




Improved herbicide selectivity in tomato by safening action of benoxacor and fenclorim

Edicarlo Castro^{1,2} , Carolina Pucci^{1,2}, Stefano Duarte^{2,3}, Nilda Roma Burgos⁴  and Te Ming Tseng⁵ 

Research Article

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Author for correspondence:

Te-Ming Tseng, Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762.
Email: t.tseng@msstate.edu

¹Graduate Student, Department of Plant Protection, São Paulo State University, Botucatu, SP 18610, Brazil; ²Visiting Research Scholar, Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS, USA; ³Graduate Student, Department of Biosystems Engineering, University of São Paulo, Pirassununga, SP 13635, Brazil; ⁴Professor, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA and ⁵Assistant Professor, Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762 USA

Abstract

Safeners have been widely used to reduce phytotoxicity to crops, thus serving as an alternative weed control strategy. Benoxacor and fenclorim safeners have the potential to protect plants from herbicide phytotoxicity by increasing glutathione S-transferase (GST) activity within the plant. The study aimed to evaluate the safening effect of benoxacor and fenclorim on tomato against selected herbicides applied POST. The experiment was conducted in a greenhouse in a completely randomized designed with four replications in a 9×3 factorial scheme, where Factor A consisted of eight herbicides including a nontreated control, and Factor B consisted of two safeners including a nontreated control. The herbicide treatments were sulfentrazone (0.220 kg ai ha⁻¹), fomesafen (0.280 kg ai ha⁻¹), flumioxazin (0.070 kg ai ha⁻¹), linuron (1.200 kg ai ha⁻¹), metribuzin (0.840 kg ai ha⁻¹), pyroxasulfone (0.220 kg ai ha⁻¹), and bicyclopyrone (0.040 kg ai ha⁻¹). Safener treatments consisted of benoxacor (0.67 g L⁻¹) and fenclorim (10 μM). Tomato seeds were immersed in safener solution before sowing and herbicides were applied when tomato plants were at the 3-leaf stage, or 25 days after sowing. Visible injury was scored at 3, 7, 14, and 21 d after application (DAA), and shoot biomass was recorded 21 DAA. Seed treatment with fenclorim reduced injury caused by imazamox and bicyclopyrone by 5.5 and 1.3 times, respectively, whereas benoxacor reduced the injury from bicyclopyrone 1.3 times. In addition, tomato plants pretreated with fenclorim showed a lesser reduction in biomass after application of imazamox, fomesafen, and metribuzin, whereas plants pretreated with benoxacor showed lesser biomass reduction after metribuzin application. Thus, the use of safeners promotes greater crop selectivity, allowing the application of herbicides with different mechanisms of action on the crop.

Introduction

The United States produces 2,707 million pounds of fresh-market tomato and 29,509 million pounds of processed tomato annually (USDA 2016). In Mississippi, tomato is grown on more than 444 acres across 627 farms, with gross sales of more than \$6 million annually (USDA 2012). A tomato crop favors the occurrence of weeds during its growing cycle due to wide plant spacing and slow development in the initial weeks after transplanting or seeding (Ronchi et al. 2010). Moreover, it is sensitive to many herbicides, and weed management is a significant constraint in tomato production (Ormeño et al. 2003). There are reports of reduced fruit yield due to interference caused by weeds (Ronchi et al. 2010). Yellow nutsedge can cause a 28% reduction in tomato dry weight (Morales-Payan et al. 2003), whereas yield reductions of up to 77% were observed with *Chenopodium album*, *Ambrosia artemisiifolia*, *Amaranthus* spp., and *Digitaria sanguinalis* (Weaver and Tan 1983). Stall and Morales-Payan (2003) observed a 50% and 81% decrease in the yield of transplanted tomato due to the infestation of *Cyperus esculentus* (50 plants m⁻²) and *Cyperus rotundus* (100 plants m⁻²), respectively. The physiological similarity between species is an essential factor to be considered in competition with weeds; the more similar their physiological characteristics, the more intense the competition suffered by the crop (Ronchi et al. 2010).

Therefore, a critical need exists to improve current weed management techniques. One of the potential methods is the use of safeners to provide selective weed control. The use of safeners improves herbicide selectivity on some crops, thus providing an additional weed control strategy to farmers. Safeners are compounds used to protect seeds of crop species from an otherwise phytotoxic herbicide (Galon et al. 2011). Safeners have been widely used to achieve herbicide selectivity in dicotyledonous plants; for example, 1,8-naphthalic anhydride protected beans in field experiments from EPTC damage (Blair 1979); soybean was protected by growth regulators,

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fungicides, and other agrochemicals from metribuzin injury (Phatak and Varvina 1989); cucumber pretreated with naptalam protected against chloramben injury (Knerr and Hopen 1989); and cotton seeds treated with disulfoton or dietholate were protected from clomazone damage (Yazbek and Foloni 2004). In *Arabidopsis*, all tested safeners increased the activity of GST, but the response was dependent on the type of protector to which the seedlings were exposed and the substrate used for GST activity assays (DeRidder et al. 2002). The GSTs and P450s enhance the defense system of plants, and upon being induced by the presence of safeners, they reduce or eliminate the phytotoxic effects of the herbicide (Riechers et al. 2010).

In tomato plants the GST genes are present in a higher number than in other plants such as *Arabidopsis* (1.6 times), sweet orange (3.8 times), lettuce (3.2 times), cotton (1.5 times), and *Capsella rubella* (1.8 times; Dong et al. 2016; He et al. 2016; Rezaei et al. 2013; Sappl et al. 2009; Yang et al. 2014). The fact that GST from *Arabidopsis* is induced by herbicide safening agents suggests that the process of recognition, signaling, and activation of genes required for the protective mechanism is present in dicotyledonous plants as well as in cereals (DeRidder et al. 2002). In maize (*Zea mays*), the expression of a GST gene protects the plant from the chloroacetanilide herbicides (i.e., alachlor, metolachlor) by encoding an enzyme lacking peroxidase activity (Karavangeli et al. 2005).

Numerous compounds have been screened as potential safeners, including fenclorim, benoxacor, flurazole, dichlormid, oxabtrinil, and fluxofenim (Galon et al. 2011). Of these six safeners, benoxacor and fenclorim can effectively increase GST activity in tomato when treated with herbicides (Islam et al. 2017). There has also been reports of increased GST activity, up to five times, with the use of benoxacor and fenclorim safeners, with the model substrate 1-chloro-2,4-dinitrobenzene (DeRidder et al. 2002).

For successful weed management, it is crucial to use herbicides with different modes of action to achieve broad-spectrum weed control. Herbicides registered or recommended for use on tomato with different modes of action include pyroxasulfone, flumioxazin, metribuzin, fomesafen, imazamox, sulfentrazone, bicyclopyrone, and linuron (Chaudhari et al. 2015; Ronchi et al. 2010). For *C. rotundus* control, sulfentrazone, a protoporphyrinogen oxidase (PPO) inhibitor, is shown to drastically reduce tuber viability (Silva et al. 2014). Sulfentrazone provides 63% to 95% and 45% to 97% control when applied PRE or POST, respectively (Grichar et al. 2003). Fomesafen, another PPO inhibitor, when applied PRE, can control *Cyperus* spp. up to 50% at 28 days after application (DAA; Miller and Dittmar 2014). In addition, fomesafen can control several biotypes of Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] by up to 96% at 21 days after treatment (Bond et al. 2006). Metribuzin, a photosystem II inhibitor, controls several broadleaf weeds, including up to 75% control of Palmer amaranth when applied PRE (Meyers et al. 2017). Steele et al. (2005), on the other hand, reported greater than 97% control of Palmer amaranth plants using pyroxasulfone PRE, whereas Geier et al. (2006) reported 85% and 99% control of Palmer amaranth at rates of 125 to 332 g ha⁻¹, respectively. Flumioxazin, a chlorophyll biosynthesis inhibitor, has been used to control several species of broadleaf weeds when applied PRE (Cranmer et al. 2000), while imazamox, an acetohydroxyacid synthase inhibitor, is used to control some broadleaf weeds and many grasses (Blackshaw 1998; Nelson and Renner 1998). Bicyclopyrone is currently registered for PRE control of broadleaf and annual grass weed species with a hydroxyphenylpyruvate dioxygenase inhibitor (Bertucci et al. 2018), while linuron, a photosystem II inhibitor, is used widely

to control grasses and broadleaf weeds in some broadleaf crops (Grenni et al. 2009). The aim of the present study was to evaluate the safening effect of benoxacor and fenclorim on tomato against herbicides such as pyroxasulfone, flumioxazin, metribuzin, imazamox, fomesafen, sulfentrazone, bicyclopyrone, and linuron applied POST.

Materials and Methods

Experiments were conducted in 2018 in a greenhouse at the R. R. Foil Plant Science Research Center at Mississippi State University, Starkville, MS (32.46936111°N, 88.78333333°E). The minimum and maximum temperatures were set at 20 C and 30 C, respectively, with 70% relative humidity. All studies were conducted with 'Better Boy', a tomato cultivar commonly grown in the southern United States. The experimental design was completely randomized with four replications, repeated twice, and arranged in a 9 × 3 factorial design, where Factor A represented herbicide treatments (eight herbicides and a nontreated control), and Factor B consisted of three safener treatments (benoxacor, fenclorim, and a nontreated safener control).

Safeners were applied to seeds after the dissolution of the safener in methanol, due to its low solubility. Tomato seeds were immersed in the safener solution for 1 h before sowing. After 1 h of treatment, seeds were air-dried for 10 min at room temperature and seeded. The rate of fenclorim and benoxacor used were 0.67 g L⁻¹ (18 g ai kg⁻¹ seeds) and 10 μM (70.5 g ai kg⁻¹ seeds), respectively (Fuerst et al. 1993; Shen et al. 2013). Seeds incubated in methanol-only solution were used as control treatments. Each experimental unit consisted of trays with six cells (50-ml cell) filled with Sunshine Professional Growing Mix (SunGro® Horticulture Sunshine Mix No. 2 Basic, Colombier, Quebec, Canada) soil, and was composed of 75% to 85% of Canadian sphagnum peat moss. Each tray was sown with 18 tomato seeds and irrigated with a surface drip system. Plants were thinned to one plant per cell before herbicide application. Herbicides were applied at the 3-leaf stage, or 25 days after sowing, using a spray chamber calibrated to deliver 200 L ha⁻¹, fitted with an AIXR 11002 nozzle (TeeJet Technologies, Wheaton, IL) and maintained at a spray pressure of 275.8 KPa. The herbicide treatments evaluated are listed in Table 1.

Crop injury was evaluated visually at 3, 7, 14, and 21 DAA, using the scale of 0% to 100%, where 0% is without injury symptoms, and 100% is complete desiccation of shoot tissues. At 21 DAA, the shoot tissues were harvested, stored in paper bags, oven-dried at 60 C for 72 h, and weighed on a precision scale.

Statistical Analysis

Data were subjected to ANOVA using the Proc Mixed Method=Type3 process in version 9.4 of SAS (SAS Institute Inc., Cary, NC 27513). Treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. The safener and herbicide treatments were considered as fixed effects, whereas replications and runs were random effects (Yang 2010). The normality of the data was investigated by plotting q-q plots for residuals of visual injury and biomass in JMP13 (SAS Institute Inc.). The q-q plots showed normal distribution of data.

Results and Discussion

Visible injury on tomato plants at 7, 14, and 21 DAA was significant ($P < 0.05$) for the interaction between herbicide and safener

Table 1. Herbicide treatments applied at R. R. Foil Plant Science Research Center at Mississippi State University, Starkville, MS.

Herbicide	Rate	Trade name	Manufacturer	Address
	kg ai ha ⁻¹			
Sulfentrazone	0.220	OSpartan 4F	FMC	Philadelphia, PA
Fomesafen	0.280	Reflex	Syngenta Crop Protection LLC	Wilmington, DE
Flumioxazin	0.070	Valor	Valent USA Corp.	Walnut Creek, CA
Linuron	1.200	Linex 4L	DuPont	Wilmington, DE
Metribuzin	0.840	Glory	Adama Agricultural Solutions	Aventura, FL
Pyroxasulfone	0.220	Zidua	BASF Corp.	Florham Park, NJ
Bicyclopyrone	0.040	Acuron	Syngenta Crop Protection LLC	Wilmington, DE

Table 2. Interaction of herbicide and safener on visual injury at 3, 7, 14, and 21 days after application in tomato plants.^a

	3 DAA	7 DAA
	Injury	Injury
CV%	40.13	24.64
F herbicide	89.623 ^b	220.113 ^b
F safener	0.301 ^c	0.695 ^c
F herbicide*safener	1.521 ^c	4.528 ^b
	14 DAA	21 DAA
	Injury	Injury
CV%	16.11	19.38
F herbicide	288.794 ^b	165.421 ^b
F safener	6.2731 ^b	3.337 ^b
F herbicide*safener	3.368 ^b	2.427 ^b

^aAbbreviations: DAA, days after application.^bSignificant at 5% probability by the Tukey's test.^cNonsignificant at 5% probability by the Tukey's test.

(Table 2). At 3 DAA, metribuzin, imazamox, and bicyclopyrone caused less than 5% injury (Table 2), whereas the most noticeable injuries were observed with flumioxazin, sulfentrazone, and linuron treatments. Pretreatment with safeners did not result in any change in injury compared with control plants, except with flumioxazin, with injury higher than 1.2 times and 1.1 times, and pyroxasulfone with injury higher than 1.9 times and 2.1 times with benoxacor and fenclorim, respectively, compared with the control (Figure 1A). At 7 DAA, flumioxazin and linuron caused greater than 77% injury regardless of safener treatment. Similar to the findings at 3 DAA, metribuzin, imazamox, fomesafen, and bicyclopyrone caused minimal lesions on tomato plants at 7 DAA. Pretreatment with fenclorim and benoxacor reduced the injury caused by sulfentrazone 50% and 44%, respectively. Benoxacor-safened plants also showed 20.6% less injury with linuron treatment compared with the control plants. On the other hand, benoxacor and fenclorim increased the injury caused by pyroxasulfone by 1.63 times and 1.6 times, respectively, whereas benoxacor increased the injury from imazamox treatment by 4.5 times (Figure 1B).

At 14 DAA, pyroxasulfone, flumioxazin, and linuron caused >86% necrosis in tomato plants (Figure 1C). The effect of sulfentrazone and bicyclopyrone was intermediate with damage greater than 35% regardless of the safener treatment. Metribuzin, imazamox, and fomesafen caused less than 24% injury across all safener treatments. Pretreatment with benoxacor reduced the injury on tomato from sulfentrazone (30%) and bicyclopyrone (40%) compared with plants without safener treatment. Fenclorim safener was able to reduce the injury caused by bicyclopyrone by 26% at

Table 3. Dry biomass at 21 days after application of herbicides in tomato plants treated with safeners before sowing.

Herbicide	Dry biomass		
	Control ^a	Benoxacor	Fenclorim
	g plant ⁻¹		
Control ^b	0.4625 aB	0.6796 aA	0.3913 abB
Pyroxasulfone	0.0979 cdB	0.2296 cdeA	0.2038 cdAB
Flumioxazin	0.1413 bcdA	0.0892 eA	0.1021 dA
Metribuzin	0.2383 bcB	0.4088 bA	0.4466 aA
Imazamox	0.2085 bcB	0.3017 bcB	0.4304 aA
Fomesafen	0.1748 bcdB	0.2421 cdeB	0.3908 abA
Sulfentrazone	0.2529 bA	0.2504 cdA	0.2633 bcA
Bicyclopyrone	0.1757 bcdA	0.1983 cdeA	0.2075 cdA
Linuron	0.0269 dA	0.1317 deA	0.0667 dA
CV%		26.88	
F herbicide		46.302 ^c	
F safener		17.159 ^c	
F herbicide*safener		5.099 ^c	

^aControl: without safener.^bControl: without herbicide.^cSignificant at 5% probability by the Tukey's test. Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other.

14 DAA (Figure 1C). Greater than 76% necrosis was observed at 21 DAA with pyroxasulfone, flumioxazin, and linuron treatments. Therefore, these herbicides are not suitable for POST application on tomato (Figure 1D). However, less injury by metribuzin (27%), bicyclopyrone (25%), and fomesafen (25%) to tomato plants occurred from benoxacor-treated seeds. Fenclorim PRE resulted in 82%, 40%, and 23% reduction in tomato injury caused by imazamox, metribuzin, and bicyclopyrone, respectively.

The dry biomass of tomato plants in this study showed significant interaction ($P < 0.05$) between herbicides and safeners (Table 3). Pretreatment with benoxacor caused a lesser reduction in tomato biomass with pyroxasulfone (2.3 times) and metribuzin (1.7 times) compared with plants without a safener. Plants that were pretreated with fenclorim had lesser biomass reduction with metribuzin (1.9 times), imazamox (2.1 times), and fomesafen (2.2 times) than plants without safener pretreatment. Across all herbicide treatments, the highest reduction in tomato biomass was caused by linuron without a safener pretreatment (Figure 2).

The safeners tested in this study reduced the injury caused by certain herbicides. Benoxacor and fenclorim have the potential to reduce injury caused by bicyclopyrone and imazamox. In a related study, fenclorim was shown to protect rice (*Oryza sativa* L.) against injuries caused by pretilachlor (Wu et al. 1996). However, due to the lack of studies on the modes of action of these safeners in crops, the mechanism of how safeners protect plants from herbicide injury is not clear. We can, however, hypothesize that safeners

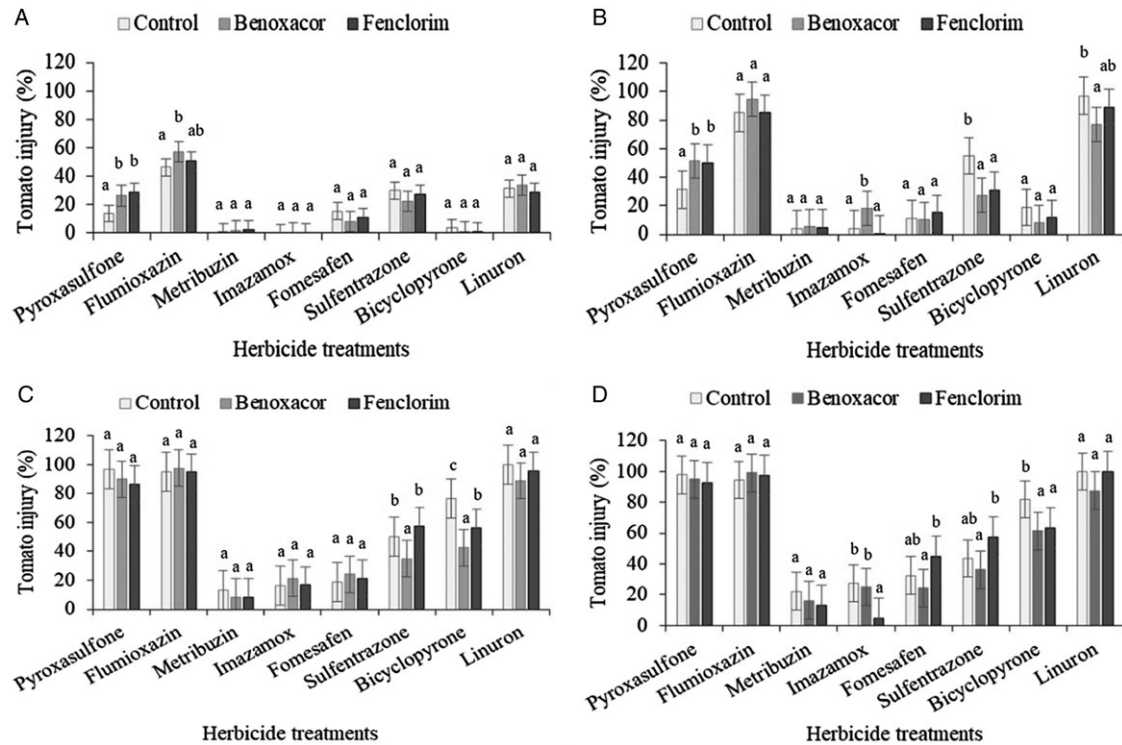


Figure 1. Visible estimates of injury at 3 (A), 7 (B), 14 (C), and 21 (D) days after application of herbicides in tomato plants treated with safeners before sowing. Means were separated within assessments using Tukey's test at $P < 0.05$. Means followed by the same lowercase letter do not differ.

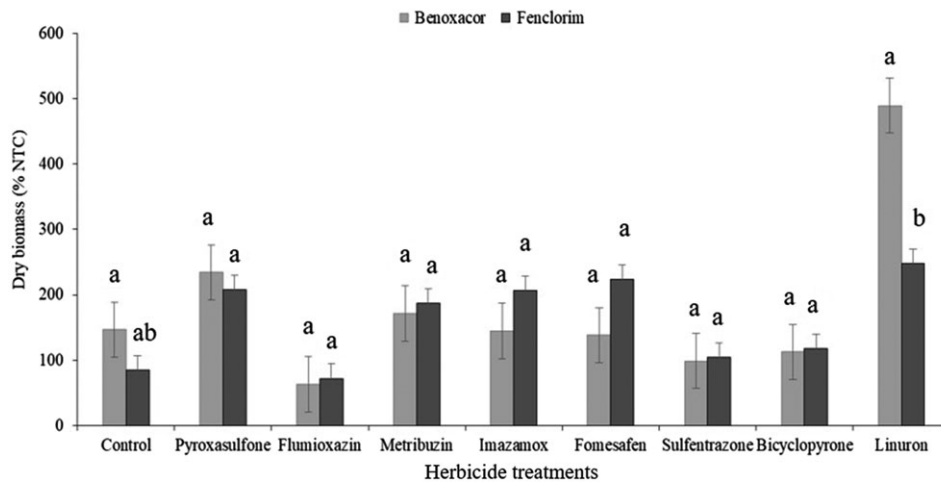


Figure 2. Dry biomass (%) of the nontreated without safener at 21 days after application of herbicides in tomato plants treated with safeners before sowing. Means were separated within assessments using Tukey's test at $P < 0.05$. Means followed by the same lowercase letter do not differ from each other.

promote rapid metabolism of the herbicide or interact with them at the sites of action (Galon et al. 2011), thus reducing the crop injury. Safeners reduce, but not completely reverse, the herbicide damage to sensitive crops.

It is likely that the rapid herbicide metabolism occurs mainly due to herbicide-glutathione conjugation and increased activity of the GST enzyme. Among the herbicides conjugated by GSTs are sulfonyleureas, imidazolinones, aryloxyphenoxypropionates, triazines, and chloroacetanilides (Galon et al. 2011). The GST activity is induced by the pretreatment of corn, rice, and grain sorghum with different safeners such as dichlormid, benoxacor,

fenclorim, and flurazole, improving their tolerance to thiocarbamate and chloroacetanilide herbicides (Riechers et al. 2010).

Using herbicides is the most effective method of controlling weeds in commercial tomato production; however, the tolerance of tomato to most herbicides is low. Safeners may alleviate this problem. The effectiveness of a safener depends on the mode of action of the herbicide, the crop to be safened, and the weeds to be controlled, because the mechanism of action of these chemical protectants is very specific (Galon et al. 2011).

Pretreatment of tomato with fenclorim reduced visual injury caused by imazamox and bicyclopyrone, whereas benoxacor

reduced visual injury from bicyclopyrone. Additionally, tomato plants showed less biomass reduction when treated with fenclorim after application of imazamox, fomesafen, and metribuzin, while pretreatment with benoxacor resulted in less biomass reduction after metribuzin application compared with plants that did not receive any safener treatment. Weeds cause significant yield losses in tomato and the use of a single method of control is not sufficient. Findings from this study show the potential use of benoxacor and fenclorim as safeners for crop protection. To expand the utility of this potential tool, studies are needed to understand how these safeners work in tomato.

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Conflicts of interest. The authors declare that there are no conflicts of interest.

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