Weed Technology 2017 31:523–535

Utilization of Chlorophyll Fluorescence Imaging Technology to Detect Plant Injury by Herbicides in Sugar Beet and Soybean

Jonas F. Weber, Christoph Kunz, Gerassimos G. Peteinatos, Hans-Joachim Santel, and Roland Gerhards*

Sensor technologies are expedient tools for precision agriculture, aiming for yield protection while reducing operating costs. A portable sensor based on chlorophyll fluorescence imaging was used in greenhouse experiments to investigate the response of sugar beet and soybean cultivars to the application of herbicides. The sensor measured the maximum quantum efficacy yield in photosystem II (PS-II) (F_v/F_m). In sugar beet, the average F_v/F_m of 9 different cultivars 1 d after treatment of desmedipham plus phenmedipham plus ethofumesate plus lenacil was reduced by 56% compared to the nontreated control. In soybean, the application of metribuzin plus clomazone reduced F_v/F_m by 35% 9 d after application in 7 different cultivars. Sugar beets recovered within few days from herbicide stress while maximum quantum efficacy yield in PS-II of soybean cultivars was reduced up to 28 d. At the end of the experiment, approximately 30 d after treatment, biomass was reduced up to 77% in sugar beet and 92% in soybean. Chlorophyll fluorescence imaging is a useful diagnostic tool to quantify phytotoxicity of herbicides on crop cultivars directly after herbicide application, but does not correlate with biomass reduction.

Nomenclature: Desmedipham; ethofumesate; flufenacet; lenacil; metamitron; metribuzin; phenmedipham; soybean, *Glycine max* (L.) Merr.; sugar beet, *Beta vulgaris* (L.) ssp. *vulgaris.*

Key words: Chlorophyll fluorescence, crop injury, F_v/F_m, imaging sensor, PS-II, stress detection.

Effective weed management is crucial in the early growth stages of sugar beet and soybean. The use of herbicides has become the primary tool for weed management in both crops. In European production systems, weed control in soybean is achieved with the combination of PRE and POST applications of selective herbicides. In soybean, metribuzin, flufenacet, dimethenamid, and clomazone are registered (Gehring et al. 2014). In sugar beet, three POST herbicide applications are commonly performed, each one stimulated by a new cohort of weed seedlings. The crop is usually treated with mixtures of metamitron, phenmedipham, desmedipham, and ethofumesate (Vasel et al. 2012).

Herbicides can damage crops and reduce crop yield when used at inappropriate rates or timings or in mixtures with several active ingredients and additives (Salzman and Renner 1992). In sugar beet, injury has been observed after applying a combination of desmedipham, phenmedipham, and triflusulfuron, resulting in a 29% reduction of sugar beet leaf area and 8% reduction of sugar beet root biomass (Wilson 1999). Three sequential herbicide applications with a mixture of desmedipham plus phenmedipham plus triflusulfuron plus clopyralid caused crop injury, with maximum yield losses of 15% in sugar beet, compared to a weed-free control (Wilson et al. 2002). Metribuzin reduced soybean yield by 38% in a study by Belfry et al. (2015). The reason for crop damage due to herbicides is always a limited capacity of the crop to metabolize the herbicide before the target is reached (Smith and Wilkinson 1974), and cultivars can vary in the rate of herbicide detoxification (Moseley et al. 1993). Phytotoxic symptoms may include temporary discoloration, reduced plant development, formation of necrotic areas on leaves, and minor changes in plant appearance (EPPO 2014). The most commonly used method for describing herbicide phytotoxicity is a simple and subjective visual estimation of the

---

DOI: 10.1017/wet.2017.22

* First, second, and third authors: Graduate Research Assistants, Department of Weed Science, University of Hohenheim, Stuttgart, Germany; Fourth and fifth authors: Weed Scientist and Professor, Department of Weed Science, University of Hohenheim, Stuttgart, Germany. Corresponding author's E-mail: j.weber@uni-hohenheim.de

observed crop injury, often expressed as percent crop damage in comparison to a nontreated control. Yet, these results depend heavily on the experience of the assessor and are difficult to compare between assessors and locations (Andújar et al. 2010). The current quantification methods for herbicide stress include assessment of crop yield and destructive sampling methods such as biomass assessment. Both can be influenced by various environmental factors during the cultivation period. More objective and rapid quantitative methods are needed for the reproducible quantification of phytotoxicity caused by herbicides.

Optical and hyperspectral sensors have been applied to measure abiotic and biotic stress in plants (Fahlgren et al. 2015; Fiorani and Schurr 2013; Thenkabail et al. 2011). For example, Donald (1998) tested a simple RGB camera as a tool for quantifying herbicide stress in plants. Optical sensors can quantify the morphological and physiological status of plants, and thus may facilitate assessment of herbicide stress (Fahlgren et al. 2015; Fiorani and Schurr 2013). Different commercial sensor systems have been developed for plant phenotyping (for example, the Phenospex® FieldScan, Lemnatec Scanalyzer3D®, or Photon Systems Instruments PlantScreen®).

A different approach for plant phenotyping is assessing chlorophyll fluorescence. This approach was introduced by Maxwell and Johnson (2000) and described by Baker (2008). Chlorophyll fluorescence measurement is a nondestructive, easy, and rapid assessment method for stress evaluation that makes it possible to assess plant response to herbicides in a short time period (Baker 2008; Burke et al. 2010; Kaiser et al. 2013; Roeb et al. 2015). Many herbicides with different modes of action cause increased chlorophyll fluorescence in crops shortly after application (Dayan and Zaccaro 2012). The F_v/F_m value provides a measure of photosystem II (PS-II) efficiency. PS-II–inhibiting herbicides directly influence the electron transport supply in PS-II, while other herbicides indirectly disturb photosynthesis in many different ways (Kaiser et al. 2013).

The objectives of this study were to determine 1) if herbicides and mixtures of different herbicides cause stress in sugar beet and soybean shortly after application, 2) if herbicide stress can be measured using chlorophyll fluorescence imaging, 3) if cultivars respond differently to herbicides, 4) if crops can recover from herbicide stress, and 5) if chlorophyll fluorescence imaging data correlates with crop dry biomass 3 to 4 wk after herbicide application.

Materials and Methods

Four experiments were carried out on sugar beet and soybean plants in the greenhouse of the University of Hohenheim, Germany (48.71°N, 9.19°E; altitude 370 m), in 2015 and 2016. Each experiment was repeated in time and set up as a randomized complete block design with four replications. The soil used was a Luvisol loamy sand, placed in 10 cm by 10 cm by 10 cm pots. In each pot, one seed of sugar beet or one seed of soybean was placed at a depth of 1 cm or 4 cm, respectively. Herbicides were applied in a precision spray chamber using a flat fan nozzle (8002EVS, TeeJet® Technologies GmbH, Ludwigsburg, Germany). The spray chamber simulated an application carrier volume of 200 L ha^{-1}. Pots were returned in the greenhouse one hour after application. The greenhouse temperature was maintained at 20 C during the day and 15 C ± 2 C at night, with a relative humidity of 70% and a 12 h light (400 µmol m^{-2} s^{-1}) and 12 h dark cycle.

Experimental Design. Two sets of experiments were conducted. In the first set of experiments (cultivar experiment), nine sugar beet cultivars and seven soybean cultivars were sprayed with two herbicide mixtures (Table 1). In sugar beet, the herbicides desmedipham plus phenmedipham plus ethofumesate (Betanal Expert®) and desmedipham plus phenmedipham plus ethofumesate plus lenacil (Betanal maxxPro®) were applied at the cotyledon (two-leaf) stage (Table 2). Soybeans were treated with the herbicides metribuzin (Sencor WG®) plus clomazone (Centium CS®) or metribuzin plus flufenacet (Artist WG®) before crop emergence (Table 3).

For the second set of experiments (herbicide experiment), two cultivars (the most tolerant and the most sensitive, as observed from the previous experiment) per crop were sprayed with several herbicides and herbicide mixtures. At the cotyledon (two-leaf) stage, sugar beet cultivars 'Capella' and 'Beta 1' were treated with five herbicides and herbicide mixtures applied as individual treatments (Table 2). In soybean, cultivars 'Gallec' and 'ES Mentor' were treated with four PRE herbicides and herbicide mixtures before crop emergence (Table 3). All the above listed applications reflect typical

Table 1. Soybean and sugar beet cultivars tested for herbicide stress response using chlorophyll fluorescence imaging.

Soybean		Sugar beet	
Cultivar	Company	Cultivar	Company
‘Sultana’	R.A.G.T Saaten, 32120 Hiddenhausen, Germany	‘Capella’	KWS SAAT AG, 3755 Einbeck, Germany
‘Solena’	Probstdorfer Saatzucht, 1011 Wien, Austria	‘Beta 1’[a]	Betaseed GmbH, 60325 Frankfurt am Main, Germany
‘ES Mentor’	Saatbau Linz eGen, 4060 Leonding, Austria	‘Beta 2’[a]	Betaseed GmbH
‘Gallec’	Saatzucht Gleisdorf Ges.mbH, 8200 Gleisdorf, Austria	‘Beta 3’[a]	Betaseed GmbH
‘Merlin’	Saatbau Linz eGen	‘Sabrina’	KWS SAAT AG
‘Lissabon’	Saatbau Linz eGen	‘Sandra’	KWS SAAT AG
‘SY Eliot’	Saatbau Linz eGen	‘SES Vanderhave’[a]	SES Vander Have N.V./S.A., 3300 Tienen, Belgium
		‘Isabella’	KWS SAAT AG
		‘Belladonna’	KWS SAAT AG

[a] Anonymous labeling; cultivar in development.

herbicide applications performed by growers in Europe. Nontreated experimental units served as references in all experiments.

Data Collection. Crop responses to herbicides were measured with the WEED-PAM® M-Series Imaging-Sensor for measuring chlorophyll fluorescence (Heinz Walz GmbH, Effeltrich, Germany). Maximum quantum efficiency of PS-II (F_v/F_m) was calculated according to Equation 1:

$$F_v/F_m = \frac{F_m - F_0}{F_m} \tag{1}$$

where F_m is the maximal fluorescence yield and F_0 is the dark fluorescence yield (Kaiser et al. 2013). The sensor WEED-PAM is a mobile version of the MAXI version of the IMAGING-PAM® fluorescence meter (Heinz Walz GmbH, Effeltrich, Germany). Chlorophyll fluorescence was induced by blue light emitting diode (LED) lights of 460 nm wavelength. An optical red long-pass filter of >680 nm wavelength was mounted in front of the camera lens. The WEED-PAM software excludes background noise with a mask, as described by Kaiser et al. (2013). Thus, only information from green leaves was processed. F_v/F_m was presented as relative values compared to nontreated control plants. After POST herbicide application, sugar beet reaction was measured 12 or 24 h after treatment, and on the 6th and 13th d after treatment (DAT), respectively, when it was at the four- to six-leaf growth stage. An extra measurement at 3 DAT was performed for the cultivar experiment. Soybean responses were assessed at the unrolled unifoliate stage and at the first and

Table 2. Herbicide rates and active ingredients tested for sugar beet stress response using chlorophyll fluorescence imaging.

Herbicide treatment	Rate	WSSA group	Formulation[a]	Company
	g or mL ai ha^{-1}			
desmedipham	47	5	OD	Bayer Crop Science AG, 40789 Monheim am Rhein, Germany; www.cropscience.bayer.com
+ phenmedipham	+ 60	5		
+ ethofumesate	+ 75	8		
+ lenacil	+ 27	5		
desmedipham	25	5	EC	Bayer Crop Science AG
+ phenmedipham	+ 75	5		
+ ethofumesate	+151	8		
metamitron	525	5	SC	ADAMA Agricultural Solution, Airport City, 7015103 Israel; www.adama.com
+ quinmerac	+ 40	4		
metamitron	700	5	SC	ADAMA Agricultural Solution
metamitron	700	5	SC	Combination[b]
+ desmedipham	+ 47	5	OD	
+ phenmedipham	+ 60	5		
+ ethofumesate	+ 75	8		
+ lenacil	+ 27	5		

[a] Formulations: EC, emulsifiable concentrate; OD, oily dispersion; SC, soluble concentrate.

[b] Combination of commercial products from ADAMA and Bayer.

Table 3. Herbicide rates and active ingredients tested for soybean stress response using chlorophyll fluorescence imaging.

Herbicide treatment	Rate	WSSA group	Formulation[a]	Company
	g or mL ai ha^{-1}			
metribuzin	262.5	5	WDG	Bayer Crop Science AG, 40789 Monheim am Rhein, D; www.cropscience.bayer.com
+ flufenacet	+ 360	15		
metribuzin	350	5	WDG	Bayer Crop Science AG
+ flufenacet (1.3×)	+ 480	15		
dimethenamid	576	15	EC, SC	BASF Corporation, 67056 Ludwigshafen, D; www.basf.com; Belchim Crop Protection, 1840 Loderzell, B; www.belchim.com
+ clomazone	+ 90	13	CS	
metribuzin	580	5	WDG	Syngenta International AG, 4002 Basel, CH; www.syngenta.com; Bechim Crop Protection
+ clomazone	+ 90	13	CS	
metribuzin	210	5	WDG	Combination[b]
+ dimethenamid	+ 576	15	EC, SC	
+ clomazone	+ 90	13	CS	

[a] Formulations: CS, capsule suspension; EC, emulsifiable concentrate; SC, soluble concentrate; WDG, water dispersible granule.

[b] Combination of three commercial products from Syngenta, BASF, and Belchim.

second trifoliate stages. Measurements were recorded 9, 13 (or 15), and 27 (or 28) DAT. Measurements were taken between 12:00 PM (noon) and 2:00 PM. Plant phytotoxicity was assessed per treatment and DAT, based on the European and Mediterranean Plant Protection Organization (EPPO) standard PP 1/135 concerning phytotoxicity assessment in crops, which identifies modifications in the development cycle (EPPO 2014). Figure 1 includes some representative images of plant phytotoxicity for both crops. For each pot, plant shoot and root biomass were measured as dry biomass 3 and 4 wk after sowing for sugar beet and soybean, respectively. Plants were washed and dried at 85 C for 48 h before dry biomass was measured.

Data Analysis. Measurement data were subjected to an ANOVA at $P \leq 0.05$. In order to evaluate the results of the experiment, a linear mixed-effect model was used. Analyses were performed with the statistics program R version 3.0.2. (R Development Core Team 2014). Years, replications (nested within years), and all interactions between these variables were considered random effects. Considering years as environmental or random effects permits conclusions about treatments to be made over a range of environments (Carmer et al. 1989). Herbicide treatments, cultivars, DAT, and their interactions were considered fixed effects. Prior to analysis, data were checked for normal distribution visually and $\log_{10}$ transformed. Only the biomass data of the sugar beet cultivar experiments were not transformed. For testing the significant differences a Tukey's honest significant difference test was applied ($\alpha = 0.05$). In addition, linear correlation analysis was performed across all four replications of all treatments in each experiment in both years using SigmaPlot (version 12.5, SYSTAT, San Jose, CA) to determine the relationship between crop biomass and F_v/F_m at 1 DAT in sugar beet and at 9 DAT in soybean.

Results and Discussion

Sugar Beet Cultivar Experiment. Both herbicide mixtures caused a significant decrease in F_v/F_m at 1 DAT. However, sugar beet plants rapidly recovered from the herbicide stress. The F_v/F_m values of the treated plants were equal to those of the nontreated control plants (100%) at 3 to 6 DAT (Table 4). Similar results were reported by Voss et al. (1984), Abbaspoor and Streibig (2007), and Roeb et al. (2015). Herbicide stress in sugar beet was strongest 1 DAT with desmedipham plus

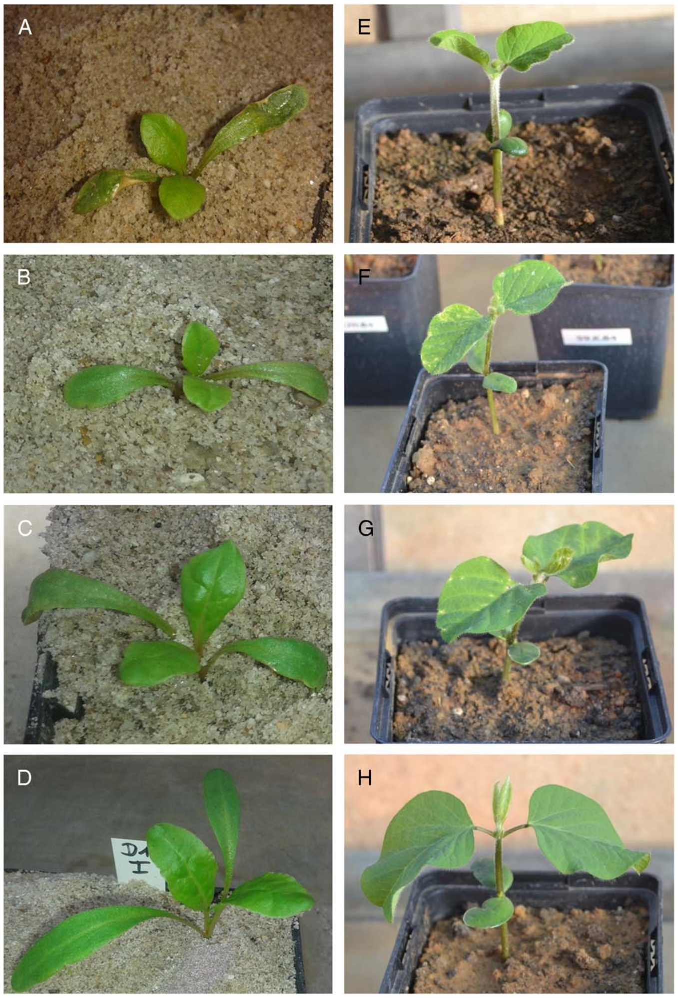

Figure 1. Representative images of sugar beet (A–D) and soybean (E–H) plants 6 and 15 days after herbicide treatment, respectively. Fitness percentage determined using the EPPO phytotoxicity guidelines: A = 50%, B = 60%, C = 90%, and D = 100% (no phytotoxicity); E = 25%, F = 50%, G = 75%, and H = 100% (no phytotoxicity).

phenmedipham plus ethofumesate plus lenacil in the cultivar 'Capella', with an F_v/F_m of only 34%. A similar herbicide mixture without lenacil had an F_v/F_m value of 76% 1 DAT, indicating that lenacil is the most phytotoxic ingredient in the mixture with desmedipham plus phenmedipham plus ethofumesate. Other cultivars, such as 'Beta 1', responded less to desmedipham plus phenmedipham plus ethofumesate plus lenacil, with an F_v/F_m of 68% 1 DAT. In general, the F_v/F_m values of 'Capella', 'Beta 3', and 'Sabrina' were lower compared to those of 'Beta 1', 'Beta 2', 'SES', and 'Isabella', when treated with desmedipham plus phenmedipham plus ethofumesate plus lenacil. Arndt and Kötter (1968) screened 29 sugar beet cultivars for selectivity to phenmedipham, and could not distinguish injury using visual estimation: visual estimation of injury was similar among varieties shortly after herbicide application. In our work, stress reactions in plants were detected using chlorophyll fluorescence imaging where visual estimation did not detect injury. The temporary reaction of fluorescence, which indicates a decrease in photosynthetic activity, may be caused by the herbicide either directly (interference in the electron transport chain) or indirectly (interference of protein restoration in the electron transport chain) affecting photosynthetic efficiency. Recovery to normal fluorescence within a few days indicates a functional protective mechanism that is able to restore normal electron flow. The likely mechanism is a detoxifying conversion of the active herbicide into an inactive metabolite, thus relieving the

Table 4. The relative maximum quantum photosystem II yield (F_v/F_m) and the relative biomass yield of nine sugar beet cultivars 1, 3 and 6 d after different herbicide applications.

	Sugar beet cultivar experiment							
	F_v/F_m[a]						Biomass	
	1 DAT	1 DAT	3 DAT	3 DAT	6 DAT	6 DAT	21 DAT	21 DAT
Cultivar	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil
	%							
'Capella'	**76 ab**[b,c]	**34 c**	**99 –**	**84 b**	99 –	97 –	**81 –**	**51 –**
'Beta 1'	**84 ab**	**68 a**	**99 –**	**94 a**	101 –	100 –	82 –	61 –
'Beta 2'	**86 a**	**64 a**	**100 –**	**96 a**	100 –	99 –	**60 –**	**52 –**
'Beta 3'	**74 b**	**47 bc**	**97 –**	**90 ab**	103 –	101 –	**79 –**	**51 –**
'Sabrina'	**75 ab**	**48 bc**	**98 –**	**90 ab**	**100 –**	**96 –**	68 –	53 –
'Sandra'	**76 ab**	**57 ab**	**99 –**	**92 a**	101 –	99 –	83 –	62 –
'SES'	**80 ab**	**66 a**	**98 –**	**91 ab**	100 –	100 –	61 –	62 –
'Isabella'	78 ab	66 a	97 –	94 a	101 –	100 –	**76 –**	**58 –**
'Belladonna'	**85 ab**	**55 ab**	101 –	96 a	101 –	99 –	65 –	60 –

[a] Abbreviations: DAT, days after treatment; F_v/F_m, the maximum quantum efficacy yield in photosystem II.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$). Means followed by a dash (–) are similar.

[c] Values in bold represent a significant difference based on a two-sided t test between the two herbicides within a cultivar at the same DAT.

inhibitory action, which is measurable as a change of fluorescence from elevated to normal levels.

Visual assessments of injury were similar among all cultivars at 1 DAT (Table 5). Plants treated with desmedipham plus phenmedipham plus ethofumesate plus lenacil (87%) appeared to recover more rapidly, as injury was less compared to that observed with desmedipham plus phenmedipham plus ethofumesate (94%) at 6 DAT. The mixture of desmedipham plus phenmedipham plus ethofumesate reduced sugar beet biomass by 27%, and the mixture of desmedipham plus phenmedipham plus ethofumesate plus lenacil reduced biomass by 43%. Biomass was reduced for most cultivars compared to the nontreated control (Table 4). The lowest and the highest biomass yields were found in cultivars

Table 5. The EPPO visual estimation of herbicide tolerance in nine sugar beet cultivars 1, 3, and 6 d after different herbicide applications.

	Sugar beet cultivar experiment					
	EPPO[a]					
	1 DAT	1 DAT	3 DAT	3 DAT	6 DAT	6 DAT
Cultivar	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil	Desmedipham, phenmedipham, ethofumesate	Desmedipham, phenmedipham, ethofumesate, lenacil
	%					
'Capella'	100 –[b,c]	100 –	**96 –**	**91 –**	**94 –**	**86 –**
'Beta 1'	100 –	100 –	96 –	93 –	**94 –**	**86 –**
'Beta 2'	100 –	100 –	**95 –**	**91 –**	**91 –**	**81 –**
'Beta 3'	100 –	100 –	94 –	95 –	**94 –**	**89 –**
'Sabrina'	100 –	100 –	95 –	93 –	**91 –**	**88 –**
'Sandra'	100 –	100 –	98 –	96 –	95 –	91 –
'SES'	100 –	100 –	**96 –**	**91 –**	93 –	88 –
'Isabella'	100 –	100 –	96 –	94 –	**94 –**	**88 –**
'Belladonna'	100 –	100 –	98 –	95 –	**96 –**	**90 –**

[a] Abbreviations: DAT, days after treatment; EPPO, values of visual estimation following the European and Mediterranean Plant Protection Organization guidelines.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$). Means followed by a dash (–) are similar.

[c] Values in bold represent a significant difference based on a two-sided t test between the two herbicides within a cultivar at the same DAT.

Table 6. The relative maximum quantum photosystem II yield (F_v/F_m) and the relative biomass yield of two sugar beet cultivars ('Capella' and 'Beta') 0.5, 6, and 13 d after different herbicide applications.

Sugar beet herbicide experiment								
	Fv/Fm						Biomass	
	0.5 DAT[a]	0.5 DAT	6 DAT	6 DAT	13 DAT	13 DAT	21 DAT	21 DAT
Herbicide	'Capella'	'Beta 1'	'Capella'	'Beta 1'	'Capella'	'Beta 1'	'Capella'	'Beta 1'
	%							
Control	100 a[b,c]	100 a	100 a	100 a	100 a	100 –	100 a	100 ab
Metamitron	76 b	71 b	96 a	98 a	98 ab	101 –	87 a	78 bc
Metamitron + quinmerac	**90 a**	**81 b**	99 a	96 ab	**98 ab**	**103 –**	**94 a**	**111 a**
Desmedipham + phenmedipham + ethofumesate + lenacil	16 c	21 c	**76 b**	**92 bc**	98 ab	100 –	38 c	50 cd
Desmedipham + phenmedipham + ethofumesate	17 c	18 c	100 a	101 a	100 ab	102 –	63 b	55 cd
Metamitron + desmedipham + phenmedipham + ethofumesate + lenacil	16 c	17 c	**66 b**	**88 c**	**95 b**	**100 –**	23 c	32 d

[a] Abbreviations: DAT, days after treatment; F_v/F_m, the maximum quantum efficacy yield in photosystem II.

[b] Means followed by a different letter within a column are significantly different according to the Tukey honest significant difference test ($P \leq 0.05$). Means followed by a dash (–) are similar.

[c] Values bold represent a significant difference based on a two-sided t test between the two cultivars within an herbicide at the same DAT.

'Capella' and 'SES' treated with desmedipham plus phenmedipham plus ethofumesate plus lenacil, which showed growth reductions of 49% and 38%, respectively, compared to the nontreated control.

Sugar Beet Herbicide Experiment. The responses of the two sugar beet cultivars to different herbicides and herbicide mixtures varied significantly. The strongest stress 0.5 DAT was measured for desmedipham plus phenmedipham plus ethofumesate plus lenacil, with 18.5% F_v/F_m; desmedipham plus phenmedipham plus ethofumesate with 17.5% F_v/F_m; and metamitron plus desmedipham plus phenmedipham plus ethofumesate plus lenacil with 16.5% F_v/F_m. Soil-active herbicides that are mostly taken up by the plant roots had much lower effects, with only 73.5% F_v/F_m after metamitron and 85.5% F_v/F_m after metamitron plus quinmerac (Goltix Titan®, ADAMA Agricultural Solution, Airport City, Israel), averaged across both cultivars (Table 6). Again, sugar beet recovered rapidly from herbicide stress, with a faster recovery of 'Beta 1' than of 'Capella'. At 13 DAT, F_v/F_m of sugar beet varieties ranged from 95% to 100%, regardless of herbicide treatment. Visual estimations of herbicide stress 6 DAT ranged from 31% crop injury with metamitron plus desmedipham plus phenmedipham plus ethofumesate plus lenacil to 1% crop injury with metamitron and metamitron plus quinmerac treatments (Table 7). Sugar beet dry biomass was reduced by up to 77% compared to that of the nontreated control in the treatment of metamitron plus desmedipham plus phenmedipham plus ethofumesate plus lenacil (Table 6). Other researchers have also observed sugar beet damage after application of desmedipham plus phenmedipham plus ethofumesate plus lenacil (Smith and Schweizer 1983; Starke and Renner 1996; Wilson 1999).

Soybean Cultivar Experiment. Metribuzin plus flufenacet reduced F_v/F_m in soybean to 78%, and metribuzin plus clomazone reduced it to 65%, compared to that of the nontreated control (Table 8). Salzman and Renner (1992) observed decreased soybean leaf area after application of a metribuzin dose of 420 g ha^{-1} compared to that observed after a lower dose of 280 g ha^{-1}. These findings are consistent with the results of our study. The lowest F_v/F_m values were measured in the cultivars 'ES Mentor' and 'Sultana' (47% and 57%, respectively), after the application of metribuzin plus flufenacet (Table 8). The F_v/F_m values of both cultivars were different from that of the nontreated control and from those of the most other cultivars in the metribuzin plus flufenacet treatment. At 18 DAT, both cultivars had slightly recovered, yet they continued to have reduced chlorophyll fluorescence (63% and 74% F_v/F_m, respectively), compared to 'Lissabon' (90%), 'SY Eliot' (92%), 'Merlin' (94%), 'Solena' (95%), and 'Gallec' (96%). Similar results for cultivars 'ES Mentor' and 'Sultana' were observed when treated with metribuzin plus

Table 7. The EPPO visual estimation of herbicide tolerance in two sugar beet cultivars ('Capella' and 'Beta 1') 0.5, 6, and 13 d after different herbicide applications.

Sugar beet herbicide experiment						
	EPPO					
	0.5 DAT[a]	0.5 DAT	6 DAT	6 DAT	13 DAT	13 DAT
Herbicide	'Capella'	'Beta 1'	'Capella'	'Beta 1'	'Capella'	'Beta 1'
	%					
Control	100 –[b,c]	100 –	100 a	100 a	100 –	100 –
Metamitron	100 –	100 –	100 a	100 a	100 –	95 –
Metamitron + quinmerac	100 –	100	100 a	100 a	94 –	100 –
Desmedipham + phenmedipham + ethofumesate + lenacil	100 –	100 –	94 ab	89 b	100 –	98 –
Desmedipham + phenmedipham + ethofumesate	100 –	100 –	81 b	76 b	98 –	91 –
Metamitron + desmedipham + phenmedipham + ethofumesate + lenacil	100 –	100 –	63 c	69 c	98 –	96 –

[a] Abbreviations: DAT, days after treatment; EPPO, values of visual estimation following the European and Mediterranean Plant Protection Organization guidelines.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$). Means followed by a dash (–) are similar.

[c] No differences were found between the two cultivars within an herbicide at the same DAT.

clomazone. Even at 18 DAT, the F_v/F_m value of ES Mentor (55%) was substantially lower than that of 'Gallec', 'Lissabon', 'SY Eliot' (all 91%) and 'Solena' (94%). At 28 DAT, all soybean cultivars still had not fully recovered, with the lowest F_v/F_m values of 81% ('Sultana') in the metribuzin plus flufenacet treatment and 69% ('ES Mentor') in the metribuzin plus clomazone treatment (Table 8).

Soybean cultivars responded differently to herbicide treatment. The chlorophyll fluorescence imaging sensor can be used to quantify herbicide tolerance in different cultivars. This is of great importance for soybean breeding and practical soybean production. Barrentine et al. (1982) and Osborne et al. (1995) also screened soybean cultivars for herbicide tolerance. They used root length and visual characteristics to determine tolerance

Table 8. The relative maximum quantum photosystem II yield (F_v/F_m) and the relative biomass yield of seven soybean cultivars 9, 18, and 28 d after different herbicide applications.

Soybean cultivar experiment								
	F_v/F_m[a]						Biomass	
Cultivar	9 DAT	9 DAT	18 DAT	18 DAT	28 DAT	28 DAT	32 DAT	32 DAT
Treatment	Metribuzin, flufenacet	Metribuzin, clomazone	Metribuzin, flufenacet	Metribuzin, clomazone	Metribuzin, flufenacet	Metribuzin, clomazone	Metribuzin, flufenacet	Metribuzin, clomazone
	%							
'Sultana'	**57 bc**[b,c]	**41 cd**	74 bc	71 ab	81 b	85 a	54 ab	16 d
'Solena'	96 a	91 a	95 a	94 a	**99 a**	**91 a**	**92 a**	**69 abc**
'ES Mentor'	**47 c**	**26 d**	63 c	55 b	87 ab	69 b	8 c	25 cd
'Gallec'	91 a	83 ab	96 a	91 a	90 ab	86 a	89 a	87 ab
'Merlin'	**84 ab**	**64 bc**	**94 ab**	**79 ab**	**91 ab**	**82 a**	50 b	49 acd
'Lissabon'	79 ab	61 bcd	90 ab	91 a	**91 ab**	**100 a**	61 ab	56 abc
'SY Eliot'	91 a	90 a	92 ab	91 a	97 a	90 a	89 ab	89 a

[a] Abbreviations: DAT, days after treatment; F_v/F_m, the maximum quantum efficacy yield in photosystem II.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$).

[c] Values bold represent a significant difference based on a two-sided t test between the two herbicides within a cultivar at the same DAT.

Table 9. The EPPO visual estimation of herbicide tolerance in seven soybean cultivars 9, 18, and 28 d after different herbicide applications.

Soybean cultivar experiment						
	EPPO[a]					
Cultivar	9 DAT	9 DAT	18 DAT	18 DAT	28 DAT	28 DAT
Treatment	Metribuzin, flufenacet	Metribuzin, clomazone	Metribuzin, flufenacet	Metribuzin, clomazone	Metribuzin, flufenacet	Metribuzin, clomazone
	%					
'Sultana'	98 ab[b,c]	97 a	**44 c**	**33 c**	**76 c**	**61 c**
'Solena'	100 a	98 a	**98 a**	**87 a**	**95 a**	**76 c**
'ES Mentor'	**99 ab**	**92 b**	**40 c**	**17 d**	**84 b**	**65 d**
'Gallec'	100 a	100 a	100 a	99 a	98 a	97 a
'Merlin'	100 a	99 a	99 a	94 a	**100 a**	**87 b**
'Lissabon'	97 b	97 a	**75 b**	**67 b**	100 a	97 a
'SY Eliot'	99 ab	100 a	97 a	88 a	98 a	96 a

[a] Abbreviations: DAT, days after treatment; EPPO, values of visual estimation following the European and Mediterranean Plant Protection Organization guidelines.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$).

[c] Values in bold represent a significant difference based on a two-sided t test between the two herbicides within a cultivar at the same DAT.

of soybean cultivars to herbicides, and reported that different soybean cultivars vary in herbicide sensitivity. EPPO assessments of herbicide damage 18 DAT showed 74% crop injury when averaged across all cultivars and herbicides (Table 9). 'Sultana' and 'ES Mentor' were the most sensitive cultivars, with 39% and 29% injury. The herbicide mixtures metribuzin plus flufenacet and metribuzin plus clomazone reduced dry biomass by 37% and 44%, respectively (Table 8).

Soybean Herbicide Experiment. Cultivar 'Gallec' was more tolerant to herbicides than was 'ES Mentor' for all herbicide treatments. F_v/F_m values for cultivar 'Gallec' were equal for all herbicide treatments (Table 10). The F_v/F_m was not reduced by the application of dimethenamid plus clomazone (Spectrum® plus Centium®). The cultivar 'ES Mentor', however, only achieved 25% F_v/F_m values 9 DAT with the higher dose of metribuzin plus flufenacet.

Table 10. The relative maximum quantum photosystem II yield (F_v/F_m) and the relative biomass yield of two soybean cultivars ('Gallec' and 'ES Mentor') 9, 13, and 27 d after different herbicide applications.

Soybean herbicide experiment[a]								
	F_v/F_m						Biomass	
	9 DAT	9 DAT	13 DAT	13 DAT	27 DAT	27 DAT	32 DAT	32 DAT
Herbicide	'Gallec'	'ES Mentor'	'Gallec'	'ES Mentor'	'Gallec'	'ES Mentor'	'Gallec'	'ES Mentor'
	%							
Control	100 –[b,c]	100 a	100 a	100 a	100 a	100 –	100 a	100 a
Dimethenamid + clomazone	100 –	99 a	98 a	98 a	99 a	99 –	90 ab	87 ab
Metribuzin + flufenacet	**95 –**	**67 b**	**86 b**	**60 bc**	88 b	96 –	**70 b**	**53 bc**
Metribuzin + flufenacet (1.3×)	**98 –**	**25 c**	**79 b**	**40 c**	89 b	96 –	**63 b**	**19 c**
Metribuzin + dimethenamid + clomazone	**98 –**	**71 b**	**97 a**	**71 abc**	97 ab	94 –	**88 ab**	**63 ab**

[a] Abbreviations: DAT, day after treatment; F_v/F_m, the maximum quantum efficacy yield in photosystem II.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$). Means followed by a dash (–) are similar.

[c] Values in bold represent a significant difference based on a two-sided t test between the two cultivars within an herbicide at the same DAT.

Also, plants treated with metribuzin plus flufenacet and metribuzin plus dimethenamid plus clomazone (Sencor® plus Spectrum® plus Centium®) had considerably lower F_v/F_m values than did plants in the nontreated control and plants treated with dimethenamid plus clomazone at 9 DAT. After a period of 28 d, soybean plants recovered and F_v/F_m values reached 88% (Table 10). In our study, treatments containing metribuzin resulted in the highest F_v/F_m yield losses in both cultivars. Poston et al. (2008) observed that plant stress increased as a result of higher metribuzin concentrations in combination with different active ingredients. It is well known that soybean cultivars exhibit different levels of tolerance to metribuzin. Cultivar-specific herbicide sensitivity stems from differing degradation capacities of cultivars (Smith and Wilkinson 1974). The metribuzin product label contains a list of sensitive crop cultivars, and for years a continuous screening of the sensitivity of new cultivars for the level of metribuzin tolerance was conducted in the United States by Mobay/Bayer Corporation. In these studies, herbicide stress was evaluated using different plant parameters such as root length, visual estimations, plant height, and comparison of yields. All the above parameters have a long assessment time span, and therefore can be affected by various environmental factors. We did not observe visual injury when assessments were recorded according to EPPO guidelines at 9 DAT (Table 11), whereas F_v/F_m at 13 DAT with the high rate of metribuzin plus flufenacet was 34% in cultivar ES Mentor and 70% in cultivar Gallec. In our study, chlorophyll fluorescence imaging could detect soybean response to herbicides more accurately than could visual estimations (see Tables 8–11). The dry biomass across both cultivars was reduced by 33% after different herbicide applications (Table 10).

Correlation Analysis. Dry biomass of different sugar beet cultivars did not correlate well with the first measurement of F_v/F_m at 1 DAT ($R^2 = 0.29$ in 2015 and $R^2 = 0.34$ in 2016) (Figure 2). In the sugar beet herbicide experiment, plant dry matter at the end of the experiment had a positive correlation ($R^2 = 0.51$ in 2015 and $R^2 = 0.64$ in 2016) with the first measurement of F_v/F_m. In the soybean cultivar experiment, the F_v/F_m values at 9 DAT correlated with the biomass yield, with R^2 values of 0.41 and 0.48 in 2015 and 2016, respectively. The highest R^2 between soybean biomass and F_v/F_m yield was calculated for the herbicide experiment at 9 DAT, with R^2 values of 0.36 and 0.67 in 2015 and 2016, respectively. Since plants recover rapidly, biomass data 3 to 4 wk after application do not confirm herbicide stress in the same way that chlorophyll fluorescence data does shortly after application. Assessing chlorophyll fluorescence enabled differentiation of herbicide influences on the photosynthetic activity of the crop. As the influence was of

Table 11. The EPPO visual estimation of herbicide tolerance in two soybean cultivars ('Gallec' and 'ES Mentor') 9, 13, and 27 d after different herbicide applications.

	Soybean herbicide experiment					
	EPPO[a]					
	9 DAT	9 DAT	13 DAT	13 DAT	27 DAT	27 DAT
Herbicide	'Gallec'	'ES Mentor'	'Gallec'	'ES Mentor'	'Gallec'	'ES Mentor'
	%					
Control	100 –[b,c]	100 –	100 a	100 a	100 a	100 a
Dimethenamid + clomazone	100 –	100 –	99 a	96 a	99 a	98 a
Metribuzin + flufenacet	99 –	97 –	70 b	53 b	79 ab	65 b
Metribuzin + flufenacet (1.3×)	98 –	97 –	**70 b**	**34 b**	61 b	46 b
Metribuzin + dimethenamid + clomazone	100 –	100 –	96 a	96 a	99 a	100 a

[a] Abbreviations: DAT, day after treatment; EPPO, values of visual estimation following the European and Mediterranean Plant Protection Organization guidelines.

[b] Means followed by a different letter within a column are significantly different according to Tukey's honest significant difference test ($P \leq 0.05$). Means followed by a dash (–) are similar.

[c] Values in bold represent a significant difference based on a two-sided t test between the two cultivars within a herbicide at the same DAT.

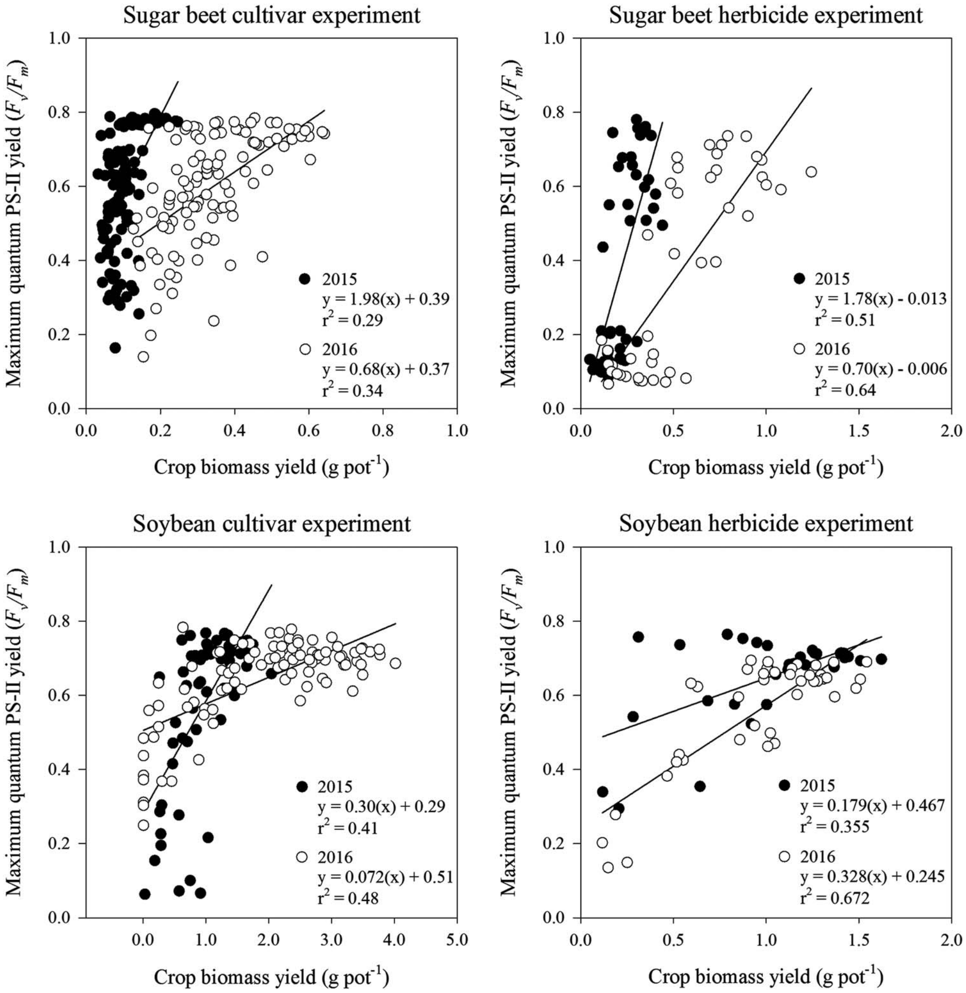

Figure 2. Correlation between the maximum quantum photosystem II yield (F_v/F_m), 1 day after treatment in sugar beet and 9 days after treatment in soybean, and crop biomass yield (grams per pot) 3 to 4 wk after treatment for cultivar and herbicide experiments. Each data point on the graph represents one replication of sugar beet or soybean within different herbicide treatments and cultivars in 2015 or 2016.

short duration, biomass often does not reflect the treatment effect because differences in biomass would only occur with a more sustained treatment effect. This effect can be reduced by decelerated crop development caused by field conditions (Wilson 1999). For rockcress [*Arabidopsis arenosa* (L.) Lawalrée] plants treated with imazapyr, Barbagallo et al. (2003) found a strong relationship ($R^2 = 0.85$) between leaf area and the F_v/F_m values of the plants.

In summary, stress was detected in both crops after herbicide application. Sugar beet had a more abrupt decrease of F_v/F_m shortly after application compared to that of soybean. However, sugar beet recovered from herbicide stress after a few days. Soybean, in contrast, needed a period of 28 d to recover. Soybean cultivars differed in their response to herbicides, while sugar beet cultivars reacted more uniformly.

Chlorophyll fluorescence imaging has the potential to evaluate crop stress caused by herbicide applications in sugar beet more effectively than do visual estimations, especially in the first few days after herbicide application (Tables 4–7). Assessments of F_v/F_m can supplement or even replace traditional estimations of phytotoxicity and herbicide selectivity in crops. The nondestructive nature of the method can make sensor measurements an important supplementary tool in herbicide evaluation. The sensor can quantify stress symptoms earlier and more accurately than can visual evaluations, according to EPPO phytotoxicity guidelines.

The current study demonstrates the use of chlorophyll fluorescence imaging to analyze the reaction of sugar beet and soybean plants to PS-II–inhibiting herbicides under controlled conditions. Since the method was successful, there are no principal obstacles preventing the use of this method on other crops or even weeds. A greater, but worthwhile, challenge may be to attempt to bring this laboratory method into the highly variable environment of practical crop production in growers' fields. The maximum quantum yield efficiency of PS-II, which is measured with the chlorophyll fluorescence imaging sensor, provides direct information on PS-II. Yet more research needs to be done, as chlorophyll fluorescence induction can be affected by external factors like plant vigor, water stress, pathogens, or environmental conditions such as temperature. As Barbagallo et al. (2003) proposed, evaluation of chlorophyll fluorescence can be used for a broad range of herbicides with different modes of action, although the method is most easily adapted to assessing crop injury with herbicides that affect light-dependent plant processes. Those potential targets include bleaching herbicides like inhibitors of phytoene-desaturase or 4-hydroxyphenylpyruvatdioxygenase, inhibitors of PS-I and PS-II, and inhibitors of protoporphyrinogen oxidase. Future work should focus on evaluating fluorescence analysis with herbicides that affect photosynthesis indirectly (Dayan and Zaccaro 2012).

Acknowledgments

We thank KWS SAAT SE, Strube GmbH & Co. KG, SESVanderHave, Betaseed GmbH, and Janina Schmid from LTZ Augustenberg for providing us with sugar beet and soybean cultivars.

Literature Cited

Abbaspoor M, Streibig JC (2007) Monitoring the efficacy and metabolism of phenylcarbamates in sugar beet and black nightshade by chlorophyll fluorescence parameters. Pest Manag Sci 63:576–585

Andújar D, Ribeiro A, Carmona R, Fernández-Quintanilla C, Dorado J (2010) An assessment of the accuracy and consistency of human perception of weed cover. Weed Res 50:638–647

Arndt F, Kötter C (1968) Zur Selektivität von Phenmedipham als Nachauflaufherbizid in Beta-Rüben [Selectivity of phenmedipham as a post-emergence herbicide in sugar beet]. Weed Res 8:259–271

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. Annu Rev Plant Biol 59:89–113

Barbagallo RP, Oxborough K, Pallett KE, Baker NR (2003) Rapid, noninvasive screening for perturbations of metabolism and plant growth using chlorophyll fluorescence imaging. Plant Physiol 132:485–493

Barrentine WL, Hartwig EE, Edwards CJ (1982) Tolerance of three soybean (*Glycine max*) cultivars to metribuzin. Weed Sci 30:344–348

Belfry KD, Soltani N, Brown LR, Sikkema PH (2015) Tolerance of identity preserved soybean cultivars to preemergence herbicides. Can J Plant Sci 95:719–726

Burke JJ, Franks CD, Burow G, Xin Z (2010) Selection system for the stay-green drought tolerance trait in sorghum germplasm. Agron J 102:1118–1122

Carmer SG, Nyquist WE, Walker WM (1989) Least significant differences for combined analyses of experiments with two-or three-factor treatment designs. Agron J 81: 665–672

Dayan FE, Zaccaro MLM (2012) Chlorophyll fluorescence as a marker for herbicide mechanisms of action. Pestic Biochem Physiol 102:189–197

Donald WW (1998) Estimated soybean (*Glycine max*) yield loss from herbicide damage using ground cover or rated stunting. Weed Sci 46:454–458

[EPPO] European and Mediterranean Plant Protection Organization. (2014) PP 1/135 (4) Phytotoxicity assessment. European and Mediterranean Plant Protection Organization Bulletin 44:265–273

Fahlgren N, Gehan MA, Baxter I (2015) Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. Curr Opin Plant Biol 24:93–99

Fiorani F, Schurr U (2013) Future scenarios for plant phenotyping. Annu Rev Plant Biol 64:267–291

Gehring K, Festner T, Gerhards R, Hüsgen K, Thyssen S (2014) Chemical weed control in soybean (*Glycine max*, L.). Pages 701–708 *in* 26th German Conference on Weed Biology and Weed Control. Braunschweig, Germany: Julius Kühn Institut, Bundesforschungsinstitut für Kulturpflanzen

Kaiser YI, Menegat A, Gerhards R (2013) Chlorophyll fluorescence imaging: a new method for rapid detection of herbicide resistance in *Alopecurus myosuroides*. Weed Res 53: 399–406

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. J Exp Bot 51:659–668

Moseley C, Hatzios KK, Hagood ES (1993) Uptake, translocation, and metabolism of chlorimuron in soybean (*Glycine max*) and morningglory (*Ipomoea* spp.). Weed Technol 7: 343–348

Osborne BT, Shaw DR, Ratliff RL (1995) Soybean (*Glycine max*) cultivar tolerance to SAN 582H and metolachlor as influenced by soil moisture. Weed Sci 43:288–292

Poston DH, Nandula VK, Koger CH, Griffin RM (2008) Preemergence herbicides effect on growth and yield of early-planted Mississippi soybean. Crop Manag. doi: 10.1094/CM-2008-0218-02-RS

R Development Core Team (2014) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 409 p

Roeb J, Peteinatos GG, Gerhards R (2015) Using sensors to assess herbicide stress in sugar beets. Pages 563–570 *in* Stafford JV, ed. Precision Agriculture´15. Netherlands: Wageningen Academic Publishers

Salzman FP, Renner KA (1992) Response of soybean to combinations of clomazone, metribuzin, linuron, alachlor, and atrazine. Weed Technol 6:922–929

Smith AE, Wilkinson RE (1974) Differential absorption, translocation and metabolism of metribuzin [4-amino-6-tert-butyl-3-(metbylthio)-as-triazine-5(4H)one] by soybean cultivars. Physiol Plant 32:253–257

Smith GA, Schweizer EE (1983) Cultivar X herbicide interaction in sugar beet. Crop Sci 23:325–328

Starke RJ, Renner KA (1996) Velvetleaf (*Abutilon theophrasti*) and sugar beet (*Beta vulgaris*) response to triflusulfuron and desmedipham plus phenmedipham. Weed Technol 10:121–126

Thenkabail PS, Lyon JG, Huete A, eds (2011) Hyperspectral Remote Sensing of Vegetation. Boca Raton, FL: CRC Press. 641 p

Vasel EH, Ladewig E, Märländer B (2012) Weed composition and herbicide use strategies in sugar beet cultivation in Germany. J für Kult 64:112–125

Voss M, Renger G, Kötter C, Gräber P (1984) Fluorometric detection of photosystem II herbicide penetration and detoxification in whole leaves. Weed Sci 32:675–680

Wilson RG (1999) Response of nine sugar beet (*Beta vulgaris*) cultivars to postemergence herbicide application. Weed Technol 13:25–29

Wilson RG, Yonts CD, Smith JA (2002) Influence of glyphosate and glufosinate on weed control and sugar beet (*Beta vulgaris*) yield in herbicide-tolerant sugar beet. Weed Technol 16: 66–73

Received December 8, 2015, and approved March 11, 2017.

Associate Editor for this paper: Ian Burke, Washington State University.