ABSTRACT

IPPOLITO, STEPHEN JAMES. Response of Stevia (*Stevia rebaudiana*) to Herbicides Applied Post-transplant (Under the direction of Dr. Katherine M. Jennings).

Stevia (*Stevia rebaudiana* Bertoni) is a zero-calorie sweetener, 200 to 400 times sweeter than sucrose. Stevia has been grown commercially as a sugar substitute in much of Asia since 1995. In addition, stevia has been consumed as a sweetener for hundreds of years; however, only recently has stevia been authorized for use as a food additive in the US by the FDA. With the authorization of stevia as a food additive, several companies have released stevia products including Coca-Cola (Truvia) and Pepsi (PureVia). Stevia is sensitive to weed competition, especially early in the season. However, few herbicides have been registered for use in stevia. As a result, post-transplant weed control options are limited in Stevia. Thus, studies were conducted to evaluate the safety of organic and conventional herbicides for use post-transplant in stevia.

Field and greenhouse studies were conducted to evaluate the efficacy and safety of organic herbicides applied over-the-top and post-directed to stevia. Treatments included caprylic acid plus capric acid, clove oil plus cinnamon oil, d-limonene, acetic acid (200 grain), citric acid, pelargonic acid, eugenol, ammonium nonanoate, and ammoniated soap of fatty acids. In field studies, D-limonene, pelargonic acid, ammonium nonanoate, and ammoniated soap of fatty acids-controlled Palmer amaranth > 90% 1 wk after treatment (WAT). Palmer amaranth was not adequately controlled (< 65%) by citric acid, acetic acid, and eugenol 1 WAT. In addition, 1 WAT d-limonene and pelargonic acid treatments resulted in over 90% control of annual sedge. Regarding crop safety, caprylic acid plus capric acid, pelargonic acid, and ammonium nonanoate caused < 30% injury 2 WAT. D-limonene, citric acid, acetic acid, and ammoniated soap of fatty acids caused less than 18 to 25% injury 2 WAT. Clove oil plus cinnamon oil and eugenol caused < 10% injury. Despite being injurious, organic herbicides did not reduce yield compared to the

nontreated check. Based upon yield data, these herbicides have potential for use in stevia; however, for established stevia these products could delay harvest. Caution should be taken before applying the majority of these organic herbicides on established stevia, if an early harvest date is desired. The application of clove oil + cinnamon oil over-the-top resulted in <10% injury 28 d after treatment (DAT) in the greenhouse and 6 WAT post-directed in the field. In addition, this treatment provided excellent control of Palmer amaranth 4 WAT. Clove oil plus cinnamon oil has potential for use for early season weed management for organic production systems.

Greenhouse studies were conducted to evaluate the safety of conventional herbicides applied over-the-top of stevia 2 wk after transplanting. Treatments included *S*-metolachlor, acifluorfen, linuron, halosulfuron, ethalfluralin, pendimethalin, metribuzin, trifloxysulfuron, pyroxasulfone, and carfentrazone. At 1 WAT, aciflourfen, metribuzin and carfentrazone caused 30 to 45% injury across both experimental runs. In contrast across both runs at 1 WAT, *S*-metolachlor, linuron, halosufluron, ethalfluralin, pyroxasulfone, pendimethalin, and tryfloxysulfuron caused 20% or less injury to stevia. By 4 WAT, injury to stevia from all treatments caused ≤ 19% injury, except metribuzin and trifloxysulfuron which caused 84 and 69%, respectively.

S-metolachlor, linuron, ethalfluralin, pendimethalin and pyroxasulfone did not cause a significant reduction in above ground biomass relative to the nontreated check 28 DAT. Below ground biomass was not impacted by linuron, ethalfluralin, pendimethalin, and pyroxasulfone. Ethalfluralin, linuron, pendimethalin, and pyroxasulfone appear to have potential for use in stevia and if registered in stevia would represent new herbicides for in-season weed management in the crop.

Response of Stevia (*Stevia rebaudiana*) to Herbicides Applied Post-transplant

by
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DEDICATION

I would like to dedicate this thesis to my grandpa and grandma Ippolito. Their support and guidance through the years has helped shape me into the person I am today. Thank you for encouraging me to pursue my dreams.

BIOGRAPHY

Stephen Ippolito was born in central Oregon May 27, 1994. Stephen was raised in Filer Idaho. Before graduate school he worked as an assistant at Agrogene for Dr. Peter Mes. He completed his bachelors at Willamette University in 2017. After college he worked as an intern at the University of Idaho’s Kimberly Research and Extension Center for Dr. Don Morishita where he assisted in the implementation of weed science studies in sugar beet, beans, and other crops. His experience there inspired him to pursue a career in weed science.

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I would like to thank my family and friends for all their support and encouragement. I cannot express enough gratitude to my parents for their love and support. Their example, persistence, and kindness molded me into who I am today. I would like to thank my siblings, because of them no matter how far the distance I have never felt separated from my family. I would also like to thank my friends Mark Dolecheck and Derrick Cox whom I consider family.

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CHAPTER 1

Response of stevia (*Stevia rebaudiana*) to organic herbicides applied post-transplant

(In the format appropriate for submission to Weed Technology)

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Abstract

Studies were conducted to determine the effect of organic herbicides applied post-transplant directed and over–the-top to stevia; as well as weed control from organic herbicides when applied in a polyethylene mulch production system. Treatments included caprylic acid plus capric acid, clove oil plus cinnamon oil, d-limonene, acetic acid (200 grain), citric acid, pelargonic acid, eugenol, ammonium nonanoate, and ammoniated soap of fatty acids. D-limonene, pelargonic acid, ammonium nonanoate, and ammoniated soap of fatty acids controlled Palmer amaranth > 90% 1 wk after treatment (WAT). Palmer amaranth was not adequately controlled (< 65%) by citric acid, acetic acid and eugenol 1 WAT. In addition, 1 WAT the application of d-limonene and pelargonic acid resulted in over 90% control of annual sedge. Caprylic acid plus capric acid, pelargonic acid, and ammonium nonanoate caused > 30% injury 2 WAT. D-limonene, citric acid, acetic acid, and ammoniated soap of fatty acids caused 18 to 25% injury 2 WAT. Clove oil plus cinnamon oil and eugenol caused < 10% injury. Despite being injurious, organic herbicides did not reduce yield compared to the nontreated check. Based upon yield data, these herbicides have potential for use in stevia; however, for established stevia these products could delay harvest. Caution should be taken before applying the majority of these organic herbicides on established stevia, if an early harvest date is desired. Clove oil plus cinnamon oil over-the-top in the greenhouse resulted in 8 and 3% injury 4 and 6 WAT POST-directed in the field, respectively. In addition, this treatment provided excellent control of Palmer amaranth 4 WAT. Clove oil plus cinnamon oil has potential for use for early season weed management for organic production systems.

Nomenclature: *Stevia rebaudiana* Bertoni; *Cyperus compressus* L*; Amaranthus palmeri* S. Watson; Caprylic acid + capric acid; Clove oil + cinnamon oil; d-limonene; Citric acid; Pelargonic acid; Ammonium nonanoate; Ammoniated soap of fatty acids; Acetic Acid; Eugenol

Key Words: Organic weed control, organic weed management

Stevia (*Stevia rebaudiana* Bertoni) is a zero-calorie sweetener, 200 to 400 times sweeter than sucrose (Lester 1999; FDA 2018). As a result, it serves as an excellent sugar substitute, especially for diabetics (Mishra et al. 2011). Stevia has been grown commercially as a sugar substitute in much of Asia since 1995 (ISO 2001). In addition, stevia has been consumed as a sweetener for hundreds of years (PCSI 2017); however, only recently has stevia been authorized for use as a food additive in the US by the FDA (Cavaliere 2009). With the authorization of stevia as a food additive, several companies have released stevia products including Coca-Cola (Truvia) and Pepsi (PureVia) (Cavaliere 2009).

In production, stevia is commonly grown from seed in tobacco float trays and then transplanted into the field 8 to 12 wk later (Koehler 2018). Stevia is a perennial, allowing multiple harvests each season and has a field life of 3 to 5 yr; however, it is typically only harvested once the first year (Koehler 2018). Diseases, insects and weeds are important pests in stevia (Stevia Technology 2022; Taak et al. 2021). Stevia's poor competitive ability with weeds can reduce yield 2 to 25%, and weed control can increase production costs (Taak et al. 2021). Stevia is particularly vulnerable to weed competition early in the season (Chriest 2019; Azimah et al. 2018). Azimah et al. (2018) reported the critical period for weed control for stevia in the greenhouse was 1 to 3 wk after planting (WAP) for a mixture of broadleaf and narrowleaf weeds. Limited herbicides are registered for use in stevia (Harrington et al. 2011; Chriest 2019). With the exception of *S*-metolachlor and clethodim, no conventional herbicide is registered for use POST-transplanting over-the-top of stevia (Chriest 2019). As a result, POST weed control options are limited in stevia.

In organic production systems, chemical weed control options are even more limited. Acetic acid has been reported to provide control of annual ryegrass (*Lolium multiflorum* Lam.), goosegrass (*Eleusine indica* Gaertn.) and redroot pigweed (*Amaranthus retroflexus* L.) (Abouziena et al. 2009). In addition, citric acid has been reported to provide control of velvetleaf (*Abutilon theophrasti* Medik.), stranglevine (*Morrenia odorata* Lindle) and black nightshade (*Solanum nigrum* Linn.) (Abouziena et al. 2009). Cinnamon oil + clove oil provided as high as 89% control when applied in studies containing redroot pigweed, common lambsquarters (*Chenopodium album* L.), and large crabgrass (*Digitaria sanguinalis* L. Scop) (O'Sullivan et al 2015). Most organic herbicides are nonselective and provide no residual weed control (Liu et al. 2021; Evans et al. 2011). As a result, over-the-top applications can cause significant crop injury (Liu et al. 2021; Evans et al. 2011). Additionally, organic herbicides are more efficacious when applied to small weeds and may require sequential applications to achieve sufficient control (Abouziena et al. 2009; Liu et al. 2021). As a result, the application of organic herbicides such as Acetic acid can cost over \$988 ha^{-1} (Evans et al. 2011). However, directed applications can require less herbicide which can result in less cost of applying organic herbicides. Prior research has shown directed applications of organic herbicides to provide effective weed control (Evans et al. 2011).

Prior studies have examined the effects of directed applications of organic herbicides in other crops (Evans et al. 2011); however, to our knowledge no peer-reviewed research has evaluated organic herbicides in stevia. In addition, characterization of weed control from organic herbicides when applied in a polyethylene mulch production system would assist growers in deciding whether or not to apply organic herbicides. Therefore, greenhouse and field studies were conducted to determine the effect of organic herbicides applied over-the-top and post-directed to transplanted stevia in a polyethylene mulch production system, respectively.

Material and Methods

Greenhouse Study

Greenhouse studies were conducted at the Marye Anne Fox Science Teaching Laboratory (35.798°N, 78.678°W) at North Carolina State University, Raleigh in 2021. Stevia was transplanted in 3 L (14 cm tall, 20 cm diam) round pots containing Fafard 4P potting mix (Conrad Fafard Inc., Agawam, MA). Treatments consisted of organic herbicides (Table 1) applied over-the-top of stevia 4 WAP with a CO_2-pressurized backpack sprayer calibrated to deliver 700 L ha^{-1} spray solution at 200 kPa utilizing a DG 8003VS nozzle (TeeJet Technologies, Wheaton IL), with the exception of eugenol which was applied at 280 L ha^{-1} to meet label recommendations (Agro Research International 2022). The study was a randomized complete block design with 6 replications, and the study was repeated twice separated in time. Data collected included visual stevia injury at 1, 3, 7, 14, and 28 d after treatment (DAT) with 0% representing no injury and 100% representing plant death (Frans et al. 1986). Yield was determined for each treatment by cutting plants 1 cm above the soil surface 28 DAT, drying them at 70 C for three d, and then measuring dry weights.

Field Study

Field studies were conducted at the Horticultural Crops Research Station in Clinton (35.023°N, 78.280°W) and Castle Hayne (34.321°N, 77.9217°W) North Carolina in 2021. Soils in Clinton and Castle Hayne were a fine-loamy, kaolinitic, thermic Typic Kandiudults with 2.4% silt and pH 6.7, and coarse-loamy, siliceous, semiactive, thermic Aeric Paleaquults with 13.6% silt and pH 6.2, respectively. Stevia seeds (Johnny’s Selected Seeds, Winslow, ME) were seeded into 50-cell trays containing potting mix (Fafard 4P, Conrad Fafard Inc., Agawam, MA) and then allowed to germinate and grow in a greenhouse for 2 mo. To establish stevia in the field, raised beds spaced 3.02 m apart were formed, polyethylene drip irrigation lines installed, covered in white polyethylene mulch and holes were punched at a 0.3 m in-row spacing. Stevia plugs were then transplanted by hand. Plots consisted of one row 12.2 m long, where the first half consisted of stevia maintained weed-free and the second half was not transplanted with stevia but was seeded with Palmer amaranth. Weeds were terminated four wk after treatment (WAT) to prevent confounding competition with the stevia.

Treatments consisted of organic herbicides (Table 1) directed to the lower third of the stevia (two passes, one to each side) and applied over-the-top of the weeds. Boom height was held constant for both halves of the plot. In addition, a nontreated check was included for comparison. All treatments were applied 2 WAP with a CO_2-pressurized backpack sprayer calibrated to deliver 700 L ha^{-1} spray solution at 200 kPa utilizing a DG 8003VS nozzle (TeeJet 8003; TeeJet Technologies, Wheaton IL) with the exception of eugenol which was applied at 280 L ha^{-1} (Agro Research International 2022). Treatments were arranged in a randomized complete block with four replications. Data collection included visual stevia injury (2 and 6 WAT) and weed control (1, 2, and 4 WAT) on a scale of 0 to 100% with 0% being no injury and 100% being plant death (Frans et al. 1986). Stevia was harvested on August 8 and September 10, 2021 in Castle Hayne and Clinton, respectively. Yield was collected by cutting plants 1 cm above the soil surface, drying them at 71 C for three d, and then measuring dry weight.

Statistical Analysis

For both the greenhouse and field studies, data were subjected to ANOVA using PROC MIXED in SAS version 9.4 (SAS Institute Inc., Cary, NC). Residuals were plotted to inspect homogeneity of variance. Herbicide treatment and experimental run were treated as fixed effects while replication nested within experimental run was considered a random effect. Means were separated using Fishers protected LSD ($\alpha = 0.05$). Injury and weed control data from the field study were transformed using arcsine square root transformations and back transformed for presentation.

Results and Discussion

Greenhouse Study

Injury was observed as necrosis. At 3 DAT, caprylic acid plus capric acid, pelargonic acid, acetic acid, and ammonium nonanoate caused >45% injury, with caprylic acid plus capric acid and ammonium nonanoate causing the greatest crop injury (> 60%) (Table 2). Although stevia regrowth occurred, injury from these herbicide treatments was still substantial by 28 DAT, with little change from 3 DAT for the majority of the treatments. Eugenol was a notable exception resulting in a 22% increase in injury from 3 to 28 DAT. Citric acid, ammoniated soap of fatty acids, and clove oil + cinnamon oil caused no more than 18% stevia injury at 3 and 28 DAT. Eugenol and d-limonene caused no more than 30% injury at 3 and 28 DAT.

Stevia yield was reduced by all herbicide treatments when compared to the nontreated check (Table 2). Consistent with the observed injury, caprylic acid plus capric acid, pelargonic acid, acetic acid, eugenol, and ammonium nonanoate reduced yield > 40% compared to the nontreated check. Citric acid and clove oil + cinnamon oil were the least injurious and reduced yield 16 to 20%, respectively. These results suggest these products are too injurious to be applied over-the-top of stevia.

Field Study

Weed control

D-limonene, pelargonic acid, ammonium nonanoate, and ammoniated soap of fatty acids all controlled Palmer amaranth (2 to 4 lf) > 90% 1 WAT (Table 4). However, citric acid, acetic acid and eugenol did not provide adequate control of Palmer amaranth (< 65%). In addition, the application of d-limonene and pelargonic acid resulted in > 90% control of annual sedge (*Cyperus compressus*). These results are similar to Abouziena et al. (2009), who reported that citric acid provided ≤ 25% control of sedges. At 2 WAT acetic acid resulted in similar Palmer amaranth control (70%) as the broadleaf weed control reported by Abouziena et al. (2009). In prior research, clove oil applied alone resulted in minimal weed control for most broadleaf and grasses (Abouziena et al. 2009), however in our studies clove oil + cinnamon oil resulted in 98 and 75% Palmer amaranth and annual sedge control 1 WAT, respectively. Although none of the herbicide treatments have residual control, by 4 WAT caprylic acid + capric acid, clove oil + cinnamon oil, pelargonic acid, ammonium nonanoate, ammoniated soap of fatty acids, and d-limonene still provided ≥ 75% Palmer amaranth control.

Crop Injury

There was a significant interaction between experimental runs (P = 0.02). However, after evaluation, the interaction was determined to be biologically uninformative; therefore, data were pooled across experimental runs. Injury was primarily characterized by contact necrosis. However, eugenol caused slight chlorosis. Similar to injury reported in peppers by Evans et al. (2011), more injurious chemicals such as pelargonic and acetic acid caused necrosis at the plant stem which resulted in stem girdling.

At 2 WAT, caprylic acid + capric acid, pelargonic acid, and ammonium nonanoate caused > 30% injury. Clove oil + cinnamon oil and eugenol caused < 10% injury. D-limonene, citric acid, acetic acid, and ammoniated soap of fatty acids caused 18 to 25 %injury 2 WAT. By 6 WAT substantial stevia regrowth and recovery occurred resulting in < 20% injury for all treatments. In particular, clove oil + cinnamon oil, citric acid, and eugenol all caused < 5% injury. However, substantial stunting was observed with caprylic acid + capric acid, pelargonic acid, d-limonene, and ammonium nonanoate all causing > 25% stunting. All other treatments caused ≤18% stunting (Table 3).

Crop Yield

The treatment-by-location interaction was not significant for stevia yield; therefore, data from both locations were combined for analysis. Despite being injurious, organic herbicides did not cause a reduction in yield relative to the nontreated check (Table 3). This is likely a result of harvesting at 120 d. Stevia is able to regrow within the same season and can be harvested more than once within a year. Based upon yield data, these herbicides have potential for use in stevia; however, for established stevia these products could delay harvest. Caution should be taken before applying the majority of these organic herbicides on established stevia, if an early harvest date is desired.

Injury to stevia from clove oil + cinnamon oil was similar to that reported by O'Sullivan et al. (2015) in tomato, corn, and pepper. The application of clove oil + cinnamon oil over-the-top resulted in <10% injury 28 DAT in the greenhouse and (3%) 6 WAT POST-directed in the field. In addition, it provided excellent control of Palmer amaranth (2 to 4 lf) (Table 4). Clove oil + cinnamon oil may have potential use for early season weed management for organic production systems. Future research is needed to explore the application of clove oil + cinnamon oil applied at later growth stages of stevia than this study's treatment timing followed by stevia harvest at various times. In addition, efficacy should be evaluated for larger weeds.

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Table 1. Herbicide treatments in stevia studies in the Marye Ann Fox greenhouse and in the field at Clinton and Castle Hayne, NC in 2021.

Active ingredient	Trade name	Concentration	Rate	Manufacturer	City, State	Website
		% v/v	L ha^{-1}			
Caprylic acid + capric acid	Homeplate	6.25	43.75	Certis Biologicals	Columbia, MD	www.certisbio.com
Clove oil + cinnamon oil[a]	Weed Zap	5	35	JH Biotech, Inc.	Ventura, CA	www.jhbiotech.com
D-limonene	Avenger	14	98	Avenger Products, LLC	Buford, GA	www.avengerorganics.com
Acetic acid 200 grain	Vinagreen	100	700	Fleischmann's Vinegar Company, Inc.	Cerritos, CA	www.fleischmannsvinegar.com
Citric acid	Ablaze	20	140	Soil Technologies Corp.	Fairfield, IA	www.soiltechcorp.com
Pelargonic acid	Scythe	5	35	Gowan Company	Yuma, AZ	www.gowanco.com
Eugenol	Weed Slayer	1.1	7.7	Agro Research International	Sorrento, FL	www.agroresearchinternational.com
Ammonium nonanoate	AXXE	12.5	87.5	BioSafe Systems LLC	Hartford, CT	www.biosafesystems.com
Ammoniated soap of fatty acids	FinalSan	10	70	Neudorff	Brentwood Bay, BC	www.neudorffpro.org

[a]Nonionic surfactant (Kinetic; Helena Agri-Enterprises, LLC, Collierville TN) was included at 0.25% v/v.

Table 2. Stevia injury and yield (dry above ground biomass) from organic herbicide treatments applied at the Marye Anne Fox Science greenhouse, Raleigh, NC in 2021. [a]

Treatment[b]	Concentration	Rate	3 DAT	28 DAT	Yield
	% v/v	L ha^{-1}	—Injury (%)[c,d]—		g $plant^{-1}$
Nontreated	-	-	-	-	17 a
Caprylic acid + capric acid	6.25	43.75	63 a	49 b	8.3 ef
Clove oil + cinnamon oil	5	35	8 de	8 ef	13.5 bc
D-limonene	14	98	27 c	27 cd	11.7 cd
Citric Acid	20	140	7 e	5 f	14.2 b
Acetic acid 200 grain	100	700	48 b	41 b	9.7 def
Pelargonic acid, related fatty acids	5	35	51 b	44 b	9.6 def
Eugenol	1.1	7.7	8 de	30 c	8.3 ef
Ammonium nonanoate	12.5	87.5	65 a	60 a	7.9 f
Ammoniated soap of fatty acids	10	70	18 cd	17 de	10.5 de

[a]Means within a column followed by the same letter are not significantly different according to Fishers protected LSD ($\alpha = 0.05$).
[b]Data were pooled across locations. The nontreated check was not included in crop injury analysis because crop injury was 0% and therefore had a variance of 0.
[c] Stevia injury was collected at 3 and 28 d after transplanting (DAT). Injury is a sum of chlorosis and necrosis.
[d]Rating scale: 0 being no injury and 100% being plant death.

Table 3. Effect of POST organic herbicides on annual sedge and Palmer amaranth control in Clinton and Castle Hayne, NC in 2021.[a,b]

Herbicide[c]	Concentration	Rate	Annual sedge 1 WAT	Palmer amaranth 1 WAT	Palmer amaranth 2 WAT	Palmer amaranth 4 WAT
			Control (%)[d]			
	% v/v	L ha^{-1}				
Nontreated	-	-	-	-	-	-
Caprylic acid + capric acid	6.25	43.75	89 ab	95 a	88 ab	81 ab
Clove oil + cinnamon oil	5	35	75 ab	98 a	97 a	95 a
D-limonene	14	98	98 a	95 a	93 a	75 ab
Citric acid	20	140	25 d	44 b	34 c	24 c
Pelargonic acid + related fatty acids	5	35	94 ab	98 a	98 a	93 a
Ammonium nonanoate	12.5	87.5	81 ab	98 a	97 a	91 ab
Ammoniated soap of fatty acids	10	70	81 ab	97a	97 a	92 a
Acetic acid 200 grain	100	700	64 bc	62 b	70 b	63 b
Eugenol	1.1	7.7	38 cd	15 c	2 c	24 c

[a]Data were pooled across locations. The nontreated check was not included in analysis because control was 0% and therefore had a variance of 0.
[b]Means within a column followed by the same letter are not significantly different according to Fishers protected LSD ($\alpha = 0.05$).
[c]Rating scale: 0 = no control and 100% = control.
[d]Herbicides were applied over-the-top of the weeds.

Table 4. Effect of organic herbicides on stevia injury, stunting, and yield in Clinton and Castle Hayne, NC in 2021.[a,b]

Herbicide	Concentration	Rate	Injury[c] 2WAT	Injury[c] 6 WAT	Stunting 6 WAT	Yield
	% v/v	L ha^{-1}	%	%	%	kg ha^{-1}
Nontreated	-	-	-	-	-	2597 a
Caprylic acid + capric acid	6.25	43.75	31 ab	16 ab	43 a	2044 a
Clove oil + cinnamon oil	5	35	9 de	3 d	2 d	2145 a
D-limonene	14	98	20 bc	11 bc	25 b	2709 a
Citric acid	20	140	18 cd	4 d	6 cd	2539 a
Pelargonic acid + related fatty acids	5	35	34 a	18 a	54 a	2866 a
Ammonium nonanoate	12.5	87.5	35 a	18 ab	51 a	2148 a
Ammoniated soap of fatty acids	10	70	21 bc	6 cd	9 bcd	2317 a
Acetic acid 200 grain	100	700	23 abc	11 bc	18 bc	2391 a
Eugenol	1.1	7.7	10 e	3 d	1 d	2971 a

[a]Data were pooled across locations. The nontreated check was not included in crop injury and stunting analysis because injury or stunting was 0% and therefore had a variance of 0.[b] Means within a column followed by the same letter are not significantly different according to Fishers protected LSD ($\alpha = 0.05$).
[c]Rating scale: 0 being no injury and 100% being plant death. Injury is the sum of chlorosis and necrosis.

CHAPTER 2

Response of stevia (*Stevia rebaudiana*) to conventional herbicides applied post-transplant

(In the format appropriate for submission to Weed Technology)

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Abstract

Stevia is susceptible to weed competition, particularly early in the season. Studies were conducted to determine the effect of conventional herbicides applied over-the-top of stevia. Treatments included *S*-metolachlor, acifluorfen, linuron, halosulfuron, ethalfluralin, carfentrazone, pendimethalin, metribuzin, trifloxysulfuron, and pyroxasulfone applied 2 wk after transplanting (WAP). At 1 wk after treatment (WAT), aciflourfen, metribuzin and carfentrazone caused 30 to 45% injury across both experimental runs. In contrast across both runs at 1 WAT, *S*-metolachlor, linuron, halosufluron, ethalfluralin, pyroxasulfone, pendimethalin, and tryfloxysulfuron caused <20% injury to stevia. By 4 WAT injury to stevia from all treatments was ≤ 19%, except metribuzin and trifloxysulfuron which was 84 and 69%, respectively. *S*-metolachlor, linuron, ethalfluralin, pendimethalin and pyroxasulfone did not cause a significant reduction in above ground biomass from the nontreated check 28 DAT. Below ground biomass was not impacted by the application of linuron, ethalfluralin, pendimethalin, and pyroxasulfone. Linuron, pendimethalin, and ethalfluralin may provide new modes of action for in-season 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: *Stevia rebaudiana* (Bertoni);

Key Words: Herbicides; Stevia; weed management; *S*-metolachlor; Acifluorfen; Linuron; Halosulfuron; Ethalfluralin; Carfentrazone; Pendimethalin; Metribuzin; Trifloxysulfuron; Pyroxasulfone

Stevia (*Stevia rebaudiana* Bertoni) is a relatively new crop in the US known for being 200 to 400 times sweeter than sugar (Lester 1999, FDA 2018). Stevia is a member of the Asteraceae family native to Paraguay, and has a long history of human consumption (Ramesh et al. 2006; PCSI 2017); however, in the US stevia was not approved for consumption in food and beverages until December of 2008 (ISO 2001; Cavaliere 2009). With the FDAs’ approval of stevia as a food additive, interest in stevia has increased in the US (Cavaliere 2009). Several companies including Coca-Cola (Truvia) and Pepsi (PureVia) have released stevia products into the market (Cavaliere 2009).

Stevia is a perennial plant with an upright growth habit, which can be harvested more than once a season depending on region and age of the stevia (Ramesh et al. 2006, Koehler 2018). It is typically planted early in the season (April-May) and has a field life of 3-5 years (Koehler 2018). Stevia is sensitive to weed pressure, especially early in the season (Ramesh et al. 2006, Azimah et al. 2018, Chriest 2019). Research conducted by Harrington et al. (2011) found that hand weeding increased stevia yield by 30-fold as compared to a weedy check. However, few herbicides have been registered for use in stevia (Chriest 2019, Harrington et al. 2011). Paraquat, linuron, and roundup are registered for use outside of the season. Clethodim, carfentrazone, and *S*-metolachlor are the only herbicides registered for use POST-transplant. Carfentrazone is registered for use in row middles.

Globally the number of herbicide resistant weed populations has increased dramatically since the 1970s (Heap 2022). The addition of more herbicides may provide growers with more options for controlling resistant weed populations. Thus, studies were conducted to evaluate crop safety of herbicides not registered for use post-transplant in stevia.

Material and Methods

Stevia seeds (Johnny's Selected Seeds, Winslow, ME) were planted into 50-cell trays in Method Road Unit 1 Greenhouse (35.788° N, 78.694° W) fall 2021. Stevia plugs were then transplanted into 6.2 L (diameter 25.4 cm, height 18.4 cm) round pots containing Sun Gro propagation mix (Sun Gro Horticulture Distribution Inc., Agawam, MA). The study was a randomized complete block design with 7 replications and repeated twice separated by time. Stevia plants were blocked by height pre-treatment. 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 of stevia with a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at 200 kPa. The boom was fitted with 2 flat fan XR 8003VS nozzles (TeeJet 8003; TeeJet Technologies, Wheaton IL).

Data collection included stevia foliar injury at 7, 14, 21, and 28 d after treatment (DAT) with 0% being no injury and 100% being plant death (Frans et al. 1986). Above ground biomass was collected at 21 and 28 DAT and then dried at 49 C for 3 d. Twenty-one DAT stevia roots were removed and washed, and then analyzed using WinRHIZO root scanning system (Regent Instruments Inc., Montreal, PQ, Canada) for root volume and projected root surface area. To quantify below ground biomass the roots were then dried for 1 d at 49 C and weighed.

Data Analysis

Data was subjected to ANOVA utilizing the PROC MIXED procedure SAS version 9.4 (SAS Institute Inc., Cary, NC). To visually examine homogeneity of variance residuals were plotted. Herbicide treatment and experimental run were treated as a fixed effect, while replication was treated as random effect. Means were separated utilizing Fishers protected LSD ($\alpha = 0.05$). Stevia injury data were transformed with an arcsine square root transformation and then back transformed for reported least squared means. Projected root surface area, root volume, and root biomass were transformed with a square root transformation for analysis and then back transformed for presented least squared means.

Results and Discussion

Injury

There was a significant interaction between treatment by experimental runs; therefore, data were separated by experimental run for analysis and presentation. Injury from the majority of the herbicides treatments appeared as chlorosis and necrosis. In particular, carfentrazone, aciflurofen, linuron, and metribuzin caused characteristic foliar necrosis. The application of halosulfuron resulted in chlorosis and necrosis at the meristem. Pyroxasulfone caused slight chlorosis along the leaf edges. Trifloxysulfuron caused initial chlorosis followed by necrosis and plant death. At 1 wk after treatment (WAT), aciflurofen, carfentrazone, and metribuzin all caused ≤ 30% injury. By 4 WAT, injury from aciflurofen and carfentrazone was reduced to 19 and 14%, respectively. In contrast, injury from metribuzin increased to 84% by 4 WAT. In prior research metribuzin applied at 350 g ai ha^{-1} resulted in 48% injury 2 WAT (Harrington et al. 2011). In the first experimental run, linuron and metribuzin caused 20 and 41% injury and in the second 5 and 30%, respectively, 1 WAT. By 4 WAT injury to stevia treated with linuron was 6%. Prior research has shown stevia to have some tolerance to linuron (Hopkins & Midmore 2015); however, when applied at a higher rate linuron has been shown to cause as high as 42% injury to stevia in a greenhouse setting (Harrington et al. 2011). The application of halosulfuron and trifloxysulfuron resulted in 16% injury in the first experimental run and 14 and 19% in the second run, respectively, 1 WAT. By 4 WAT the injury from halosulfuron was reduced to 7%; however, injury from trifloxysulfuron increased to 69%. Treatment with *S*-metolachlor, pyroxasulfone, and pendimethalin resulted in ≤11% injury at 1 WAT and ≤4% by 4 WAT.

Above ground biomass

Due to a lack of experimental run-by-treatment interaction, data for above biomass was combined across both runs. Above ground biomass collected at 21 DAT was generally higher than that collected at 28 DAT. At 21 DAT ethalfluralin and pendimethalin did not cause a significant reduction from the nontreated check (Table 3). By 28 DAT the stevia had further recovered from the herbicide treatment, as a result *S*-metolachlor, linuron, ethalfluralin, pendimethalin and pyroxasulfone did not cause a reduction in above ground biomass. Harrington et al. (2011) also reported a non-significant reduction in above ground biomass for stevia treated with linuron at 900 g ai ha^{-1} in the greenhouse. However, a significant reduction in above ground biomass was observed for stevia treated with metribuzin. At a lower rate (350 g ai ha^{-1}) Harrington et al. (2011) did not see a significant reduction in above ground biomass from the nontreated check for stevia treated with metribuzin. Our results support those of Hopkins and Midmore (2015) in which they did not observe a reduction in stevia above ground biomass from treatment with pendimethalin.

Projected root surface area, root volume, and below ground root biomass

For the projected root surface area and root volume there was a significant interaction between treatment by experimental runs; therefore, data were separated by experimental run for analysis and presentation. For below ground root biomass, there was not a significant interaction between treatment by experimental runs, therefore data were pooled. In the first experimental run the application of ethalfluralin, pendimethalin, and pyroxasulfone did not cause a significant reduction in projected root surface area compared to the nontreated check (Table 4). In the second experimental run linuron, and ethalfluralin did not cause a significant reduction in projected root surface area from the nontreated check. Root volume followed a similar trend in, in which for the first experimental run ethalfluralin and pendimethalin also did not cause a significant reduction from the nontreated. However, in contrast to projected root surface area in the first run, there was a significant reduction in root volume from the application of pyroxasulfone. Root volume in the second experimental run was not impacted by treatment with linuron, ethalfluralin, and pyroxasulfone. Similar to projected root surface area and root volume, below ground biomass was not impacted by linuron, ethalfluralin, pendimethalin, and pyroxasulfone (Table 4).

At present, the only options for weed control in stevia post-transplant are *S*-metolachlor clethodim, and carfentrazone. However, application of linuron, pendimethalin, ethalfluralin, and pyroxasulfone did not cause a reduction in above or below ground biomass. In prior research at 1 WAT, linuron has been shown to provide as high as 98% Palmer amaranth control (Moore et al. 2021). Linuron, pendimethalin, and ethalfluralin may provide new modes of action for in-season weed management in stevia. However, further research is needed to evaluate the effect of these herbicides on stevia growth and quality in the field. In addition, the soil type utilized in this study contains a high concentration of organic matter. Thus, future research is needed to determine the effect of herbicides with residual control on stevia growth when applied in field soils with lower concentrations of organic matter.

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Table 1. Herbicide treatments applied to stevia in the Method Road Unit 1 greenhouse 2 wk after transplanting in 2021.

Active ingredient	Trade name	Rate	Manufacturer	City, State	Website
		g ai/ha			
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 (Kinetic; Helena Agri-Enterprises, LLC, Collierville TN) was included at 0.25% v/v.

Table 2. Injury from herbicides applied over-the-top to stevia 2 wk after transplanting in the Method Road Unit 2 Greenhouse, Raleigh, NC in 2021.

		1 WAT[a]		4WAT[b]
Treatment[c]	Rate	Run1	Run2	
	g ai/ha	Injury (%)[d]		
Nontreated	-	-	-	-
S-metolachlor	1070	1 d	1 f	1 f
Acifluorfen	280	35 a	32 a	19 c
Linuron	560	20 b	5 e	6 de
Halosulfuron	26.3	16 b	14 cd	7 de
Ethalfluralin	1260	3 cd	1 f	0 f
Pyroxasulfone	59.5	7 c	11 d	4 e
Pendimethalin	800	1 d	0	3 f
Metribuzin	420	45 a	30 ab	84 a
Trifloxysulfuron	5.5	16 b	19 bc	69 b
Carfentrazone	17.5	41 a	36 a	14 cd

[a]At 1 WAT (wk after treatment), the interaction between treatment and experimental run was significant; therefore, data were separated by experimental run.
[b]At 4 WAT, the interaction between treatment and experimental run was not significant; therefore, data were pooled across experimental runs.
[c]Means within a column followed by the same letter are not significantly different according to Fishers protected LSD ($\alpha = 0.05$). The nontreated check was not included in crop injury and stunting analysis because injury or stunting was 0% and therefore had a variance of 0. Data was transformed using a square root transformation for analysis, and then back transformed for reported least squared means.
[d]Rating scale: 0 being no injury and 100% being plant death. Injury is a sum of chlorosis, necrosis, and stunting.

Table 3. Effect of herbicides on stevia dry above ground biomass at 21 and 28 DAT applied in Method Road Unit 1 Greenhouse, Raleigh, NC in 2021.[a]

Herbicide[b]	Rate	21 DAT[c]	28 DAT
	g ai/ha	——— 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] Data were pooled across experimental runs.
[b] Treatments were applied over-the-top of stevia 2 wk after transplanting.
[c] Means within a column followed by the same letter are not significantly different according to Fishers protected LSD (α = 0.05).

Table 4. Effect of herbicides on stevia projected root surface area, root volume, and below ground root biomass in Method Road Unit 2 Greenhouse, Raleigh, NC 21 DAT in 2021.[a]

Treatment[b]		Projected root surface area		Root volume		Root biomass
	Rate	Run1	Run2	Run1	Run2	
	g ai/ha	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; therefore, data were separated by experimental run. Data was transformed using a square root transformation for analysis, and then back transformed for reported least squared means. Plant roots were harvest 21 d after treatments.

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