initial irrigation event after application had little effect on soil azoxystrobin concentrations. The only influence occurred with

the combination of 131 L ha⁻¹ and 414 kPa for samples taken after the second irrigation (Table 3). In this case, the delayed irrigation resulted in a lower azoxystrobin concentration in soil (39% reduction) than the 40-hr irrigation treatment.

Conclusions

The soil concentration in the spanish peanut field which was watered within hours of the applications contained a range of 0.11 to 0.82 ppm, depending on treatment combination and application date. In the virginia field, only an average of 0.040 to 0.1725 ppm of azoxystrobin was found in the soil, depending on treatment combination and application date. The difference in azoxystrobin concentration in soil between these two fields was much larger than that obtained by carrier volume or pressure. It is possible that the differences in peanut architecture between spanish which is a more upright plant than virginia type peanuts, played a role in the distribution of product to the soil. However, that is not a fungicide spray parameter than can be manipulated. It is possible that the

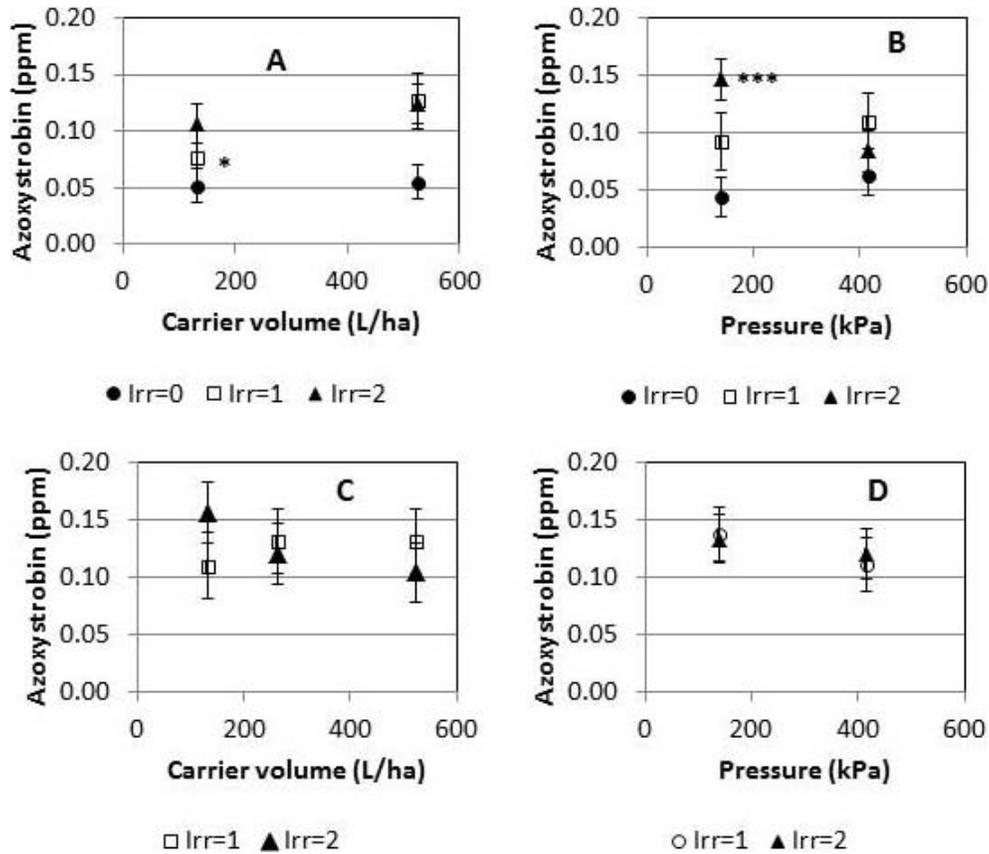


Fig. 5. Effect of carrier volume and application pressure on soil azoxystrobin concentrations (ppm). A and B) First irrigation occurred approximately 107 hr after application; C and D) First application occurred approximately 40 hr after application. There were no significant ($P > 0.10$) interactions between carrier volume and pressure in either trial. Error bars represent the standard error within an irrigation event. Samples taken after application, but before the first irrigation event are Irr=0; after the first irrigation, but before the second irrigation event are Irr=1; and after the second irrigation event are Irr=2. ***F-test for treatment was significant at $P < 0.01$; * F-test for treatment was significant at $P < 0.10$.

largest difference was in the immediate watering of the spanish peanut field (3-5 hr) versus the delayed watering in the virginia peanut field (40 and 107 hr). The decision of when to water the fields was based on the producer's needs for his crop growth, and was out of our control; hence the "quicker" initial irrigation in 2014 was not as quick as anticipated when making that application.

In all five experiments, plant foliage was large enough (lapping physically down the row, and wider than the spray band width) to block fungicide

deposition to soil (Jensen and Spliid, 2003). In 2013, the earliest application when plants were small had a lower concentration of fungicide deposited in the soil than the two later applications. Plant height increased by 9 and 16% for the next two applications, and width increased by 18 and 13% for the next two applications. A more likely explanation of azoxystrobin concentration differences probably has to do with the intensity, duration, or amount of water that fell on the plants after application. Soil deposition of azoxystrobin was greatest (7 Aug,

Table 3. T-Test values for comparison of azoxystrobin concentration after fungicide tests differed in timing of initial irrigation (40 hr vs 107 hr) after application.

Volume (L ha ⁻¹)	Pressure (kPa)	Foliage		Soil	
		Irr=1 ^b	Irr=2	Irr=1	Irr=2
131	138	2.94 ^a	4.67 ^{**}	1.24	1.93
131	414	5.44 ^{**}	4.23 ^{**}	0.92	3.48 ^{**}
524	138	2.14 [*]	1.37	1.56	1.87
524	414	0.35	0.77	1.64	1.21

^aT-test comparison with 6 degrees of freedom were significant at $P=0.05$ with a T-test value of 1.943 (*) and were significant at $P=0.01$ with a value of 3.143 (**).

^bSamples were taken for both tests after one and two irrigation events (Irr=1 or 2).

2013) for the carrier volumes $< 300 \text{ L ha}^{-1}$ and when the trial was rained on rather than irrigated.

Lower carrier volume, combined with 276 or 414 kPa did result in some situations with a higher concentration of azoxystrobin in the soil. It was assumed going into this study that the higher carrier volume would push more product to the soil, but that was not the case. Higher carrier volumes do complicate applications typically by slowing down the application time, both in the field, and in time necessary to load the tank. It is an advantage that the lower carrier volume of 131 L ha^{-1} (2014) or 262 L ha^{-1} (2013) resulted in equal or better movement of the product to the soil. In some studies an increase in carrier volume has resulted in better disease control in the lower part of the plant canopy. Armstrong-Cho *et al.* (2008), reported that the best foliar disease control when increasing carrier volume from 100 to 200 or 300 L ha^{-1} . In our studies, foliar concentration of azoxystrobin were best for carrier volumes $< 300 \text{ L ha}^{-1}$ and decreased with higher (505 and 1038 L ha^{-1}) carrier volumes. Higher carrier volumes were tested using different application methods (which somewhat confounds the results), where 1700 L ha^{-1} resulted in lower foliar concentrations of chlorothalonil than 120 L ha^{-1} carrier volume (Brenneman *et al.*, 1990). These and other studies were focused on foliar distribution of fungicides, and did not measure soil deposition of the fungicide. There were no comparable studies measuring soil deposition such as in this study. The lower overall concentration of fungicide on foliage and soil in our study associated with higher carrier volumes may indicate more splashing outside of the tested area. Fungicide that was deposited outside of the banded area of the row, or in soil a distance from the center of the bed would not be useful in pod rot control.

This project was designed to measure concentration of azoxystrobin in the soil, which was present at low levels. The use of disease as a bio-indicator of significant levels of fungicide has often been utilized (Armstrong-Cho *et al.*, 2008; Augusto and Brenneman, 2011; Egel and Harmon, 2001; Tompkin *et al.*, 1983; Woodward *et al.*, 2012). While disease suppression is the goal of a fungicide application, it is also important to know the fungicide concentration necessary to achieve disease suppression or control. The concentration of azoxystrobin in soil that will control pod rot caused by *Pythium* spp. or *R. solani* is not known (or not published), and is an area of research that needs additional work. However, *in-vitro* work has been published for these pathogens. The concentration of azoxystrobin found to inhibit mycelial growth of *P. volutum* (EC50) on agar media was 0.052 ppm

(Kerns *et al.*, 2009). The EC50 for *P. aphanidermatum* was 0.05 ppm to azoxystrobin on agar media (Mihajlović *et al.*, 2013). Isolates of *P. irregulare* and *P. ultimum* obtained from corn and soybean fields grew well at 10 and 100 ppm of azoxystrobin on agar media (Broders *et al.*, 2007). These values give some indication of the potential concentrations necessary to inhibit mycelial growth of *Pythium* species in soil. Azoxystrobin is considered particularly effective on spore stages of Oomycetes (Matheron and Porchas, 2000; Sudisha *et al.*, 2010). *Pythium* species that readily produce zoospores may be more vulnerable to azoxystrobin than those that do not. *Pythium* species that produce sporangia and zoospores include: *P. dissotocum*, *P. myriotylum* (Van der Plaats-Niterink, 1981), and *P. vexans* (Biesbrock and Hendrix, 1967). *P. irregulare* and *P. ultimum* seldom produce sporangia or zoospores (Van der Plaats-Niterink, 1981). *P. spinosum* does not form sporangia and zoospores (Van der Plaats-Niterink, 1981). The soil concentrations of azoxystrobin measured in this work as a result of carrier volume $< 300 \text{ L ha}^{-1}$ were in range that would control certain *Pythium* species, if the *in-vitro* results can be transferrable to field situations. However, they were also below EC50 values for some species. It is important to maximize soil concentration of azoxystrobin with an appropriate carrier volume and possibly timely irrigation after application.

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