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Acetic acid; ammoniated soap of fatty acids; ammonium nonanoate; caprylic acid plus capric acid; clove oil plus cinnamon oil; d-limonene; citric acid; eugenol; pelargonic acid; *Amaranthus palmeri* S. Watson; stevia, *Stevia rebaudiana* Bertoni

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Response of stevia to reduced-risk synthetic and nonsynthetic herbicides applied post-transplant

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Abstract

Greenhouse trials were conducted to determine the response of stevia to reduced-risk synthetic and nonsynthetic herbicides applied over-the-top post-transplant. In addition, field trials were conducted with stevia grown in a polyethylene mulch production system to determine crop response and weed control in planting holes to reduced-risk synthetic and nonsynthetic herbicides applied post-transplant directed. 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. Stevia yield (dry aboveground biomass) in the greenhouse was reduced by all herbicide treatments. Citric acid and clove oil plus cinnamon oil were the least injurious, reducing yield by 16% to 20%, respectively. In field studies, d-limonene, pelargonic acid, ammonium nonanoate, and ammoniated soap of fatty acids controlled Palmer amaranth (>90% 1 wk after treatment (WAT). In field studies caprylic acid plus capric acid, pelargonic acid, and ammonium nonanoate caused >30% injury to stevia plants at 2 WAT, and 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, herbicides applied in the field did not reduce yield compared to the nontreated check. Based upon yield data, these herbicides have potential for use in stevia; however, these products could delay harvest if applied to established stevia. In particular, clove oil plus cinnamon oil has potential for use for early-season weed management for organic production systems. The application of clove oil plus cinnamon oil over-the-top resulted in <10% injury 28 d after treatment (DAT) in the greenhouse and 3% injury 6 WAT postemergence-directed in the field. In addition, this treatment provided 95% control of Palmer amaranth 4 WAT.

Introduction

Stevia is used to produce a zero-calorie sweetener, containing steviol glycosides, which are 200 to 400 times sweeter than sucrose (FDA 2018; Lester 1999). As a result, it serves as an excellent sugar substitute, especially for diabetics (Mishra et al. 2011). Stevia has been consumed as a sweetener for hundreds of years (PCSI 2017). 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 during 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 up to 25%, and weed control can increase production costs (Taak et al. 2021). Stevia is particularly vulnerable to weed competition early in the season (Azimah et al. 2018; Chriest 2019). Azimah et al. (2018) reported that the critical period for weed control for stevia in the greenhouse was 1 to 4 wk after planting for a mixture of dicotyledonous and monocotyledonous weeds. Few herbicides are registered for use in stevia (Chriest 2019; Harrington et al. 2011). Ethalfluralin may be applied pre-transplant incorporated for residual weed control; however, *S*-metolachlor and clethodim are the only conventional herbicides registered for use in postemergence-transplanting over-the-top of stevia (Chriest 2019). As a result, postemergence weed control options are limited in stevia. Nonsynthetic herbicides may be applied in stevia; however, these herbicides have not been evaluated to determine if injury from these herbicides will significantly affect stevia yield.

In organic production systems, chemical weed control options are limited to biological or botanical (nonsynthetic) herbicides for food crops and herbicidal soaps (synthetic) that can be used only for maintenance of noncrop areas of the farm and for fields used only for ornamental crops. In organic production systems, a biological or botanical substance (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.), stranglervine (*Morrenia odorata* Lindle), and black nightshade (*Solanum nigrum* Linn.) (Abouziena et al. 2009). Cinnamon oil plus clove oil provided as much 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). The herbicides that are permitted for use in organic production are nonselective and provide no residual weed control (Evans et al. 2011; Liu et al. 2021). As a result, over-the-top applications can cause significant crop injury (Evans et al. 2011; Liu et al. 2021). Additionally, organic herbicides are more efficacious when applied to small weeds and may require sequential applications to achieve effective control (Abouziena et al. 2009; Liu et al. 2021). However, directed applications can require less herbicide, which can reduce the cost of applying nonsynthetic herbicides. Prior research has shown that directed applications within the crop canopy of nonsynthetic herbicides provided effective weed control in bell pepper and broccoli (*Brassica oleracea* L. var. italica) (Evans et al. 2011).

Prior studies have examined the effects of directed applications of nonsynthetic herbicides in other crops (Evans et al. 2011); however, to our knowledge no peer-reviewed research has evaluated nonsynthetic or reduced-risk synthetic herbicides in stevia. In addition, Although polyethylene mulch can reduce weed pressure, weeds within the planting holes may affect crop yield; characterization of weed control from reduced-risk synthetic and nonsynthetic herbicides would assist organic growers in deciding whether or not to apply reduced-risk synthetic and nonsynthetic herbicides. Therefore, greenhouse and field studies were conducted to determine the effect of reduced-risk synthetic and nonsynthetic herbicides applied over-the-top and postemergence-directed to transplanted stevia in a polyethylene mulch production system, respectively.

Materials and Methods

Greenhouse Study

Greenhouse trials were conducted at the Marye Anne Fox Science Teaching Laboratory (35.787°N, 78.674°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). Stevia did not receive supplemental light; greenhouse temperature ranged from 18 C to 24 C. The plants were hand-watered twice daily to maintain consistent soil moisture. Treatments consisted of reduced-risk synthetic and nonsynthetic herbicides (Table 1) applied over-the-top of stevia 4 WAT 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 instructions (Agro Research International 2022). The study was arranged in a randomized complete block design with six replications, and the study was repeated twice with two experimental runs that were separated in time. Data collected included visible stevia injury at 3 and 28 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 3 d, and then measuring dry weights.

Field Study

Field trials were conducted under conventional production practices at the Horticultural Crops Research Station in Clinton (35.023°N, 78.280°W) and Castle Hayne (34.321°N, 77.9217°W), NC, in 2021. Soils in Clinton and Castle Hayne were a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) with 2.4% silt and pH 6.7, and Stallings fine sand (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 (110 mL) 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 1.5-m beds spaced 3.02 m apart were formed, polyethylene drip irrigation lines were installed, and the beds covered in 0.25 mm thick white on black polyethylene mulch (TriEast Ag Group, Greenville, SC). Stevia plugs were transplanted by hand May 10 in Clinton and Castle Hayne, NC, at a density of 0.3 plants m^{-1} of row. Plots consisted of one row 12.2 m longer, in which the first 6.1 m consisted of stevia maintained weed free and the second 6.1 m consisted of holes punched into the plastic to allow weeds to emerge. Weedy sections were seeded at stevia transplanting with Palmer amaranth at a rate of 5 to 10 seeds per hole. Due to their proximity to the stevia, weeds in the weedy section of each plot were terminated 4 WAT to prevent confounding competition with the stevia.

Treatments consisted of the herbicides (Table 1) used in the greenhouse study directed to the lower third of the stevia (two passes, one to each side). Weeds were less than 7 cm tall at application and thus were fully covered by the treatment application, as the 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 wk after planting 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). Stevia was 25.4 to 30.5 cm tall at application. Treatments were arranged in a randomized complete block with four replications. Data collection included visible 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 3 d, and then measuring dry weight.

Statistical Analysis

For both the greenhouse and field studies, data were subjected to ANOVA using the MIXED procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). Residuals were plotted to inspect

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	Spray concentration	Percent ai formulated	Manufacturer	City, state	Website
		% v/v	%			
Caprylic acid + capric acid	Homeplate	6.25	44 + 36	Certis Biologicals	Columbia, MD	www.certisbio.com
Clove oil + cinnamon oil[a]	Weed Zap	5	45 + 45	JH Biotech, Inc.	Ventura, CA	www.jhbiotech.com
D-limonene	Avenger	14	70	Avenger Products, LLC	Buford, GA	www.avengerorganics.com
Acetic acid 200 grain	Vinagreen	100	20	Fleischmann's Vinegar Company, Inc.	Cerritos, CA	www.fleischmannsvinegar.com
Citric acid[b]	Ablaze	20	–	Soil Technologies Corp.	Fairfield, IA	www.soiltechcorp.com
Pelargonic acid[c]	Scythe	5	57	Gowan Company	Yuma, AZ	www.gowanco.com
Eugenol	Weed Slayer	1.1	6	Agro Research International	Sorrento, FL	www.agroresearchinternational.com
Ammonium nonanoate	AXXE	12.5	40	BioSafe Systems LLC	Hartford, CT	www.biosafesystems.com
Ammoniated soap of fatty acids	FinalSan	10	22	Neudorff	Brentwood Bay, BC, Canada	www.neudorffpro.org

[a]Nonionic surfactant (Kinetic; Helena Agri-Enterprises, LLC, Collierville TN) was included at 0.25% v/v.
[b]Ablaze does not list percent active ingredient in the formulated product on the label.
[c]Pelargonic acid is not permitted in organic production and thus not OMRI certified. Axxe and FinalSan are OMRI listed but only for use as herbicides for farmstead maintenance and fields used only for ornamental crops.

homogeneity of variance. Herbicide treatment and experimental run were treated as fixed effects, whereas replication nested within experimental run was considered a random effect. Least squared 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

As a significant interaction between experimental runs was not observed, data were pooled across experimental runs. 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 stevia injury from 3 to 28 DAT. Citric acid, ammoniated soap of fatty acids, and clove oil plus 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 plus cinnamon oil were the least injurious and reduced yield 16% to 20%, respectively. These results suggest that all products evaluated 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 (two- to four-leaf) >90% 1 WAT (Table 3). In addition, the application of d-limonene and pelargonic acid resulted in >90% control of annual sedge (*Cyperus compressus* L.). However, citric acid, acetic acid, and eugenol did not provide adequate control of Palmer amaranth and annual sedge (<65%). These results are similar to those of Abouziena et al. (2009), who reported that citric acid provided ≤25% control of sedges. Treatment with either d-limonene or pelargonic acid resulted in ≥94% control of annual sedge 1 WAT. At 2 WAT acetic acid resulted in Palmer amaranth control (70%) similar to 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 plus cinnamon oil resulted in 98% and 75% Palmer amaranth and annual sedge control 1 WAT, respectively. Although none of the herbicide treatments have residual effects, by 4 WAT caprylic acid plus capric acid, clove oil plus cinnamon oil, pelargonic acid, ammonium nonanoate, ammoniated soap of fatty acids, and d-limonene still provided ≥75% Palmer amaranth control.

Crop Injury

There was not an interaction between experimental run and herbicide; therefore, data were pooled across experimental runs. Injury was primarily characterized by contact necrosis. However, eugenol caused slight chlorosis. Similar to injury reported in bell pepper by Evans et al. (2011), more injurious chemicals such as pelargonic and acetic acid caused necrosis at the plant stem, which resulted in some stem girdling.

At 2 WAT, caprylic acid plus capric acid, pelargonic acid, and ammonium nonanoate caused >30% injury. In contrast, clove oil plus cinnamon oil and eugenol caused <10% injury. d-Limonene, citric acid, acetic acid, and ammoniated soap of fatty acids caused 18% to 25% injury by 2 WAT. By 6 WAT substantial stevia regrowth and recovery occurred, resulting in <20% injury for all treatments. In particular, clove oil plus cinnamon oil, citric acid, and eugenol all caused <5% injury. However, substantial stunting was observed, with caprylic acid plus capric acid, pelargonic acid, d-limonene, and ammonium nonanoate all causing 25% to 54% stunting. All other treatments caused ≤18% stunting (Table 3).

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Table 2. Stevia injury and yield (dry above ground biomass) from reduced risk synthetic and nonsynthetic herbicide treatments applied over-the-top of stevia at the Marye Anne Fox Science greenhouse, Raleigh, NC in 2021.[a]

Treatment	Spray concentration	Percent ai formulated	Stevia injury 3 DAT	Stevia injury 28 DAT	Yield
	% v/v	%	%[c,d]		g plant^{-1}
Nontreated[b]	–	–	–	–	17 a
Caprylic acid + capric acid	6.25	44 + 36	63 a	49 b	8.3 ef
Clove oil + cinnamon oil	5	45 + 45	8 de	8 ef	13.5 bc
D-limonene	14	70	27 c	27 cd	11.7 cd
Citric acid	20	20	7 e	5 f	14.2 b
Acetic acid 200 grain	100	–	48 b	41 b	9.7 def
Pelargonic acid, related fatty acids	5	57	51 b	44 b	9.6 def
Eugenol	1.1	6	8 de	30 c	8.3 ef
Ammonium nonanoate	12.5	40	65 a	60 a	7.9 f
Ammoniated soap of fatty acids	10	22	18 cd	17 de	10.5 de

[a]Least squared means within a column followed by the same letter are not significantly different according to Fishers protected LSD (α = 0.05).
[b]Data were pooled across experimental runs. The nontreated check was not included in the crop injury analysis because crop injury was 0% and therefore had a variance of 0.
[c]Stevia injury was assessed 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 reduced risk synthetic and nonsynthetic herbicides applied post transplanted directed to stevia on annual sedge and Palmer amaranth control in Clinton and Castle Hayne, NC in 2021.[a,b]

Herbicide[c]	Spray concentration	Percent ai formulated	Annual sedge control 1 WAT	Palmer amaranth control 1 WAT	Palmer amaranth control 2 WAT	Palmer amaranth control 4 WAT
	% v/v	%	%[d]			
Caprylic acid + capric acid	6.25	44 + 36	89 ab	95 a	88 ab	81 ab
Clove oil + cinnamon oil	5	45 + 45	75 ab	98 a	97 a	95 a
D-limonene	14	70	98 a	95 a	93 a	75 ab
Citric acid	20	20	25 d	44 b	34 c	24 c
Pelargonic acid + related fatty acids	5	–	94 ab	98 a	98 a	93 a
Ammonium nonanoate	12.5	57	81 ab	98 a	97 a	91 ab
Ammoniated soap of fatty acids	10	6	81 ab	97 a	97 a	92 a
Acetic acid 200 grain	100	40	64 bc	62 b	70 b	63 b
Eugenol	1.1	22	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]Least squared means within a column followed by the same letter are not significantly different according to Fishers protected LSD (α = 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 reduced risk synthetic and nonsynthetic herbicides applied post transplanted direct to stevia on crop injury, stunting, and yield in Clinton and Castle Hayne, NC in 2021.[a,b]

Herbicide	Spray concentration	Percent ai formulated	Injury[c] 2WAT	Injury[c] 6 WAT	Stunting 6 WAT	Yield
	% v/v	%	%		%	kg ha^{-1}
Nontreated	–	–	–	–	–	2597 a
Caprylic acid + capric acid	6.25	44 + 36	31 ab	16 ab	43 a	2044 a
Clove oil + cinnamon oil	5	45 + 45	9 de	3 d	2 d	2145 a
D-limonene	14	70	20 bc	11 bc	25 b	2709 a
Citric acid	20	20	18 cd	4 d	6 cd	2539 a
Pelargonic acid + related fatty acids	5	–	34 a	18 a	54 a	2866 a
Ammonium nonanoate	12.5	57	35 a	18 ab	51 a	2148 a
Ammoniated soap of fatty acids	10	6	21 bc	6 cd	9 bcd	2317 a
Acetic acid 200 grain	100	40	23 abc	11 bc	18 bc	2391 a
Eugenol	1.1	22	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]Least squared means within a column followed by the same letter are not significantly different according to Fishers protected LSD (α = 0.05).
[c]Rating scale: 0 being no injury and 100% being plant death. Injury is the sum of chlorosis and necrosis.

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 4). This is likely a result of harvesting later in the season. 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, when applied to 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. In addition, sequential applications of these herbicides may be required for continued weed suppression, which could increase injury as well as add to the cost of production.

Injury to stevia from clove oil plus cinnamon oil was similar to that reported by O'Sullivan et al. (2015) in tomato (*Solanum lycopersicum* L), corn (*Zea mays* L.), and bell pepper. The application of clove oil plus cinnamon oil over-the-top resulted in <10% injury 28 DAT in the greenhouse and 3% injury 6 WAT postemergence-directed in the field. In addition, it provided excellent control of Palmer amaranth (two- to four-leaf) (Table 3). Further evaluation of weed control from these herbicides on other weed species common in stevia is needed. Clove oil plus cinnamon oil may be potentially useful for ear-ly-season weed management for organic production systems; however, because this study was conducted in a conventional production system, additional research is needed to evaluate the effect of these herbicides when applied in an organic production system. Future research is needed to explore the application of clove oil plus cinnamon oil applied at later growth stages of stevia than this study's treatment timing followed by stevia harvest at various maturities. In addition, stevia tolerance to sequential application of organic herbicides should be evaluated.

Practical Implications

At present, there are few options available for weed management in organically grown stevia. Based on the results from this study, several nonsynthetic herbicides could potentially be used to supplement current weed management practices in stevia. In particular, directed applications such as the method used in this study target weeds within the planting holes that are often competitive and difficult to control with current practices.

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Competing Interests. No conflicts of interest have been declared.

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