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Vegetable Soybean Tolerance to Bentazon, Fomesafen, Imazamox, Linuron, and Sulfentrazone

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Poor weed control, resulting from limited herbicide availability and undeveloped integrated weed management systems, is a major hurdle to production of vegetable soybean in the United States. Vegetable soybean, the same species as grain-type soybean, has few registered herbicides because of unknown crop tolerance. Tolerance of as many as 128 vegetable soybean entries to a 2X registered rate of bentazon, fomesafen, imazamox, linuron, and sulfentrazone were quantified within 4 wk after treatment in field trials. Several grain-type soybean entries were included for comparison, including entries with known herbicide tolerance or sensitivity. Injury and seedling growth reduction to all vegetable entries was comparable to all grain-type entries for fomesafen, linuron, and sulfentrazone; and less than all grain-type entries for bentazon and imazamox. Responses of ten of the more widely used vegetable soybean entries were comparable to grain-type entries with known herbicide tolerance. Bentazon, fomesafen, imazamox, linuron, and sulfentrazone pose no greater risk of adverse crop response to vegetable soybean germplasm than the grain-type soybean to which they have been applied for years. Since initiation of this research, fomesafen, imazamox, and linuron are now registered for use on the crop in the United States. Development of integrated weed management systems for vegetable soybean would benefit from additional herbicide registrations.

Nomenclature: Bentazon; fomesafen; imazamox; linuron; sulfentrazone; vegetable soybean, [*Glycine max* (L.) Merr.].

Key words: Crop injury, edamame, herbicide tolerance, minor crop.

Control de malezas deficiente como resultado de una disponibilidad limitada de herbicidas y de sistemas de manejo integrado de malezas poco desarrollados, es un obstáculo importante a la producción de soya tipo-hortaliza en los Estados Unidos. La soya tipo-hortaliza, que es la misma especie que la soya tipo-grano, tiene pocos herbicidas registrados porque se desconoce su tolerancia. La tolerancia de 128 accesiones de soya tipo-hortaliza a 2X de la dosis de registro de bentazon, fomesafen, imazamox, linuron, y sulfentrazone fue cuantificada a 4 semanas después del tratamiento en experimentos de campo. Varias accesiones de soya tipo-grano fueron incluidas para comparación, incluyendo accesiones con tolerancia o susceptibilidad a herbicidas conocidas. El daño y la reducción del crecimiento de plántulas de todas las accesiones tipo-hortaliza fueron comparables a todas las accesiones tipo-grano para fomesafen, linuron, y sulfentrazone, y fueron menores que para las accesiones tipo-grano para bentazon e imazamox. La respuesta de diez de las accesiones tipo-hortaliza más ampliamente usadas fueron comparables con las accesiones tipo-grano con tolerancia a herbicidas conocida. Bentazon, fomesafen, imazamox, linuron, y sulfentrazone no representan un riesgo mayor de una respuesta adversa del cultivo de soya tipo-hortaliza que la soya tipo-grano, a la cual estos herbicidas han sido aplicados por años. Desde que se inició esta investigación, fomesafen, imazamox, y linuron fueron registrados para el uso en el cultivo en Estados Unidos. El desarrollo de sistemas de manejo integrado de malezas para soya tipo-hortaliza se beneficiaría de registros de herbicidas adicionales.

Vegetable soybean, commonly known as edamame, is differentiated from the more widely grown grain-type soybean in that vegetable soybean cultivars are harvested and eaten at an immature seed stage and have larger seeds with a sweet, nutty flavor (Shurtleff and Aoyagi 2009). Historically a food of East Asian cultures, vegetable soybean consumption has grown in other parts of the world, including the United States. Sams et al. (2012) reported a four-fold increase in U.S. consumption of vegetable soybean between 2000 and 2008. Moreover, the United Soybean Board forecasts vegetable soybean will grow larger than other soy products by 2020 (Shockley et al. 2011). Although the United States is the leading producer of soybean worldwide, a majority of the vegetable soybean consumed in the United States is imported from

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Asia. Fueled in part by increasing consumer interest in the crop and in locally grown products, the U.S. vegetable industry seeks to produce the crop domestically.

Poor weed control is a major hurdle to vegetable soybean production in the United States. Grain-type soybean sensitivity to weed interference is well established (see review by Zhimdahl 2004); weed interference in vegetable soybean has not been researched. Integrated weed management (IWM) systems are currently limited. Hand weeding, the most common form of weed control in East Asian countries that grow and export vegetable soybean (Pornprom et al. 2010; Q. Zhang, personal communication), is prohibitively expensive in the United States. Other physical weed control tactics, as well as cultural practices, are key components of soybean IWM (Green 2012); however, tailoring these tactics to vegetable soybean remains undeveloped. Although numerous herbicides are registered for use on grain-type soybean in the United States, criteria for registering products on vegetable soybean differ (IR-4 2012). As a result, chemical weed control tactics are very limited, since few herbicides are registered on the crop. Judicious use of herbicides, in combination with a comprehensive IWM system, facilitates production of high-quality food to the general public at reasonable prices (IR-4 2009).

The U.S. vegetable industry needs additional herbicides available for use on vegetable soybean, particularly certain products that have been used for years on grain-type soybean (Altemose et al. 2011). In order for existing herbicides to be registered on new crops, the herbicide manufacturer must support the proposed use. The primary reason manufacturers are reluctant to support such 'minor use' registrations of their products is potential liability, particularly action taken as the result of crop injury (personal observation). Is vegetable soybean more susceptible than grain-type soybean to certain herbicides? Metolachlor was registered for use on edamame in the United States in 2010 and crop sensitivity to metolachlor has not been reported. Several other products primarily lack only manufacturer support for registration on vegetable soybean, including bentazon and sulfentrazone. At the initiation of this research, imazamox, fomesafen, and linuron also fit this category, but have since been registered (Anonymous 2012, 2013, 2014). Quantifying vegetable soybean tolerance to these herbicides will provide herbicide manufacturers, regulatory agencies, food processors, and vegetable growers an understanding of the potential risk of crop injury from these herbicides. Therefore, the objective of this research was to determine vegetable soybean tolerance to bentazon, fomesafen, imazamox, linuron, and sulfentrazone.

Materials and Methods

Germplasm. Vegetable soybean entries were obtained from both private and public sources. Seed from nearly every commercial cultivar available in the United States were procured from 24 different seed companies or individuals. In a few cases, seed of an individual cultivar was not available for all years. Seed of available cultivars developed from university vegetable soybean breeding programs was obtained from four universities. In addition, the following criteria were used to select entries from the USDA Soybean Germplasm Collection: parental lines of U.S.-developed vegetable soybean and large-seeded entries with names associated with vegetable soybean. There were 122, 128, and 126 vegetable soybean entries in 2011, 2012, and 2013, respectively.

Grain-type soybean entries included lines with known sensitive or tolerant responses to the herbicides in the study, as well as several maturity group checks. Total number of grain-type soybean entries was 33, 14, and 14 in 2011, 2012, and 2013, respectively. Duplicate maturity group checks were dropped in 2012 and 2013; hence the lower number of grain-type entries compared to 2011.

Experimental Approach. Experiments were conducted in a different field each year at the University of Illinois Vegetable Crop Farm near Urbana, Illinois, USA. The soil was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) averaging 3.5% organic matter and a pH of 5.8. Trials followed the soybean year of a sweet corn (*Zea mays* L.)-soybean rotation. Prior to planting, fields received two passes of a field cultivator.

Each herbicide trial was a separate experiment with three replications of entries arranged in a randomized complete block. An experimental unit was a single 2.5 m row planted with 50 seeds. Trials were planted June 3, May 18, and May 22 of 2011, 2012, and 2013, respectively. Immediately after

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planting, metolachlor was applied throughout the trials at a rate of 1.8 kg ai ha^{-1} to control most grasses and small-seeded broadleaf weeds. Emerged weeds were removed by hand-weeding, as necessary. All herbicide trials were repeated each year, with one exception. Linuron was registered for use on vegetable soybean in 2012; therefore, the trial was not repeated in 2013.

All herbicides were applied perpendicular to crop rows, such that 1.3 m of row was treated and 1.3 m was left as the metolachlor control. PRE herbicides (linuron and sulfentrazone) were applied immediately after planting. POST herbicides (bentazon, fomesafen, and imazamox) were applied when a majority of plants had two fully-emerged trifoliate leaves. To simulate application overlap, herbicides were applied at twice the registered use rate: bentazon at 2.2 kg ai ha^{-1}, fomesafen at 841 g ai ha^{-1}, imazamox at 70 g ai ha^{-1}, linuron at 4.5 kg ai ha^{-1}, and sulfentrazone at 420 g ai ha^{-1}. Consistent with labeled recommendations on grain-type soybean, adjuvants included 1% crop oil concentrate in the bentazon trial, and 0.25% nonionic surfactant in the fomesafen and imazamox trials. Treatments were applied in 187 L ha^{-1} of spray volume. Daily minimum and maximum air temperature and rainfall was obtained from a weather station located within 1 km of the trials (Illinois State Water Survey, Champaign, IL).

Data Collection. Response to POST herbicides was assessed visually one and 2 wk after treatment (WAT). Response to PRE herbicides was assessed visually four WAT. Relative to the metolachlor control, injury was scored on the following scale: 0 = no visible symptoms, 10 = slight chlorosis or necrosis, 20 = chlorosis/necrosis with possible stunting/stand reduction, 30 = chlorosis/necrosis with stunting/stand reduction, 40 = chlorosis/necrosis with significant stunting/stand reduction, 50 = 50% plant stunting/stand reduction, 60 = 60% plant stunting/stand reduction, 70 = 70% plant stunting/stand reduction, 80 = 80% plant stunting/stand reduction, 90 = 90% plant stunting/stand reduction, and 100 = all plants dead or not emerged.

Plant growth was evaluated two and four WAT with POST and PRE herbicides, respectively. Plant height and canopy width were measured on three randomly selected plants per treated and metolachlor subplots. Growth reduction was calculated using the equation:

$$(1 - Htrt \times Wtrt / Hcon \times Wcon)100 \qquad [1]$$

where H and W is mean plant height and canopy width, respectively, for treated (*trt*) and metolachlor control (*con*) plants.

Data Analysis. Two hypotheses were tested: (1) overall response of vegetable entries was comparable to overall response of grain-type entries, and (2) individual vegetable entries currently used by food processors have herbicide responses comparable to each other as well as to grain-type entries with known herbicide tolerance or sensitivity. The first hypothesis evaluates the complete collection of vegetable entries, while the second hypothesis focuses on a select group of vegetable entries used in current U.S. vegetable soybean production.

To address hypothesis (1), the Kolmogorov-Smirnov test of frequency distributions was used to determine if crop injury and growth reduction differed overall between vegetable and grain-type entries. To address hypothesis (2), 10 vegetable entries currently used by food processors and four grain-type entries with known herbicide tolerance or sensitivity were selected for additional analysis. Vegetable entries included: AGS292 (Rupp Seed, Wauseon, OH), IA1010 (Iowa State University, ISU), IA2076 (ISU), Misono Green (Snow Brand, Sapporo, Japan), Mojo Green (Wannamaker, Saluda, NC), Sayamusume (Territorial, Cottage Grove, OR), Sunrise (Wannamaker), WSU729 (Washington State University, WSU) and WSU910a (WSU). Grain-type entries, from the USDA Soybean Germplasm Collection, included: Clark 63, previously identified as bentazon tolerant (Wax et al. 1974); L78-3263, a Clark 63 near-isogenic line with bentazon sensitivity from PI 229342 (USDA-ARS 2014); Manokin, sulfentrazone tolerant (Swantek et al. 1998); and KS4895, sulfentrazone sensitive (Swantek et al. 1998). Entries with no variance in herbicide response (e.g. injury of 100%) were removed prior to analysis. Data were found to comply with ANOVA assumptions of homogeneity of variance, based on the modified Levene's test (Neter et al. 1996), and normality, based on diagnostic test of residuals. To determine if selected cultivars varied in their herbicide response, crop injury and growth reduction data were subjected to ANOVA. Means of

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Table 1. Weekly rainfall, minimum, maximum, and mean average daily air temperature, and departure from 30-yr average temperature for the first 4 wk after planting in 2011, 2012, and 2013.

Year	Weeks after planting	Rainfall	Average daily temperature			
			Min	Max	Mean	Departure from average
		cm	C			
2011	1	2.7	20.0	33.3	26.7	5.6
	2	3.6	14.7	26.3	20.5	−1.7
	3	1.2	18.9	27.1	23.0	−0.3
	4	3.9	17.1	26.9	22.0	−1.9
2012	1	2.3	12.5	28.2	20.3	2.5
	2	5.4	17.6	29.3	23.4	4.0
	3	0.8	11.9	24.1	18.0	−2.6
	4	1.6	14.6	29.4	22.0	−1.3
2013	1	3.1	10.6	21.4	16.0	−1.8
	2	5.0	14.3	24.5	19.4	0.0
	3	0.1	14.3	27.0	20.6	0.0
	4	0.9	17.2	29.7	23.5	0.2

selected cultivars were compared using protected, Bonferroni-corrected multiple comparisons (Neter et al. 1996). Confidence intervals (95%) were constructed around means that were to be compared to entry means with zero variance so that significant differences could be determined. All analyses were conducted in SYSTAT 13 and hypotheses were tested at $\alpha = 0.05$.

Results and Discussion

Weather. Within the first week of planting, fields received 2.3 to 3.1 cm of rainfall each year and total rainfall the first 4 wk ranged from 9.0 to 11.4 cm (Table 1). Initial rainfall events were sufficient to incorporate PRE herbicide treatments into the soil profile and favored seed germination and growth. Air temperatures also were adequate for soybean seedling growth and development. Within the first week of planting, mean air temperature was 16.0 C or higher (Table 1). The largest deviations from the 30-yr average air temperature were within the first week of the 2011 trials (+5.6 C) and third week of the 2012 trials (–2.6 C).

Bentazon. Injury from bentazon on all vegetable entries was less than injury to all grain-type entries. Mean injury on grain-type entries 1WAT was 48%, while mean injury to vegetable entries was 27 % (Table 2). A similar trend, albeit at lower levels of crop injury, were observed 2WAT (data not shown). This observation is the result of bentazon-sensitive grain-type entries included in the trial. Bernard and Wax (1975) showed bentazon sensitivity in soybean is conditioned largely by a single recessive gene, *hb*. Two entries, L75-6631 and L78-3263, carry the *hb* allele (USDA-ARS 2014). Four grain-type entries from Japan previously identified as bentazon-sensitive, presumably with the *hb* allele, were included in these trials: PI 86504, PI 243525, PI 360839, and PI 86098 (Wax et al. 1974). All bentazon-sensitive controls were killed by bentazon.

Vegetable entries exhibited levels of injury comparable to grain-type entries with known tolerance to bentazon. The allele for bentazon tolerance, *Hb*, is known to occur in Clark 63, which is the recurrent parent of the near-isolines L75-6631 and L78-3263 (Bernard and Wax 1975; USDA-ARS 2014). Injury 1WAT observed in the ten widely used vegetable soybean entries ranged from 13 to 41%, comparable to Clark 63 (Table 2). Unlike grain-type entries carrying the *hb* sensitivity allele (e.g. L78-3263), no vegetable entries were killed by bentazon.

Similar results were observed in plant growth responses to bentazon 2WAT. Growth reductions from bentazon were higher in grain-type entries (mean of 26%) than vegetable entries (mean of 14%) (Table 3). While some stunting was observed among individual vegetable entries (up to 28%), growth reductions were comparable to the bentazon-tolerant Clark 63 (18%). While a 2X application rate of bentazon resulted in some early-season injury and stunting in vegetable soybean, risk of an

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Table 2. Mean injury 1 wk after POST (bentazon, fomesafen, and imazamox) or 4 wk after PRE (linuron and sulfentrazone) herbicide application. Differences in plant type were determined by the Kolmogorov-Smirnov test of frequency distributions. Entry means separation was determined by protected, Bonferroni-corrected multiple comparisons at $P < 0.05$.

Type	Entry	Bentazon	Fomesafen	Imazamox	Linuron	Sulfentrazone
		%				
Grain	—	48	11	27	1	30
Vegetable	—	27	12	19	1	24
KS statistic		0.288	0.038	0.245	0.035	0.103
p-value		< 0.001	1.000	< 0.001	1.000	0.417
Grain	Clark 63[a]	28 bc	12	24 b	0	18
	L78-3263[b]	100 a	10	50 a	0	32
	Manokin[c]	18 c	9	17 b	0	23
	KS4895[d]	29 bc	13	9 b	0	21
Vegetable	AGS292	20 bc	13	14 b	0	20
	BeSweet 292	33 bc	10	23 b	0	15
	IA1010	27 bc	16	13 b	0	24
	IA2076	26 bc	11	8 b	5	19
	Misono Green (2)	41 b	14	19 b	0	24
	Mojo Green	22 bc	14	11 b	0	18
	Sayamusume	13 c	13	20 b	0	42
	Sunrise	30 bc	17	18 b	5	14
	WSU729	23 bc	9	21 b	0	28
	WSU910a	34 bc	10	23 b	0	28
F-ratio		3.303	0.559	7.233	0.845	0.306
p-value		0.002	0.877	< 0.001	0.614	0.989

[a] Identified as bentazon-tolerant by Wax et al. (1974).
[b] Identified as bentazon-sensitive by USDA-ARS (2014).
[c] Identified as sulfentrazone-tolerant by Swantek et al. (1998).
[d] Identified as sulfentrazone-sensitive by Swantek et al. (1998).

adverse crop response is no greater than in grain-type soybean.

Fomesafen. Only low levels of injury were observed from fomesafen. Mean injury 1WAT was comparable across all grain-type and vegetable entries, averaging 12% (Table 2). Of the subset of widely used vegetable entries, injury 1WAT also was comparable to individual grain-type entries, ranging from 9 to 17%. Similarly, growth reductions 2WAT were minimal and comparable between grain-type and vegetable entries (Table 3).

Vegetable soybean tolerance to fomesafen has been reported by others. Altemose et al. (2011) reported excellent crop tolerance to fomesafen among five vegetable entries. Soybean tolerance to fomesafen is based on rapid herbicide detoxification by glutathione transferase enzymes (Andrews et al. 1997; Evans et al. 1987).

Imazamox. Injury from imazamox to all vegetable entries was less than injury to all grain-type entries. Mean injury to grain-type entries 1WAT was 27%, while mean injury to vegetable entries was 19 % (Table 2). Similar results were observed 2 WAT; however, at lower levels of crop injury (data not shown). Growth reductions from imazamox also were higher in all grain-type entries (mean of 29%) than all vegetable entries (mean of 17%) (Table 3).

The ten vegetable entries used in commercial production had responses to imazamox that were comparable to most grain-type entries. One exception was L78-3263, a grain-type entry that had injury 1WAT significantly greater than all other entries (Table 2). This entry was used in the trials because it carries the bentazon sensitivity *hb* allele; the present work appears to be the first report of cross-sensitivity to imazamox. Other *hb* entries and bentazon-sensitive entries, all of which were killed by bentazon, also had moderate to high levels of injury from imazamox (data not shown). Perhaps soybean sensitivity to imazamox and bentazon is conditioned by a common genetic basis. In any

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Table 3. Mean growth reduction 2 wk after postemergence (bentazon, fomesafen, and imazamox) or 4 wk after PRE (linuron and sulfentrazone) herbicide application. Differences in plant type were determined by the Kolmogorov-Smirnov test of frequency distributions. Entry means separation was determined by protected, Bonferroni-corrected multiple comparisons at $P < 0.05$.

Type	Entry	Bentazon	Fomesafen	Imazamox	Linuron	Sulfentrazone
		%				
Grain	—	26	4	29	−4	31
Vegetable	—	14	8	17	−1	21
KS statistic		0.148	0.130	0.201	0.109	0.140
p-value		0.023	0.158	< 0.001	0.797	0.116
Grain	Clark 63[a]	18 b	7	31	−7	18
	L78-3263[b]	100 a	−2	56	−32	46
	Manokin[c]	6 b	−7	18	7	35
	KS4895[d]	16 b	20	7	−1	23
Vegetable	AGS292	8 b	−2	28	−7	−3
	BeSweet 292	21 b	−2	20	−23	13
	IA1010	24 b	11	17	−7	33
	IA2076	12 b	−6	10	0	32
	Misono Green (2)	28 b	9	23	−5	48
	Mojo Green	−11 b	8	12	−11	13
	Sayamusume	−3 b	5	22	0	35
	Sunrise	7 b	17	24	−19	15
	WSU729	18 b	−7	30	19	41
	WSU910a	5 b	12	20	23	3
F-ratio		3.614	0.801	1.504	0.473	1.036
p-value		< 0.001	0.657	0.104	0.910	0.430

[a] Identified as bentazon-tolerant by Wax et al. (1974).
[b] Identified as bentazon-sensitive by USDA-ARS (2014).
[c] Identified as sulfentrazone-tolerant by Swantek et al. (1998).
[d] Identified as sulfentrazone-sensitive by Swantek et al. (1998).

event, tolerance to imazamox comparable to grain-type soybean has been confirmed previously in five vegetable soybean entries by Altemose et al. (2011).

Linuron. Of all herbicides tested, linuron was safest. Overall crop injury 1WAT was 1% in both grain and vegetable entries (Table 2). Most individual entries exhibited no adverse response to linuron, and of those where injury was observed, injury 1 WAT was $\leq 5\%$. These results are consistent with unpublished data on six vegetable soybean cultivars tested in Washington State (R. Boydston, personal communication).

Sulfentrazone. Sulfentrazone resulted in widespread crop response; however, vegetable entries were adversely affected no more than grain-type entries. Mean crop injury 1 WAT from sulfentrazone to grain-type entries was 30%, while mean injury to vegetable entries was 24% (Table 2). Growth reduction data showed a similar trend (Table 3).

Grain-type soybean cultivar sensitivity to soil-applied sulfentrazone is well documented (Dayan et al. 1997; Hulting et al. 2001; Taylor-Lovell et al. 2001). Previous research indicated soybean sensitivity to sulfentrazone may be conditioned by a single recessive gene (Swantek et al. 1998). Two grain-type entries previously identified by Swantek et al. (1998) as homozygous for sulfentrazone-sensitive alleles, KS4895 and A4715, were included in the present study. In addition, one grain-type entry previously identified by Swantek et al. (1998) as homozygous for sulfentrazone-tolerance alleles, Manokin, served as the sulfentrazone-tolerant check. Interestingly, Manokin appeared to be no more tolerant to sulfentrazone than KS4895 (Tables 1 and 2) and A4715. Li et al. (2000) concluded differential response among soybean cultivars is due primarily to differential sulfentrazone absorption during the earliest stages of seedling development. While soybean was injured by sulfentrazone in this work, apparently conditions favoring differential absorption among cultivars did not occur. In any

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event, a 2X application rate of sulfentrazone resulted in some early-season injury and stunting in vegetable soybean; however, adverse crop responses were no greater than in grain-type soybean.

In order to quantify vegetable soybean tolerance to two PRE and three POST herbicides now being used or considered for use on the crop in the near future, this work assembled a collection of as many as 128 private and public vegetable soybean entries. Responses of entries to the herbicides were compared to responses of grain-type soybean, including certain grain-type entries with documented herbicide tolerance or sensitivity. Bentazon, fomesafen, imazamox, linuron, and sulfentrazone pose no greater risk of adverse crop response to vegetable soybean germplasm than the grain-type soybeans to which they have been applied for years. Additional herbicide registrations will provide the vegetable industry with valuable, cost-effective tools for use in the development of IWM systems for this emerging crop.

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