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Running title: Stevia tolerance to herbicides

Response of stevia to herbicides applied post-transplant in the greenhouse

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Abstract

Greenhouse studies were conducted to determine the response of stevia to several herbicide modes of action applied 2 wk after transplanting (WAP). At 1 wk after treatment (WAT), aciflourfen, metribuzin, and carfentrazone injured stevia 34 to 39%. In contrast, *S*-metolachlor, linuron, halosufluron, ethalfluralin, pyroxasulfone, pendimethalin, and tryfloxysulfuron injured stevia <20%, 1 WAT. By 4 WAT, stevia injury was ≤ 19% regardless of treatment, except metribuzin and trifloxysulfuron with 84 and 69% injury, respectively. *S*-metolachlor, linuron, ethalfluralin, pendimethalin, and pyroxasulfone did not reduce aboveground biomass compared to the nontreated check, 4 WAT. Linuron, ethalfluralin, pendimethalin, and pyroxasulfone did not reduce belowground biomass. Linuron, pendimethalin, and ethalfluralin may provide new modes of action for POST-transplant weed management in stevia. However, further research is needed to evaluate the effect of these herbicides on stevia growth and quality in the field.

Nomenclature: Acifluorfen; carfentrazone; ethalfluralin; halosulfuron; linuron; metribuzin; pendimethalin; pyroxasulfone; *S*-metolachlor; trifloxysulfuron; stevia, *Stevia rebaudiana* Bertoni.

Keywords: Crop response; specialty crop; weed management; Stevia, *Stevia rebaudiana* Bertoni.

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Introduction

Stevia is a relatively new specialty crop in the U.S. With the FDA's approval of stevia as a food additive, interest in stevia has increased in the U.S. (Cavaliere 2009). Being 200 to 400 times sweeter than sugar, stevia is used as a nonnutritive sweetener (FDA 2018; Lester 1999). Stevia, a member of the Asteraceae family, is native to Paraguay and has a long history of human consumption (PCSI 2017; Ramesh et al. 2006). However, stevia was not approved for food and beverage consumption in the U.S. until December 2008 (Cavaliere 2009; ISO 2001). Several companies, including Coca-Cola (Truvia) and Pepsi (PureVia), have commercially available stevia products (Cavaliere 2009).

Stevia is a perennial plant with an upright growth habit (Figure 1) and can be harvested more than once a season, depending on the region and its age (Koehler 2018; Ramesh et al. 2006). In North Carolina, stevia is typically planted in April through May and has a field life of 3 to 5 years (Koehler 2018). In field production stevia is typically harvested with a combine before drying, baling, and shipment to an extraction facility (Koehler 2018). Stevia does not compete well with weeds (Chreist 2019), especially early in the season (Azimah et al. 2018; Ramesh et al. 2006), although weed loss studies have not been conducted. Research conducted by Harrington et al. (2011) found that hand weeding increased stevia yield by 30-fold compared to a weedy check. However, few herbicides have been registered for use in stevia (Chriest 2019; Harrington et al. 2011). Glyphosate and ethafluralin are registered PRE-transplant, and clethodim, carfentrazone, *S*-metolachlor, and glyphosate are the only herbicides registered for POST-transplant application. *S*-metolachlor can be applied over the top of stevia for residual control of small-seeded grass and broadleaf weed species (Anonymous 2023). In addition, clethodim can be applied over-the-top of stevia to control emerged grass species (Anonymous 2017). Carfentrazone and glyphosate are registered only for directed applications between rows after stevia transplanting for controlling emerged weeds. Therefore, few herbicides are registered for use over-the-top of stevia, especially for broadleaf weed control.

With few herbicides registered for use POST-transplant in stevia, the addition of more herbicides for use over-the-top may help to prevent weed interference in the crop. In addition, adding alternative modes of action to those registered for use in stevia can help to prevent the development of herbicide resistant weed populations. Thus, greenhouse studies were conducted to determine stevia tolerance to POST-transplant applied herbicides not registered for stevia.

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Material and Methods

Stevia seeds (Johnny's Selected Seeds, Winslow, ME) were planted in 50 square cell trays (T.O. Plastics, Inc., Clearwater, MN) at North Carolina State University's Method Road Greenhouse Unit 1, Raleigh, NC (35.788° N, 78.694° W) in fall 2021. Stevia seedlings (8-10 cm tall) were then each transplanted into 6.2 L (diameter 25.4 cm, height 18.4 cm standard round pots (HC Companies, Twinsburg, OH) containing propagation mix (Sun Gro Horticulture Distribution Inc., Agawam, MA). The stevia received water three times daily from overhead irrigation and supplemental lighting to prevent stevia from flowering. The greenhouse was kept at 29 C ± 5 C. The treatments were arranged as a randomized complete block design with 7 replications and consisted of 2 experimental runs. Each plot consisted of two pots. Treatments consisted of herbicides, listed in Table 1, applied 2 wk after transplanting (WAP) to 25.4 to 30.5 cm tall stevia plants. Treatments were applied over-the-top with a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at 124 kPa. The boom was equipped with 2 flat fan XR 8003VS nozzles (TeeJet 8003; TeeJet Technologies, Wheaton IL) spaced 51 cm apart.

At 1, 2, 3, and 4 wk after treatment (WAT), stevia injury including chlorosis and necrosis was evaluated on a percent scale with 0% being no injury and 100% being plant death (Frans et al. 1986). At 3 WAT, one pot of stevia per plot was randomly selected for destructive root analysis. Stevia roots were excavated, washed, and then analyzed using WinRHIZO root scanning system (Regent Instruments Inc., Montreal, PQ, Canada) for root volume and projected root surface area. The roots were then dried for 1 d at 49 C and weighed. Aboveground biomass was dried at 49 C for 3 d then weighed. The remaining pot of stevia per plot was allowed to grow for 1 additional week, then aboveground biomass was collected and dried at 49 C for 3 d then weighed.

Data Analysis

Data was subjected to ANOVA utilizing the MIXED procedure SAS version 9.4 (SAS Institute Inc., Cary, NC). Residuals were plotted to visually examine homogeneity of variance. Herbicide treatment and experimental run were treated as fixed effects, while replication nested within experimental run was treated as a random effect. Means were separated utilizing Fishers protected LSD ($\alpha = 0.05$). Stevia foliar injury data required an arcsine square root transformation

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for analysis. Projected root surface area, root volume, and root biomass required a square root transformation for analysis. Data were presented as back-transformed least-squares means.

Results and Discussion

Stevia Injury

At 1 and 2 WAT, there was a significant (P < 0.05) experimental run by treatment interaction. Interactions means were plotted and, following assessment, it was determined that the interactions were biologically uninformative; therefore, data were pooled across experimental runs for analysis. No interactions were significant (P > 0.05) at 4 WAT; therefore, data were pooled across experimental runs. Stevia injury from herbicide treatments across the study appeared as chlorosis and necrosis. In particular, carfentrazone, aciflurofen, linuron, and metribuzin caused foliar necrosis of stevia. Halosulfuron resulted in chlorosis and necrosis at the meristem. Pyroxasulfone caused slight chlorosis along the leaf edges of stevia. Trifloxysulfuron caused initial chlorosis followed by necrosis and stevia death. At 1 WAT, acifluorfen, carfentrazone, and metribuzin injured stevia ≥ 34% (Table 2). By 4 WAT, stevia injury from acifluorfen and carfentrazone was reduced to 19 and 14%, respectively. In contrast, injury from metribuzin increased to 84% by 4 WAT. In prior research, metribuzin at 350 g ai ha^{-1} injured stevia 48% at 2 WAT (Harrington et al. 2011). At 1 WAT, linuron injured stevia 13%, which was reduced to 6% by 4 WAT. Prior research has shown stevia to have some tolerance to linuron (Hopkins and Midmore 2015). When applied at a higher rate, it has been reported that linuron can injure greenhouse grown stevia as high as 42% (Harrington et al. 2011). At 1 WAT, halosulfuron and trifloxysulfuron injured stevia 15 and 18%, respectively. Halosulfuron injured stevia 23% at 2 WAT, an 8% increase compared to 1 WAT. By 4 WAT, the injury from halosulfuron was reduced to 7%; however, injury from trifloxysulfuron increased to 69%. *S*-metolachlor, pyroxasulfone, and pendimethalin injured stevia ≤9% at 1 and 2 WAT, and ≤4% by 4 WAT.

Aboveground biomass

Due to a lack of experimental run-by-treatment interactions, data for aboveground biomass were combined across experimental runs. In the first year of field production, stevia is typically harvested approximately 110 d after transplanting; therefore, data from this study best quantifies

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stevia's early recovery from the herbicides applied. Aboveground biomass collected at 4 WAT was generally higher than that collected at 3 WAT. At 3 WAT, ethalfluralin and pendimethalin did not reduce aboveground biomass when compared to the nontreated check (Table 3). By 4 WAT, stevia had further recovered from the herbicide treatment, and as a result, *S*-metolachlor, linuron, ethalfluralin, pendimethalin, and pyroxasulfone did not reduce aboveground biomass. Harrington et al. (2011) also reported a non-significant reduction in aboveground biomass for stevia treated with linuron at 900 g ai ha^{-1} in the greenhouse. Metribuzin reduced stevia aboveground biomass by 87%, 4 WAT (Table 3). However, at a lower rate (350 g ai ha^{-1}) Harrington et al. (2011) did not see a reduction in aboveground biomass for stevia treated with metribuzin relative to the nontreated check. Our results support those of Hopkins and Midmore (2015) in which they reported no aboveground biomass reduction with pendimethalin.

Projected root surface area, root volume, and belowground root biomass

For the projected root surface area and root volume a significant treatment by experimental run interaction was observed; therefore, the data were separated by experimental run for analysis and presentation. No interactions were present for belowground root biomass data; therefore, data were pooled across experimental runs. In the first experimental run, ethalfluralin, pendimethalin, and pyroxasulfone did not reduce stevia projected root surface area compared to the nontreated check (Table 4). In the second experimental run linuron, and ethalfluralin did not reduce stevia projected root surface area from the nontreated check. Root volume followed a similar trend, where in the first experimental run ethalfluralin and pendimethalin did not reduce stevia aboveground biomass compared to the nontreated check. However, in contrast to projected root surface area in the first run pyroxasulfone reduced root volume. Linuron, ethalfluralin, and pyroxasulfone did not reduce root volume and belowground biomass in the second run. (Table 4).

At present, the only options for weed control in stevia post-transplant are *S*-metolachlor clethodim, glyphosate, and carfentrazone. However, linuron, pendimethalin, ethalfluralin, and pyroxasulfone did not reduce above or belowground biomass, and if registered could help to control broadleaf weeds such as Palmer amaranth (*Amaranthus palmeri* S. Watson) in stevia production. In prior research, linuron applied POST-transplant controlled 98%, 1 WAT (Moore et al. 2021). Linuron, pendimethalin, and ethalfluralin may provide new modes of action for

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POST-transplant weed management in stevia. However, further research is needed to evaluate the effect of these herbicides on stevia growth and quality in the field, as these studies were limited to the greenhouse. In addition, soil utilized in this study contains a high concentration of organic matter. Thus, future research is needed to determine the safety of these herbicides applied to stevia grown in fields across various soil types and textures. In addition, while *S*-metolachlor is registered for use in stevia and did not affect aboveground biomass at 4 WAT, it did reduce root biomass, projected root surface area, and root volume. As a perennial crop, root growth is important to stevia establishment and overwintering. Further research is needed to determine the long-term effect of *S*-metolachlor application on stevia overwintering and regrowth the following season.

Practical Implications

There are few herbicides registered for use in stevia production. With only *S*-metolachlor registered for use over-the-top of stevia for broadleaf weed control; growers have few options for controlling broadleaf weeds. Identifying herbicides that may be safe for use POST-transplant in stevia may help control weeds that emerge after the effective residual activity of *S*-metolachlor has worn off.

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Competing Interests

Competing interests: The authors declare none.

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Table 1. Herbicide treatments applied to stevia 2 wk after transplanting in 2021.

Active ingredient	Trade name	Rate	Manufacturer	City, State	Website
		g ai ha^{-1}			
S-metolachlor	Dual Magnum	1070	Syngenta	Greensboro, NC	www.syngetna.com
Acifluorfen	Ultra Blazer	280	UPL AgroSolutions Canada Inc.	King of Prussia, PA	www.upl-ltd.com
Linuron	Linex 4L	560	NovaSource	Phoenix, AZ	www.novasource.com
Halosulfuron[a]	Sandea	26.3	Gowan Company	Yuma, AZ	www.gowanco.com
Ethalfluralin	Curbit EC	1260	Loveland Products Inc.	Greeley, Colorado	www.lovelandproducts.com
Carfentrazone	Aim EC	17.5	FMC Corporation	Philadelphia, PA	www.ag.fmc.com
Pendimethalin	Prowl H20	800	BASF Corporation	Research Triangle Park, NC	www.agriculture.basf.us.com
Metribuzin	Tricor DF	420	UPL AgroSolutions Canada Inc.	King of Prussia, PA	www.upl-ltd.com
Trifloxysulfuron	Envoke	5.5	Syngenta	Greensboro, NC	www.syngetna.com
Pyroxasulfone	Zidua WG	59.5	BASF Corporation	Research Triangle Park, NC	www. agriculture.basf.us.com

[a]Nonionic surfactant (Chemwet 1000; Victorian Chemical Company Pty, LLC, Coolaroo, Victoria, AU) was included at 0.25% v/v.

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Figure 1. Field (A) and greenhouse (B) grown stevia. In field production, stevia is typically harvested by cutting approximately 3 cm above the soil line with a combine.

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Table 2. Stevia injury[a] at 1, 2, and 4 wk after treatment following herbicide applications at 2 wk after transplanting.

Herbicide[b]	Rate	1 WAT[c]		2 WAT		4 WAT	
	g ai ha^{-1}	——— % ———					
S-metolachlor	1070	1	e	4	e	1	f
Acifluorfen	280	34	a	30	c	19	c
Linuron	560	13	cd	10	d	6	de
Halosulfuron	26.3	15	bc	23	c	7	de
Ethalfluralin	1260	2	e	0		0	f
Carfentrazone	17.5	39	a	28	c	14	cd
Pendimethalin	800	1	e	0		3	e
Metribuzin	420	38	a	82	a	84	a
Trifloxysulfuron	5.5	18	b	54	b	69	b
Pyroxasulfone	59.5	9	d	5	d	4	cd

[a]Rating scale: 0 being no injury and 100% being plant death. Injury includes chlorosis, necrosis, and stunting.

[b] At 1 and 2 WAT, there was a significant ($P < 0.05$) experimental run by treatment interaction. Interactions means were plotted and, following assessment, it was determined that the interactions were biologically uninformative; therefore, data were pooled across experimental runs for analysis. No interactions were significant ($P > 0.05$) at 4 WAT; therefore, data were pooled across experimental runs

[c] Least squared means within a column followed by the same letter are not significantly different according to Fishers protected LSD (α = 0.05). Data were transformed using a square root transformation for analysis. Back transformed least squared means are presented.

Table 3. **S**tevia dry aboveground biomass at 3 and 4 WAT following herbicide applications 2 wk after transplanting.[a]

Herbicide[b]	Rate	3 WAT		4 WAT	
	g ai ha^{-1}	——— g plant^{-1} ———			
Nontreated	-	3.7	a	4.2	Ab
S-metolachlor	1070	2.4	d	3.7	Bc
Acifluorfen	280	1.5	e	1.6	E
Linuron	560	3	bc	4.4	A
Halosulfuron	26.3	2.2	d	2.8	Cd
Ethalfluralin	1260	3.5	ab	4.5	A
Carfentrazone	17.5	1.5	e	2.1	De
Pendimethalin	800	3.6	a	3.8	Ab
Metribuzin	420	0.4	f	0.7	F
Trifloxysulfuron	5.5	1.1	e	0.7	F
Pyroxasulfone	59.5	2.6	cd	3.6	Bc

[a]There were no significant experimental run interactions ($p > 0.05$); therefore; data were pooled across experimental runs.

[b]Least squared means within a column followed by the same letter are not significantly different according to Fishers protected LSD ($\alpha = 0.05$).

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Table 4. Stevia projected root surface area, root volume, and belowground root biomass following herbicide applications 2 wk after transplanting.[a]

Treatment[b]	Rate	Projected root surface area				Root volume				Root biomass	
		Run 1		Run 2		Run 1		Run 2			
	g ai ha^{-1}	cm^2				cm^3				g $plant^{-1}$	
Nontreated	-	239.6	a	220.6	a	9.3	a	7.5	a	0.92	a
S-metolachlor	1070	116.8	c	126.7	cd	3.7	d	4.2	bcd	0.29	b
Acifluorfen	280	60.6	d	88.6	d	1.8	e	3	d	0.21	c
Linuron	560	146.8	bc	204.1	ab	4.8	cd	6.9	a	0.76	a
Halosulfuron	26.3	114.7	c	126.5	cd	3.4	d	4	cd	0.38	b
Ethalfluralin	1260	234.7	a	171.7	abc	8	ab	6.2	ab	0.8	a
Carfentrazone	17.5	52.8	d	89.4	d	1.7	e	2.9	d	0.12	c
Pendimethalin	800	230.8	a	141.7	c	8.6	ab	4.8	bc	0.8	a
Metribuzin	420	5.8	e	14.4	f	0.22	f	0.6	f	0.02	d
Trifloxysulfuron	5.5	49.4	d	34.3	e	1.5	f	1.2	e	0.03	d
Pyroxasulfone	59.5	177.8	ab	153.7	bc	6.2	bc	5.2	abc	0.67	a

[a]The interaction between treatment and experimental run was significant ($P < 0.05$) for projected root surface area and root volume; therefore, data were separated by experimental run. Data were transformed using a square root transformation for analysis, and then back transformed for reporting the least-squared means. There were no significant experimental run interactions ($p > 0.05$) for root biomass; therefore, data were pooled across experimental runs.

[b]Least-squared means within a column followed by the same letter are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$).

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