Progress Report – R-25

Title: Effects of multiyear applications of 2,4-D choline on Muscadine Grape

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Public Abstract: Field studies were conducted in commercial muscadine vineyards in western North Carolina in 2018 and eastern North Carolina in 2019, 2020, and 2021 to determine tolerance of younger (< 9 yr) and older (≥ 9 yr) bearing muscadine grapevines to 2,4-D choline postemergence-directed beneath the crop canopy. Treatments included 2,4-D choline at 0, 0.53, 1.06, 1.60, and 2.13 kg ha^{-1} (1, 2, 3, and 4 pt/A) applied as a single treatment in May or June (spring) at immediate pre-bloom, and sequential treatments at 0.53 followed by (fb) 0.53, 1.06 fb 1.06, 1.6 fb 1.6, or 2.13 fb 2.13 kg ha^{-1}. The first application of the sequential treatments was applied in spring followed by another application of the same amount in July (summer) at pre-veraison. No differences were observed for injury on muscadine grapevines from 2,4-D choline treatments. Differences among treatments were not observed for yield of younger vines. However, with respect to yield of older vines, a difference due to 2,4-D choline rate was observed in 2018 where yield was higher for the 1.6 kg ha^{-1} 2,4-D choline treatment compared to the nontreated, 0.53 and 2.13 kg ha^{-1} treatments. A rate by timing interaction was observed in 2019 with lower yield from the 0.53 kg ha^{-1} 2,4-D choline summer treatment compared to all other summer treatments but similar to the nontreated. However, no biological pattern was observed from either of these differences. No differences among treatments were observed for fruit pH, titratable acidity, or soluble solid content of either young or older vines.

Introduction: Muscadine grape is native to the southeast United States with commercial production primarily in Georgia, Florida, North Carolina and South Carolina (Hoffmann et al. 2020). Production is increasing in North Carolina with an estimated 4600 ha planted commercially, valued at approximately $400 to 600 per ton if used for processing, and a higher market value if used as a fresh-market crop (M Hoffmann, personal communication). Most muscadine production occurs between the Coastal Plains and North Carolina Piedmont region (M Hoffmann, personal communication). Approximately 20 muscadine cultivars are currently used for fresh market production, and 4 to 5 cultivars are used commercially for processing (Hoffmann et al. 2020). Muscadine production in the southeast can be approached with lower pesticide inputs compared to other fruit crops (Hickey et al. 2019; Hoffmann et al. 2021). However, as a perennial crop, weed management options are limited compared to annual cropping systems, and strategies should begin during vineyard establishment. Basinger et al. (2018) found management of under-vine vegetation in *V. vinifera* L. 'Cabernet Franc' affects vine growth, berry composition and yield in vigorous growing vines. In muscadine vineyards, weed management recommendations include maintaining a vegetation-free strip under the vine with the use of herbicides, control of woody perennial weeds, and mowing vegetative grass row middles (Hoffmann et al. 2020). Over ten PRE herbicides and six POST herbicides are registered for use in muscadine vineyards in North Carolina, but only carfentrazone has selective control on broadleaf weeds without injury to the grass vineyard cover (Cline 2020). The lack of selective broadleaf POST herbicides increases interest in the use of 2,4-D choline for weed management in muscadine grape vineyards.

2,4-D is a synthetic auxin in the phenoxy-carboxylic acid family and is in WSSA Group 4 (Shaner 2014). Inside the plant, 2,4-D mimics indole acetic acid (IAA), disrupting processes in the cell wall and altering nucleic acid metabolism (Shaner 2014). Applied as a POST herbicide, 2,4-D affects cell division and growth in meristematic regions (Shaner 2014); when contact is made with foliage of muscadine grapevines, leaf strapping occurs (Figure 3.1).

Grape species (*Vitis* spp.) are sensitive to synthetic auxins, and foliar injury from low rates of 2,4-D amine has been documented on 'Concord' (*V. lubrusca* L.) (Comes et al. 1984; Ogg et al. 1991) and European wine grape cultivars (*V. vinifera* L.) (Bhatti et al. 1997; Mohseni-Moghadam et al. 2016). Rossouw et al. (2019) conducted a study on potted grapevines (*V. vinifera* L.) to evaluate effects of 2,4-D, MCPA, dicamba and glyphosate applied at simulated drift rates on vegetative and reproductive grapevine development. By the third and fourth harvest, primary bud necrosis, which is related to next year's fruit yield, was observed in 50 to 60% of the buds treated with 2,4-D, respectively. The current season's yield was reduced 34% and titratable acidity (TA) increased 9% due to 2,4-D simulated drift.

The 2,4-D choline salt formulation has "ultra-low volatility," which reduces vapor particle movement (Anonymous 2012). The choline salt is less volatile than the amine salt formulation due to higher stability and less disassociation from 2,4-D acid (Peterson et al. 2016). These characteristics should reduce the potential for volatility from the application site, and therefore reducing drift into muscadine vineyards, lowering any potential off-target injury effects. The choline formulation provides similar weed control efficacy as the amine formulation and will control weeds common to muscadine grape vineyards such as cutleaf eveningprimrose (*Oneothera laciniata* Hill), curly dock (*Rumex crispus* L.), horseweed [*Conyza canadensis* (L.) Cronq.], lettuce species (*Lactuca* spp.), morningglory species (*Ipomoea* spp.), and wild garlic (*Allium vineal* L.) (Anonymous 2018).

With limited studies on weed management in muscadine vineyards and only one POST selective broadleaf herbicide option in muscadine, a study was conducted to determine the effect of 2,4-D choline applied POST-directed underneath the vine canopy on muscadine grape tolerance, and fruit yield and quality.

Materials and Methods:

Field trials were conducted at commercial muscadine vineyards in eastern and western North Carolina in 2018, 2019, 2020, and 2021 with older (≥ 9 yr) and younger (< 9 yr) bearing vines of ‘Nesbitt’ or ‘Carlos’ cultivars (Table 3.1). Soils in western North Carolina were a sandy clay loam, and soils in eastern North Carolina were primarily a loamy sand with pH between 5.6 and 6.4, and organic matter (OM) between 0.65 and 3 (Table 3.2). The crop at each study site was managed by commercial vineyard operations using best management practices (Hoffmann et al. 2020).

The study design for all trials was a two (application timing) by four (herbicide rate) factorial plus a nontreated control in a randomized complete block design with treatments replicated four times. All plots consisted of a single planted row 1.5-m wide by 12.2-m long with two vines spaced 6.1-m apart. Between row spacing was approximately 3.4-m in all trials. A weed-free strip 1.5-m wide was maintained under all vines, and the herbicide program included glyphosate, paraquat, and indaziflam. Treatments included 2,4-D choline (Embed Extra, Corteva Agriscience, Indianapolis, IN) at 0.53, 1.06, 1.6 and 2.13 kg ha^{-1} applied as single treatments in May or June (spring) at immediate pre-bloom and as sequential treatments applied in May or June followed by (fb) an application in July (summer) at pre-veraison (Table 1) (Figure 2). Treatments were directed under the crop canopy using a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at 138 kPa equipped with two TeeJet 8003 VS nozzles (TeeJet Technologies, Springfield, IL).

Data recorded included visual crop injury 1, 2 to 3, 4, and 8 wk after treatment (WAT) for the single application, and 1, 2 to 3, and 4 WAT for the sequential application. Crop injury was characterized by stunting of the shoots or leaf deformation (epinasty or strapping) rated on a scale of 0 (no injury) to 100% (whole plant stunting or leaf deformation). A drift event in 2021 occurred before the 4 WAT of the single application rating. This obscured the remainder of the single application and following sequential application ratings; therefore, injury data for 2021 were not included in analysis.

Fruit was harvested in September for all trials by stripping all fruit from three randomly selected 30-cm sections per vine for a total of six sections per plot (Table 1) (Basinger et al. 2019). Total harvested fruit weight was taken per vine using a FG-150KBM kg scale (A&D Company Limited, Tokyo, Japan) and averaged across the plot to determine average yield per vine. Fifty ripe berries were collected from each plot, randomly selecting 25 berries per vine. Berries were weighed using a Scout SPX421 g scale (Ohaus Corporation, Parsippany, NJ) to calculate average ripe berry weight. Ten samples of 50 berries randomly selected across the plots were collected and weighed using a Scout SPX421 g scale to calculate average unripe berry weight. Unripe berry weight was converted to estimated ripe berry weight using the mean sample weight of ripe and unripe berries and average vine yield per plot, a similar equation used for blueberry yield (Equation 1) (Aldridge et al. 2019; Coneybeer-Roberts et al. 2016; Meyers et al. 2016).

$$(\text{mean ripe fruit weight} / \text{mean unripe fruit weight}) * \text{mean vine yield plot}^{-1} \quad [1]$$

The 50-ripe berry samples collected from each plot were stored at -20 C in 1 L sealed polyethylene (PE) bags until berries were analyzed for pH, titratable acidity (TA) [percent citric acid equivalents (v/v)], and total soluble solid content (SSC). Frozen berry samples were thawed to room temperature, then homogenized by hand crushing the berries in the PE bag. The berry juice was extracted from the PE bag using an 8 ml disposable transfer pipet (VWR International LLC, Radnor, PA). The pH of each fruit sample was measured using a PC800 pH meter (Apera Instruments, Columbus, OH) standardized to pH 4 and 7. Soluble solid content and TA were determined by the PAL-BX|ACID F5 pocket Brix-acidity meter (Atago Company, Limited, Bellevue, WA) on setting 2 for grape.

Response variables of crop injury, yield, and fruit quality (SSC, TA, and pH) were subjected to ANOVA and analyzed in SAS PROC MIXED (SAS 9.4, SAS Institute, Cary, NC). Herbicide application timings, herbicide rates, and their interactions were considered fixed effects. Year and replication within year were considered random effects when data were not separated by maturity stage; otherwise, year was considered a fixed effect. The nontreated control was not included in visual crop injury analysis, but it was included in fruit yield and fruit quality analyses.

Results: Visible crop injury from 2018, 2019, and 2020 were combined to determine the effect of 2,4-D choline on shoot and leaf injury by application timing (spring, summer). No difference in injury was observed for any rate of 2,4-D choline at either application timing (Table 3.3). The maximum observable injury from the single application was 8% 8 WAT from 1.06 kg ha^{-1} and from the sequential applications was 7% 4 WAT from 1.06 fb 1.06 and 1.6 fb 1.6 kg ha^{-1}. Other studies report higher injury on *V. vinifera* from simulated drift of 2,4-D. Al-Khatib et al. (1993) saw 75% foliar injury 2 WAT from 374 g ha^{-1} of 2,4-D simulated drift (1/3 the label rate) in a field study. Mohseni-Moghadam et al. (2016) conducted a simulated drift greenhouse study and saw 66% foliar injury 6 WAT from 28 g ha^{-1} of 2,4-D amine (1/30 the label rate). This could indicate a higher sensitivity of *V. vinifera* grapevines to 2,4-D than muscadine grapevines. However, the amount of 2,4-D choline that contacted the muscadine grapevines in our study is unknown and could have been lower than the rates used in the simulated drift studies previously mentioned.

Yield and fruit quality data were separated by vine maturity (older and younger) to determine if there were differences in response of grapevine to treatment by age of vine. No year by rate by timing interaction with yield was observed for the younger vines, so data were combined across years. No differences were observed in yield for the younger vines (Table 4). A three-way interaction between rate, timing, and year for older vine yield was observed, so data were analyzed separately by year (Table 4). In 2018, differences were observed by rate where the 1.6 kg ha^{-1} 2,4-D choline treatment had a higher yield than the nontreated, 0.53, and 2.13 kg ha^{-1} treatments. In 2019, there was a rate by timing interaction where yield from 0.53 kg ha^{-1} 2,4-D choline summer treatment was lower than all other summer treatments and 0.53, 1.06, and 1.6 kg ha^{-1} spring treatments, but it was not different from the nontreated. In contrast to these findings, Rossouw et al. (2019) found yield of *V. vinifera* treated with 2,4-D amine applied as a simulated drift at 7% of the label rate decreased 34% compared the nontreated.

The differences in yield in 2018 and 2019 may be attributed more to pruning technique than an effect of 2,4-D choline. It has been documented that pruning techniques can affect muscadine grape yield in the coming season where increasing the node count of the vines increases yield to a point (Sims et al. 1990). The vineyards used in these studies mechanically prune and hand prune the vines in the winter, but if the vines were not standardized to a set node count per vine, it could explain the inconsistency in yield across treatments.

No interaction by year with older or younger vine fruit quality was observed, so data were combined across year by maturity stage (Table 5). No difference was observed in berry chemistry traits (pH, TA, and SSC) for fruit of either older or younger vines.

The results from these trials indicate 2,4-D choline applied POST-directed beneath the muscadine vine does not affect crop growth or fruit quality when applied sequentially in spring and summer. Although no differences were observed for visual crop injury, muscadine grape growers will need to be informed that minor injury may occur. Differences in yield of older vines were observed, but it is inconclusive if that was an effect of pruning technique or 2,4-D exposure. Future research should include a multi-year study to take into consideration grape flower development as floral buds injured during the growing season before they bloom directly affect yield the year after application (Srinivasan and Mullins 1981). As a perennial crop, a multi-year study on muscadine grape will also determine if minor injury is compounded over multiple years and affects shoot growth and yield as was seen by Ogg et al. (1991) on Concord grapes.

Table 1. Year, location, cultivar, crop age, treatment application dates and harvest date for studies evaluating effect of 2,4-D on muscadine grape, 2018-2021.

Year	Location (GPS coordinates)	Cultivar	Crop age (yr)	Treatment application dates[a]		Harvest date
				Spring	Summer	
2018	Vale, NC (35.510823°N, 81.479545°W)	Nesbitt	9	Jun 6, 2018	Jul 27, 2018	Sep 18, 2018
2019	Rose Hill, NC (34.853444°N, 77.976812°W)	Carlos	18	May 16, 2019	Jul 18, 2019	Sep 3, 2019
2020	Teachey, NC (34.761510°N, 77.987174°W)	Carlos	2-3	May 13, 2020	Jul 7, 2020	Sep 7, 2020
2021	Teachey, NC (34.761260°N, 77.987162°W)	Carlos	3-4	May 17, 2021	Jul 11, 2021	Sep 6, 2021

[a] Spring application included single plus first sequential treatments, and summer applications included second sequential treatments.

Table 2. Soil characteristics by site for studies evaluating effect of 2,4-D on muscadine grape in North Carolina, 2018-2021.

Year	Soil series	Soil characteristics				
		pH	OM	Sand	Clay	Silt
			--------------%--------------			
2018	Cecil sandy clay loam (Fine, kaolinitic, thermic Typic Kanhapludults)	5.4	3.16	63.6	15.2	20.8
2019	Noboco loamy fine sand (Fine-loamy, siliceous, subactive, thermic Oxyaquic Paleudults)	6.4	3.00	58	12.2	29.6
2020	Goldsboro loamy sand (Fine-loamy, siliceous, subactive, thermic Aquic Paleudults)	5.6	0.65	76.4	7.2	16.4
2021	Goldsboro loamy sand (Fine-loamy, siliceous, subactive, thermic Aquic Paleudults)	5.6	0.65	72.8	10.8	16.4

Table 3. Effect of 2,4-D choline applied POST-directed beneath the canopy of muscadine grape on visual crop injury by application timing in North Carolina in 2018-2020.[a]

Rate	Application timing[b]				
	Single			Sequential	
	WAT[c]			WAT	
	1	4	8	1	4
----kg ha^{-1}----	---------------------------------------%---------------------------------------				
0.53	0	4	7	6	4
1.06	0	4	8	5	7
1.6	0	2	3	5	7
2.13	0	3	6	5	5
p-value	--	0.3120	0.5005	0.7599	0.3632

[a] Muscadine grape injury was recorded on a scale of 0 (no injury) to 100 (whole plant stunting or leaf deformation).

[b] Single applications of 2,4-D choline were made in June 2018, May 2019, 2020, or 2021. Sequential applications were first made in June 2018, May 2019, 2020, or 2021 followed by July 2018, 2019, 2020 or 2021.

[c] Abbreviation: WAT, wk after treatment.

Table 4. Effect of 2,4-D choline applied POST-directed beneath the canopy of muscadine grape on estimated total yield by maturity stage in North Carolina in 2018-2021.[a]

Timing (T)[b]	Rate (R) (kg ha^{-1})	Yield[c]		
		Older		Younger
		2018	2019	
		------------------------kg ha^{-1}-------------------------		
	0	17,482 a		
	0.53	22,439 bc		
	1.06	19,455 ab		
	1.6	23,345 c		
	2.13	18,122 a		
	p-value	0.0183		
Nontreated			16,416 ab	20,147 a
Spring	0.53		22,120 c	21,639 a
	1.06		19,614 bc	20,787 a
	1.6		22,386 c	20,947 a
	2.13		18,922 a-c	20,414 a
Summer	0.53		15,031 a	19,348 a
	1.06		23,078 c	22,600 a
	1.6		22,279 c	20,734 a
	2.13		21,960 c	21,160 a
T X R	p-value		0.0057	0.4991

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[a] Means within a column followed by the same letter are not different according to Fisher’s protected LSD (α=0.05).

[b] Spring applications of 2,4-D choline were made in June 2018, May 2019, 2020, or 2021. Summer applications were made in July 2018, 2019, 2020 or 2021.

[c] Estimated yield is based on 533 muscadine grape vines ha^{-1}.

Table 5. Effect of 2,4-D choline applied POST-directed beneath the canopy of muscadine grape on fruit pH, titratable acidity (TA), and soluble solid content (SSC) of mature and young vines, combined across years by maturity stage, in North Carolina in 2018-2021.

Timing (T)[a]	Rate (R) (kg ha^{-1})	Fruit quality					
		Older			Younger		
		pH	TA[b]	SSC[c]	pH	TA	SSC
Nontreated		3.49	0.40	14.4	3.36	0.46	14.9
Spring	0.53	3.48	0.41	14.3	3.30	0.51	14.7
	1.06	3.42	0.42	14.3	3.33	0.49	14.7
	1.6	3.40	0.43	14.3	3.33	0.49	14.7
	2.13	3.45	0.43	14.4	3.31	0.48	14.3
Summer	0.53	3.42	0.43	14.4	3.36	0.51	14.7
	1.06	3.44	0.42	14.5	3.30	0.53	14.3
	1.6	3.40	0.45	14.6	3.33	0.50	14.4
	2.13	3.47	0.42	14.4	3.31	0.51	14.2
T X R	p-value	0.6170	0.8108	0.9014	0.3587	0.6284	0.7026

[a] Spring applications of 2,4-D choline were made in June 2018, May 2019, 2020, or 2021. Summer applications were made in July 2018, 2019, 2020 or 2021.

[b] TA is measured in percent citric acid equivalents (v/v).

[c] SSC is expressed in °Brix.