Weed Science 2014 62:217–229

Foliar Application of Glyphosate Affects Molecular Mechanisms in Underground Adventitious Buds of Leafy Spurge (*Euphorbia esula*) and Alters Their Vegetative Growth Patterns

Münevver Doğramacı, James V. Anderson, Wun S. Chao, and Michael E. Foley*

Long-term control of leafy spurge with glyphosate requires multiple applications because the plant reproduces vegetatively from abundant underground adventitious buds, referred to as crown and root buds. Determining the molecular mechanisms involved in controlling vegetative reproduction in leafy spurge following foliar glyphosate treatment could identify limiting factors or new targets for manipulation of plant growth and development in invasive perennial species. Thus, we treated leafy spurge plants with 0 or 2.24 kg ai ha^{-1} glyphosate to determine its impact on selected molecular processes in crown buds derived from intact plants and plants decapitated at the soil surface 7 d after glyphosate treatment. New shoot growth from crown buds of foliar glyphosate-treated plants was significantly reduced compared with controls after growth-inducing decapitation, and had a stunted or bushy phenotype. Quantification of a selected set of transcripts involved in hormone biosynthesis and signaling pathways indicated that glyphosate had the most significant impact on abundance of *ENT-COPALYL DIPHOSPHATE SYNTHETASE 1*, which is involved in a committed step for gibberellin biosynthesis, and auxin transporters including *PINs, PIN-LIKES*, and *ABC TRANSPORTERS*. Foliar glyphosate treatment also reduced the abundance of transcripts involved in cell cycle processes, which would be consistent with altered growth patterns observed in this study. Overall, these results suggest that interplay among phytohormones such as auxin, ethylene, and gibberellins affect vegetative growth patterns from crown buds of leafy spurge in response to foliar glyphosate treatment.

Nomenclature: Glyphosate; leafy spurge, *Euphorbia esula* L.

Key words: Auxin transporters, endoplasmic reticulum, hormones, stunted and bushy phenotype, transcript abundance.

Leafy spurge is an invasive perennial weed infesting rangelands, right-of-ways, and noncultivated areas in the Great Plains of the United States and Canada. Leafy spurge reproduces vegetatively from an abundance of underground adventitious buds (UABs), commonly referred to as crown and root buds, which exhibit well-defined phases of seasonal dormancy (Anderson et al. 2005). Integrated biological, chemical, cultural, and mechanical control measures have reduced infestations of leafy spurge in some areas, although these measures sometimes are impractical for economic reasons or lack efficacy in certain environments. For example, in sandy soil, leafy spurge is often difficult to manage with biological control agents such as flea beetles (*Aphthona* spp.) (Lym and Nelson 2002). Thus, leafy spurge control generally entails a long-term management program requiring consistent follow-up. Some integrated leafy spurge management approaches incorporate the use of herbicides such as glyphosate for long-term control (Lym and Messersmith 2013).

Glyphosate is a broad-spectrum herbicide used worldwide in agricultural systems. It translocates in a source-to-sink manner to meristematic tissues, including organs capable of vegetative reproduction in perennial plants (Shaner 2009). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is a nuclear-encoded and chloroplast-localized enzyme (Della-Cioppa et al. 1986) of the shikimate biosynthetic pathway; inhibition of EPSPS results in the accumulation of shikimate (Amrhein et al. 1980; Steinrucken and Amrhein 1980). More specifically, glyphosate inhibits EPSPS-catalyzed conversion of shikimate-3-phosphate (S3P) to EPSP by forming an EPSPS–S3P–glyphosate

DOI: 10.1614/WS-D-13-00156.1

* Postdoctoral Plant Molecular Biologist, Research Chemist (ORCID: 0000-0002-1801-5767), Research Molecular Geneticist, and Supervisory Research Plant Physiologist, USDA-Agricultural Research Service, Biosciences Research Lab, Sunflower and Plant Biology Research Unit, 1605 Albrecht Boulevard N., Fargo, ND 58102. Corresponding author's E-mail: munevver.dogramaci@ars.usda.gov. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

complex that competes for phosphoenolpyruvate binding (Boocock and Coggins 1983; Franz et al. 1997; Gruys et al. 1992). These are critical steps for the production of chorismate, which is a precursor of aromatic amino acids (phenylalanine, tyrosine, and tryptophan), auxin, and many other secondary products essential for plant growth and development. The lack of downstream aromatic amino acids required for protein synthesis and other metabolic processes is generally considered the primary effect leading to plant death (Duke and Powles 2008); however, it has also been suggested that inhibition of EPSPS likely causes shikimate to accumulate faster than it can be consumed in other metabolic pathways, leading to lethality (Herrmann and Weaver 1999).

Leafy spurge and several other laticiferous perennial weeds such as hemp dogbane (*Apocynum cannabinum* L.) (Wyrill and Burnside 1976) are somewhat tolerant to glyphosate. Glyphosate (0.8 kg ha^{-1}) can provide ~80–90% leafy spurge control 12 mo after the first treatment, but follow-up applications are required for long-term control (Lym 2000; Lym and Messersmith 2013). Maxwell et al. (1987) reported that glyphosate was readily absorbed and glyphosate or its metabolites translocate and accumulate in roots and UABs of leafy spurge plants when applied at all phenological stages (i.e., prebloom, full bloom, and senescence), and high concentrations (~2–6 kg ha^{-1}) induced uncontrolled growth patterns from UABs in subsequent years. Because UABs are generally in a paradormant state during the active growing season and remain relatively quiescent unless they are released from paradormancy by sectioning of roots or decapitation of the aerial portion of the plant (Foley et al. 2009; Raju et al. 1964), the induction of uncontrolled growth of UABs in response to glyphosate could provide new insights on molecular mechanisms affecting vegetative reproduction.

So far, the impact of glyphosate treatment on global gene expression has mainly focused on leaf tissues of annuals such as soybean (*Glycine max* L.) (Zhu et al. 2008) and horseweed [*Conyza canadensis* (L.) Cronq.] (Peng et al. 2010; Yuan et al. 2010), or perennials such as turf grasses (*Festuca* spp.) (Cebeci and Budak 2009). In the case of turf grasses, Cebeci and Budak (2009) reported that glyphosate treatment led to decreased abundance of transcripts involved in the detoxification of reactive oxygen species, and major changes in transcript abundance were observed in over 20 functional categories, including photosynthesis, metabolism, protein synthesis and fate, transport and mechanisms, and energy. Additionally, ABC transporters, cytochrome P450 mono-oxygenases, and glycosyltransferases have been shown to be overexpressed in response to glyphosate treatment, and these gene families have been proposed to play some role in glyphosate resistance in horseweed (Peng et al. 2010), tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Riggins et al. 2010), and several other weeds (reviewed by Yuan et al. 2007).

In this study, the effects of foliar glyphosate treatment on molecular mechanisms and vegetative growth responses from crown buds of leafy spurge plants are reported. The objectives of this study are to evaluate vegetative growth from crown buds of leafy spurge plants treated with sublethal concentrations of foliar-applied glyphosate, and to monitor changes in transcript abundance in these crown buds. Selection of transcripts used in this study is based on (1) previously reported modes of glyphosate action, (2) its impact on molecular mechanisms or pathways involved in phytohormone biosynthesis and signaling, and (3) transcripts known to be involved in regulating dormancy in leafy spurge (Doğramacı et al. 2010, 2011, 2013). The outcomes from this discovery-driven approach will help to identify molecular mechanisms and pathways affected by glyphosate, or its impact on the regulation of bud dormancy, which should lead to testable hypothesis-driven studies. Identifying insights into regulatory networks that could be novel targets for reducing the bud bank of invasive perennial weeds or make them more susceptible to alternative control techniques will contribute to next-generation approaches for weed management.

Materials and Methods

Plant Material, Glyphosate Treatments, and Vegetative Growth. Leafy spurge plants were propagated from a uniform biotype (1984-ND001) in cone-tainers and maintained in a greenhouse as described by Anderson and Davis (2004). In preliminary studies (data not shown) to determine a sublethal dose, 4-mo-old greenhouse-grown leafy spurge plants were treated with 2.24, 4.48, or 6.72 kg ai ha^{-1} rates of glyphosate (Buccanneer Plus®, 480 g ai L^{-1}, Tenkoz, Inc., Alpharetta, GA) plus 0.25% of surfactant (Class Act® NG® surfactant Agrisolutions—Winfield Solutions, LLC, St. Paul, MN) using a SS8001 nozzle delivering 95 L ha^{-1} solution in a positive airflow hood (DeVries Manufacturing Co., Hollandale, MN). In this study, 2.24 kg ha^{-1} glyphosate rate was chosen for further studies, with 0 kg ha^{-1}

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

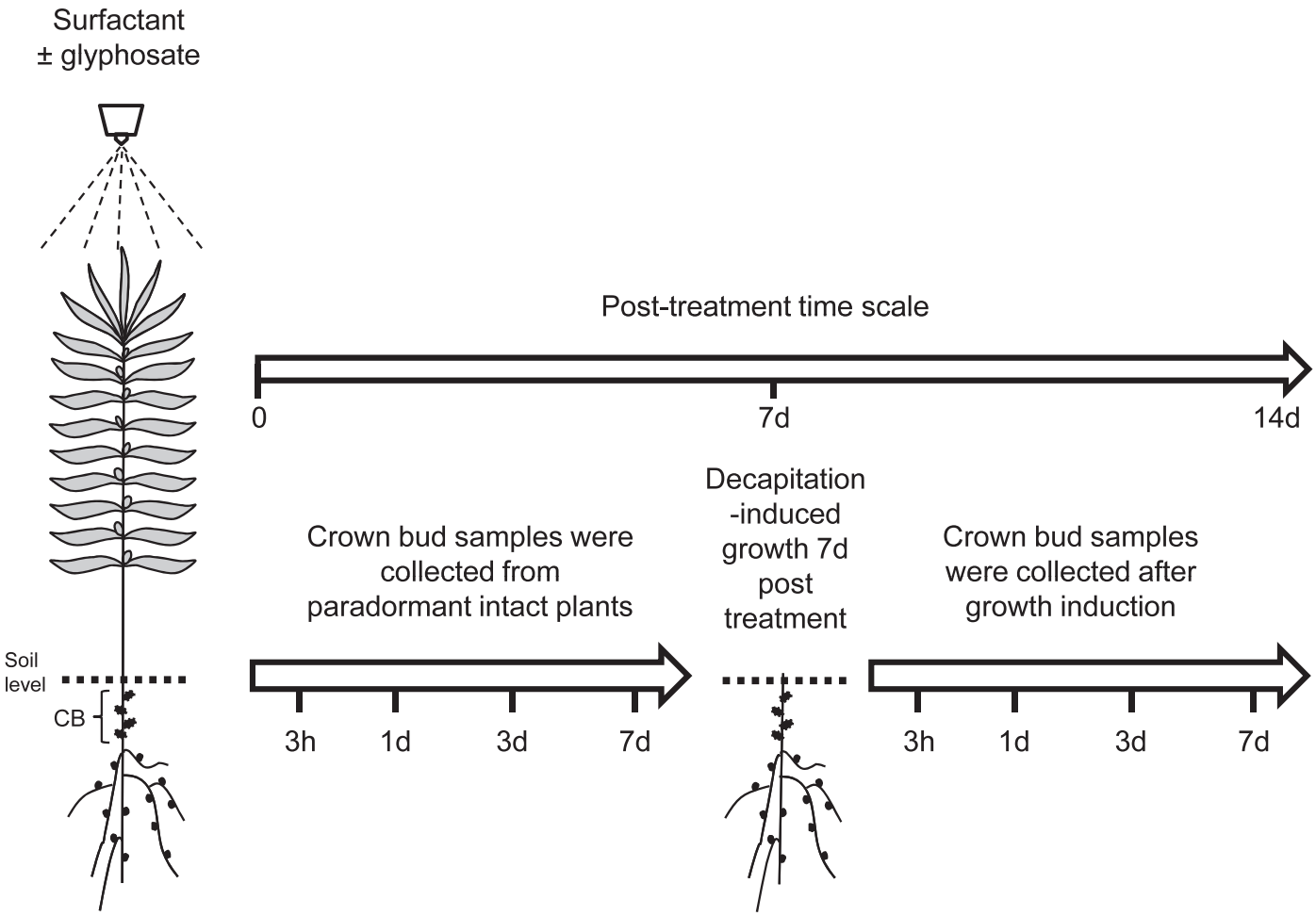

Figure 1. Leafy spurge plants were treated with surfactant ± 2.24 kg ha^{-1} glyphosate, and were kept under growth-conducive conditions until crown bud (CB) collection; CB samples were collected over a 14-d timescale from intact or decapitated plants. Paradormant CB were collected from intact plants at 3 h and 1, 3, and 7 d after foliar treatment, whereas growth-induced CB were collected at 3 h and 1, 3, and 7 d after decapitation. These CB were used to quantify changes in transcript abundance; values obtained from glyphosate-treated plants (surfactant + glyphosate) were compared with control plants (surfactant alone) at each time point.

as the control, and both rates included surfactant. Plants were returned to growth-conducive conditions in the greenhouse after glyphosate treatment until time of crown bud collection. Each experiment included four biological replicates containing 86 plants. Six plants from each replicate were decapitated 7 d postglyphosate treatment, and kept in the greenhouse under growth-conducive conditions to determine the vegetative growth rate as previously described by Foley et al. (2009). New vegetative shoot growth (height) was recorded weekly for 6 wk; results were analyzed using the generalized linear mixed model (PROC GLIMMIX) procedure of SAS 9.2, and 95% confidence intervals for treatment-by-week means were generated.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Analysis. To study changes in gene expression by qRT-PCR, crown buds were collected and pooled for each time point and stored at −80 C. We examined transcript abundance for ~200 genes in two separate sets of crown buds from glyphosate-treated leafy spurge plants (Figure 1). The first set included paradormant crown buds collected from intact plants at 3 h, 1 d, 3 d, and 7 d after the glyphosate treatments (0 and 2.24 kg ha^{-1}). For the second set, glyphosate-treated and control plants were maintained under growth-conducive conditions for 7 d, then aerial tissues were decapitated to induce vegetative growth, and crown buds were collected from the decapitated plants after 3 h, 1 d, 3 d, and 7 d. RNA extraction and complementary (c)DNA synthesis was done as described by Doğramacı et al. (2010). Sequences from a leafy spurge EST database (Anderson et al. 2007) were used for designing primer pairs using the Primer-Select of Lasergene 8 software program (DNASTAR, Inc., Madison, WI). These primer

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

pairs were used to quantify abundance of transcripts associated with various hormonal pathways (i.e., abscisic acid [ABA], auxin, cytokinin, ethylene, gibberellic acid [GA]), cell cycle processes, and bud dormancy and vegetative growth regulation (see Supplemental Material-1; http://dx.doi.org/10.1614/WS-D-13-00156.SM1). Many of these pathways can be viewed at http://pmn.plantcyc.org/PLANT/class-tree?object=Pathways (Plant Metabolic Network 2008). In brief, 20 µg of total RNA was treated with DNase2 amplification grade (Invitrogen), and reverse transcribed using Super Script First-Strand Synthesis Kit3 (Invitrogen) in a 80-µl volume according to the manufacturer's instructions. After cDNA synthesis, each reaction was diluted to 2,400 µl total volume and stored at −80 C. For qRT-PCR reactions, 1 µl of total cDNA was added to 10 µl of PCR reaction mixture containing 5 µl of LightCycler® 480 SYBR Green I Master and 0.5 µl of each primer set. Transcript abundance was measured from three technical and four biological replicates using a LightCycler 480 II (Roche). All transcript values were normalized using the reference genes *ARF2*, *PTB*, *SAND*, and *ORE9*/*MAX2* identified by Chao et al. (2012). QbasePLUS version 2.4 software (Biogazelle, Ghent, Belgium) was used to normalize expression values and to perform statistical analyses. Values from four biological replicates were averaged and data from untreated samples (0 kg ha^{-1}) for each time period were used for baseline expression. Gene abbreviations and descriptions of all putative homologous leafy spurge genes included throughout this report were obtained from an *Arabidopsis* (*Arabidopsis thaliana* L.) website (www.arabidopsis.org) and are presented in SM-1.

Results and Discussion

Effects of Glyphosate on Phenotype and Vegetative Growth. Foliar glyphosate treatment (2.24 kg ha^{-1}) had a sublethal effect on leafy spurge plants. This treatment caused yellowing and desiccation of the apical meristem within 2–3 d, and within 7 d after the treatment the apical meristem was completely desiccated. Although the remaining aerial tissues survived 7 d posttreatment, the surviving leaves eventually died off over a 3–4-wk period (Figure 2). Generally, physiological factors outside the buds maintain paradormancy, as often illustrated by the auxin theory of apical dominance, or by unknown signals from the leaves or stem (Lang et al. 1987). Thus, new vegetative growth from paradormant crown buds of glyphosate-treated plants did not occur until surviving aerial tissue was decapitated. After foliar glyphosate treatment, crown buds were still viable and decapitation of surviving aerial tissues from glyphosate-treated plants (7 d posttreatment) induced vegetative growth, but the growth of new shoots from crown buds was reduced significantly ($P < 0.05$) compared with the untreated controls (Figures 3 and 4a). Most of the UABs initiated shoot growth (release from paradormancy) simultaneously, but the shoot growth was suspended after reaching ~2–3 cm and only a few extended beyond the soil surface (Figure 4b). The appearance of new shoots was represented by stunted and bushy phenotypes, often with variegated leaves (Figures 4c, d). These phenotypic patterns in vegetative growth from crown buds of glyphosate-treated plants remained similar after several additional decapitations (data not shown). Thus, the initiation of uncontrolled vegetative growth from UABs in response to glyphosate treatment (Figure 4b) is a significant event, in that release of paradormancy under field conditions normally results in only a few crown buds initiating new vegetative growth. Understanding the molecular mechanisms associated with glyphosate-induced bud growth could provide new strategies for reducing the bud bank of invasive perennial weeds or make them more susceptible to alternative control techniques.

Changes in Transcript Abundance of Leafy Spurge Crown Buds after Foliar Glyphosate Treatment. The abundance of ~200 transcripts was monitored for crown buds of glyphosate-treated and untreated leafy spurge plants that were either in a paradormant (from intact plants) or vegetative growth phase (after growth-inducing decapitation). There were no significant changes of transcript abundance in paradormant crown buds of leafy spurge 3 h after foliar glyphosate treatment (SM-1; Tables 1–5). Gottrup et al. (1976) reported that between 0.5 and 1% of ^{14}C-labeled glyphosate translocated into the root system of leafy spurge within 12 h. Thus, it is not surprising that no significant changes in transcript abundance in paradormant crown buds were observed 3 h after application of glyphosate. However, 1 d after foliar glyphosate treatment significant changes in transcript abundance were observed in paradormant crown buds, and glyphosate had a more prominent effect on transcript abundance 3–7 d posttreatment. Growth-inducing decapitation also resulted in rapid and major changes in transcript abundance in crown buds compared with untreated controls.

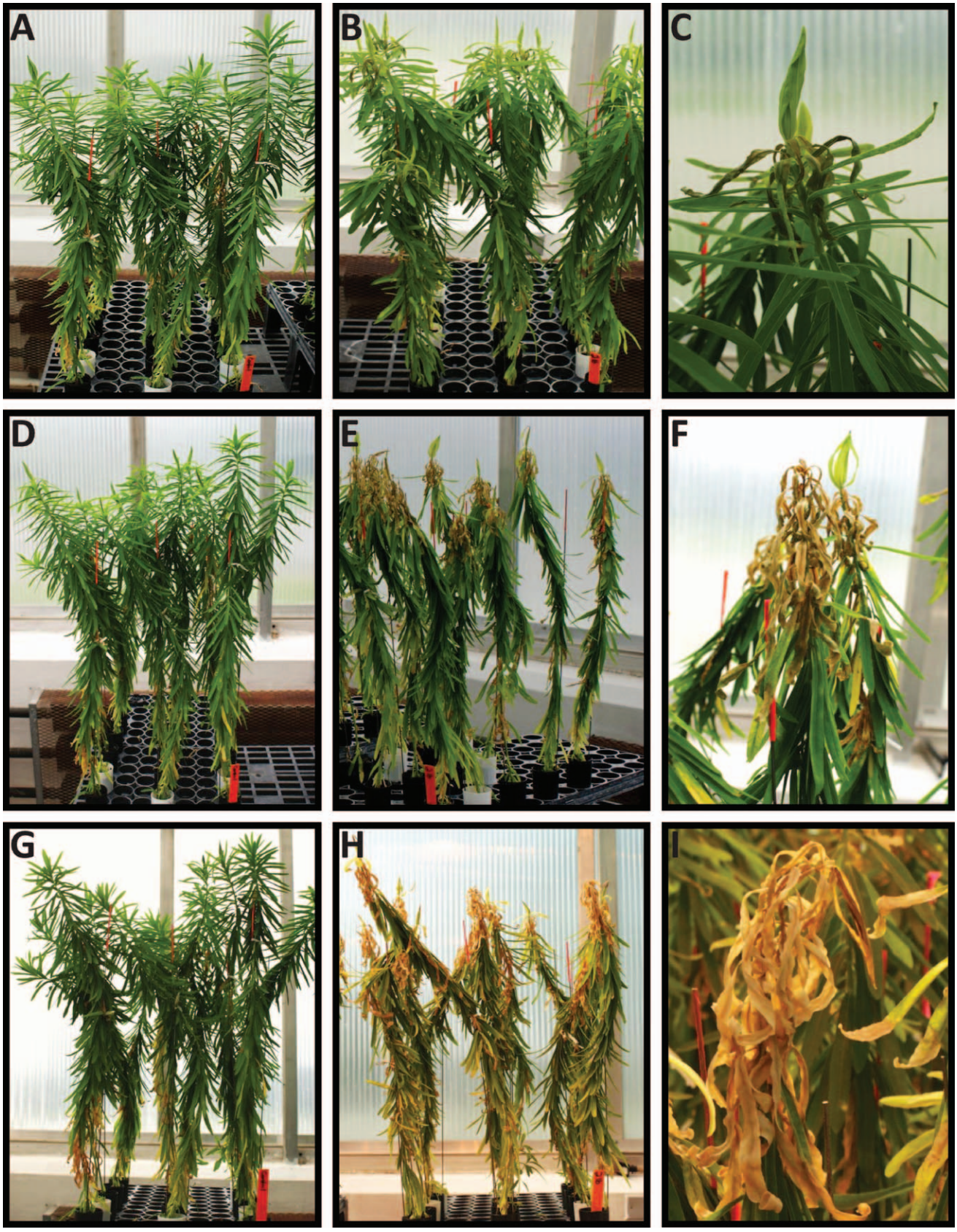

Figure 2. Effect of foliar glyphosate treatment on leafy spurge plants, which were treated with 0 kg ha^{-1} (controls) or 2.24 kg ha^{-1} (treated) of glyphosate. A, D, and G represent control plants 7, 14, and 21 d posttreatment, respectively. B, E, and H represent treated plants 7, 14, and 21 d posttreatment, respectively. C, F, and I, represent close-ups of treated plants 7, 14, and 21 d posttreatment, respectively. (Color for this figure is available in the online version of this paper.)

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

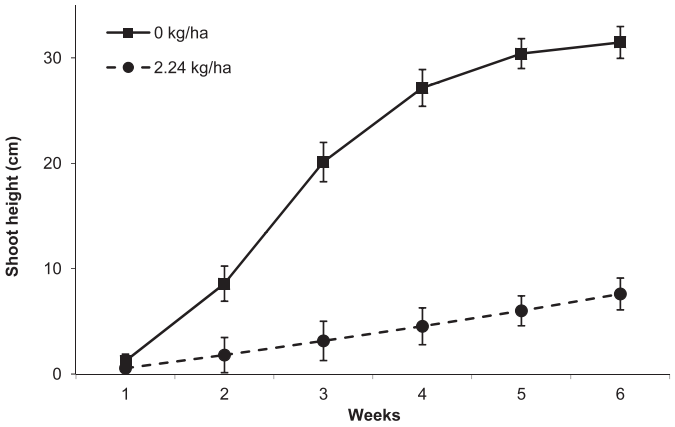

Figure 3. Vegetative growth of shoots from crown buds of leafy spurge after foliar glyphosate treatment and growth-inducing decapitation. Vertical bars indicate 95% confidence limits.

For example, within 3 h postdecapitation numerous transcripts associated with various hormonal pathways and cell processes in crown buds showed significant changes in abundance, and the effect of glyphosate continued to affect transcript abundance in growth-induced crown buds 1–7 d postdecapitation (Tables 1–5; SM-1).

Impact of Glyphosate on the Shikimate Biosynthesis Pathway. Changes in abundance of transcripts involved in various stages of the shikimate biosynthetic pathway (Table 1) were observed at 3 d (*EMB1144*, *MEE32*) and 7 d posttreatment (*MEE32*) in paradormant crown buds and 1 d after growth-inducing decapitation (*DHS1*, *EMB1144*). Although changes in abundance of these transcripts were statistically significant, their changes in amplitude were minor (Table 1). Similar to the results observed for homologues of *EPSPS* in leafy spurge (Table 1), expression of *EPSPS* in glyphosate-resistant and -susceptible Palmer amaranth (*Amaranthus palmeri* S. Wats.) did not change significantly with or without glyphosate treatment (Gaines et al. 2010). These results suggest that glyphosate treatment does not induce drastic changes in abundance of transcripts known to be involved in the shikimate pathway; previous studies have shown similar results in horseweed (Peng et al. 2010) and soybean [*Glycine max* (L.) Merr.] (Zhu et al. 2008).

Impact of Glyphosate on ABA Biosynthesis, Signaling, and Response. ABA is responsive to environmental stresses and is involved in many developmental processes; therefore, we monitored abundance of numerous transcripts involved in ABA biosynthesis, signaling, and response. Only a few transcripts (*NCED3, CYP707A1*) had significant changes in abundance. Because transcript abundance for *NCED3* (involved in biosynthesis) was decreased and for *CYP707A1* (involved in catabolism) was increased, these results suggest that ABA biosynthesis and signaling are negatively affected in crown buds of foliar glyphosate-treated plants (Table 2; SM-1). Thus, our results suggest that any stress-induced effects of glyphosate do not appear to involve direct ABA signaling. However, the increased abundance of *LEA5*, a putative ABA-responsive gene, could suggest the presence of reactive oxygen species in these crown buds, which would be consistent with similar results obtained from glyphosate-treated turf grasses (Cebeci and Budak 2009).

Impact of Glyphosate on Ethylene Biosynthesis, Signaling, and *AP2/ERF* Transcription Factors. Ethylene is involved in numerous processes throughout the plant's life cycle including growth, senescence, and stress responses (Bleecker and Kende 2000). It is also believed to be involved in induction of endodormancy in crown buds of leafy spurge (Doğramacı et al. 2010, 2013). Therefore, we monitored changes in abundance of transcripts involved in ethylene biosynthesis, perception, and signaling in crown buds of foliar glyphosate-treated leafy spurge (Table 3). Abundance of transcripts involved in ethylene biosynthesis changed little in paradormant crown buds 3 h–7 d posttreatment. However, *ACO4*, which encodes the enzyme for the biosynthesis of the ethylene precursor 1-aminocyclopropane-1-carboxylate oxidase (ACC), had increased abundance 3–7 d posttreatment and its abundance declined after decapitation. The abundance of several other transcripts involved in ethylene biosynthesis (e.g., *ACS10*, *ACS8*, *SAM2*) were mainly increased 1–7 d after growth-inducing decapitation. Among the ethylene receptors evaluated (Table 3; SM-1), only *ETR2* increased in abundance 1–7 d posttreatment in paradormant crown buds. These transcripts did not change in crown buds after growth-inducing decapitation relative to nontreated controls. A transcription factor (*CTR1*) downstream of the ETR2 receptor had increased transcript abundance 3 d posttreatment in paradormant crown buds and 1 d after growth-inducing decapitation. Most other transcripts involved in ethylene responses and signaling had little change in abundance (SM-1). However, increased abundance of a transcript encoding an

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

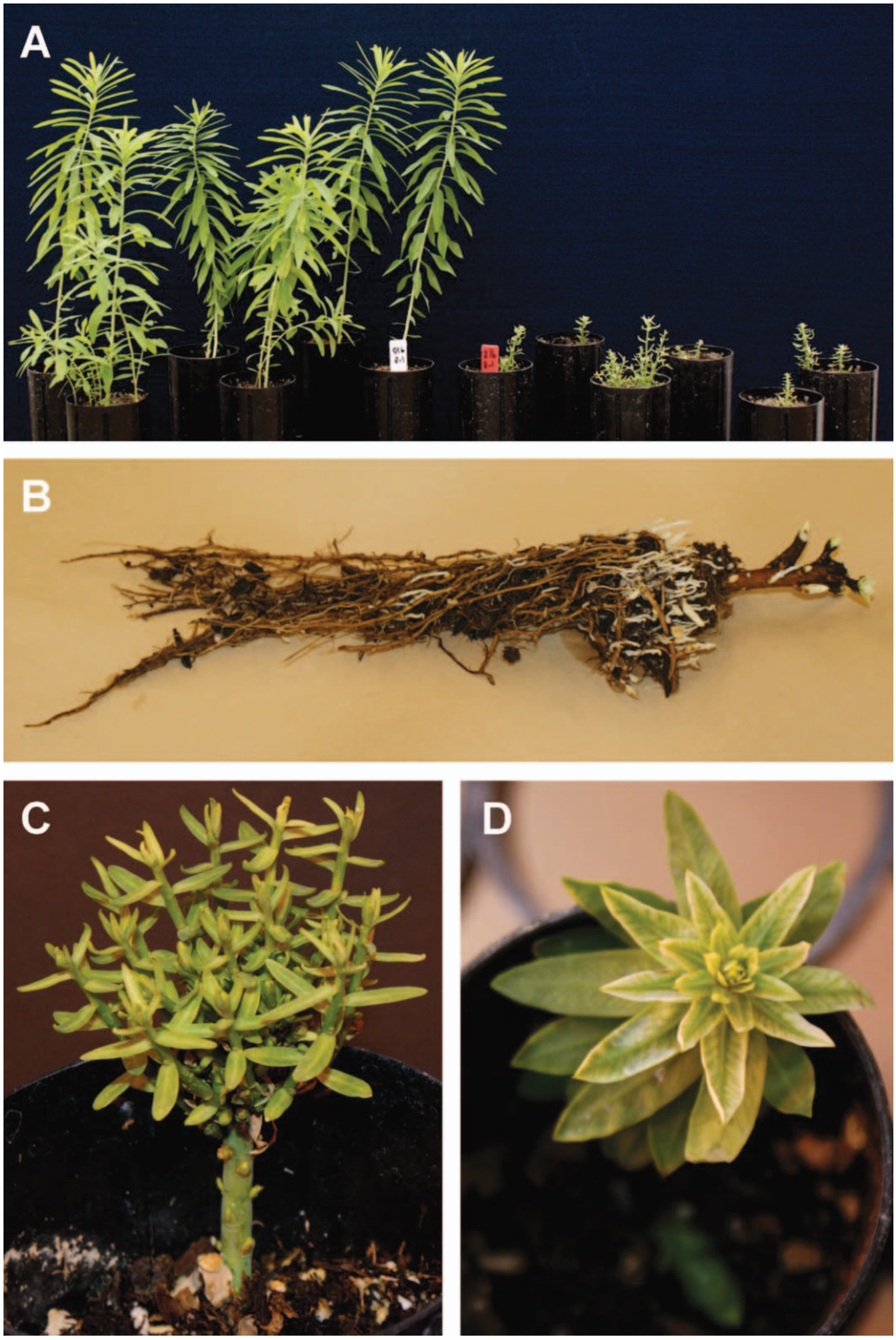

Figure 4. Vegetative shoot growth from crown buds of foliar glyphosate-treated (0 or 2.24 kg ha^{-1}) leafy spurge; plants were kept under growth-conducive conditions for 7 d posttreatment, and then decapitated to induce vegetative growth. (A) New shoot growth from crown buds of treated plants (right) were significantly shorter compared with controls (left) 6 wk postdecapitation. (B) Numerous underground adventitious buds initiated shoot growth simultaneously from glyphosate-treated plants, but shoot growth was suspended after reaching ~2–3 cm and only a few extended beyond the soil surface. (C) The appearance of new shoots from crown buds of glyphosate-treated plants was often represented by stunted and bushy phenotypes. (D) Phenotypical patterns from crown buds of glyphosate-treated plants often included variegated leaves. (Color for this figure is available in the online version of this paper.)

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

Table 1. Changes in abundance of transcripts involved in shikimate biosynthesis in crown buds (CB) of leafy spurge after foliar glyphosate treatment (surfactant + 2.24 kg ha^{-1}) compared with untreated controls (surfactant only). Paradormant CB were collected from intact plants at various time points after glyphosate treatment. Growth-induced CB were collected at various time points from treated plants that were decapitated 7 d after glyphosate treatment. The *Arabidopsis* information resource (TAIR) identifications (IDs) represent *Arabidopsis* genes used to annotate homologues of leafy spurge transcripts. Values represent fold changes; bold values represent $P < 0.05$ (*) or $P < 0.1$ (**).

		Paradormant CB				Growth-induced CB			
Gene ID & category	TAIR ID	3 h	1 d	3 d	7 d	3 h	1 d	3 d	7 d
Shikimate biosynthesis									
CM1	At3g29200	−0.04	−0.17	−0.02	0.11	0.13	0.47	0.02	−0.11
DHS1	At4g39980	−0.05	−0.02	0.07	−0.22	−0.32	**0.37***	−0.19	−0.07
DHS2	At4g33510	−0.18	−0.20	0.18	0.18	0.28	0.53	0.13	−0.13
EMB1144	At1g48850	−0.04	−0.13	**0.33****	−0.07	−0.03	**0.68***	**−0.17****	−0.35
EPSPS	At2g45300	−0.06	−0.01	0.15	0.07	0.016	0.57	−0.15	−0.22
MEE32	At3g06350	0.35	−0.39	**−0.69***	**−0.56***	**−0.68***	−0.77	−0.44	−0.39
SK1	At2g21940	0.03	−0.18	0.42	−0.06	0.06	−0.07	−0.13	0.11

auxin-inducible ACC synthase (*ACS8*) in growth-induced crown buds could also suggest a potential for cross-talk among ethylene and auxin signaling pathways in response to glyphosate treatment (Table 3).

Some of the *AP2/ERF* transcription factors responsive to ethylene signaling in leafy spurge (Doğramacı et al. 2013) are also known to be involved in transcriptional regulation of downstream stress-responsive pathways (Mizoi et al. 2012). Indeed, exogenous application of the ethylene precursor ACC increased transcript abundance of some *AP2/ERFs* (e.g., *DREB26*, *ERF1*, and *ABR1*) in leafy spurge crown buds and resulted in a stunted vegetative regrowth following decapitation (Doğramacı et al. 2013). In this study, *ABR1* also had increased transcript abundance in paradormant crown buds 1–3 d posttreatment (Table 3), whereas *DREB26* and *ERF1* had increased transcript abundance in growth-induced crown buds 3 h–7 d postdecapitation. Thus, our data support glyphosate having an impact on ethylene signaling pathways, given the similarity observed in transcript abundance and growth patterns in this study and previous research (Doğramacı et al. 2013). Indeed, overexpression of several *AP2/ERF*s, including *DREB26*, has been shown to result in dwarfed phenotypes (Krishnaswamy et al. 2011; Mizoi et al. 2012), and overexpression of some members of the DREB1 class is known to induce dwarfism through regulation of GA metabolism (Magome et al. 2008; Tong et al. 2009). Some DREBs are also known to bind to cis-acting elements of *LEA5* and might explain the increased transcript abundance observed for a homologue of *LEA5* (Table 2).

Table 2. Changes in abundance of transcripts involved in abscisic acid (ABA) biosynthesis, signaling, and response in crown buds (CB) of leafy spurge after foliar glyphosate treatment (surfactant + 2.24 kg ha^{-1}) compared with untreated controls (surfactant only). Paradormant CB were collected from intact plants at various time points after glyphosate treatment. Growth-induced CB were collected at various time points from treated plants that were decapitated 7 d after glyphosate treatment. The *Arabidopsis* information resource (TAIR) identifications (IDs) represent *Arabidopsis* genes used to annotate homologues of leafy spurge transcripts. Values represent fold changes; bold values represent $P < 0.05$ (*) or $P < 0.1$ (**); n, no data.

		Paradormant CB				Growth-induced CB			
Gene ID & category	TAIR ID	3 h	1 d	3 d	7 d	3 h	1 d	3 d	7 d
ABA biosynthesis									
AAO3	At2g27150	−0.19	0.61	0.41	0.15	−0.53	0.29	−0.17	0.37
ABA1	At5g67030	−0.09	**−0.47****	−0.27	−0.36	−0.06	−0.02	0.04	−0.22
ABA2	At1g52340	0.005	−0.16	−0.05	−0.21	−0.17	0.19	−0.11	0.07
CYP707A1	At4g19230	−0.18	0.59	**1.17***	0.42	**1.10***	**1.33***	0.32	**0.40****
NCED3	At3g14440	0.77	−0.21	n	−0.16	**−1.77***	−0.72	**−0.81***	−0.34
ABA signaling/response									
LEA5	At4g02380	0.22	0.29	0.80	**1.34***	**10.90***	**0.65****	**2.59***	**1.17***
ABI1	At4g26080	0.16	−0.42	**−0.48****	−0.44	**−0.33***	**−0.50****	−0.14	**−0.14****
RCAR3	At5g53160	−0.11	−0.62	−0.33	**−0.41****	−0.14	0.11	0.04	0.04

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

Table 3. Changes in abundance of transcripts involved in ethylene biosynthesis, signaling, and AP2/ERF transcription factor family members in crown buds (CB) of leafy spurge after foliar glyphosate treatment (surfactant + 2.24 kg ha^{-1}) compared with untreated controls (surfactant only). Paradormant CB were collected from intact plants at various time points after glyphosate treatment. Growth-induced CB were collected at various time points from treated plants that were decapitated 7 d after glyphosate treatment. The *Arabidopsis* information resource (TAIR) identifications (IDs) represent *Arabidopsis* genes used to annotate homologues of leafy spurge transcripts. Values represent fold changes; bold values represent $P < 0.05$ (*) or $P < 0.1$ (**); n, no data.

		Paradormant CB				Growth-induced CB			
Gene ID & category	TAIR ID	3 h	1 d	3 d	7 d	3 h	1 d	3 d	7 d
Ethylene biosynthesis									
ACO4	At1g05010	−0.31	−0.33	**1.80***	**2.30***	**2.03***	**0.81****	0.24	0.08
ACS8	At4g37770	n	−0.12	**−0.47****	1.01	0.72	**1.50***	**2.30***	**2.09***
ACS10	At1g62960	0.69	−0.02	0.51	0.08	0.03	0.05	−0.003	**0.70****
SAM2	At4g01850	0.02	0.53	0.12	0.17	−0.49	**0.93****	−0.37	0.31
Ethylene receptors									
ETR2	At3g23150	0.07	**1.05***	**1.68***	**0.89***	−0.19	0.96	−0.21	0.24
Ethylene signaling									
CTR1	At5g03730	−0.05	0.18	**0.64***	0.06	0.16	**0.74***	−0.09	0.03
AP2/ERF TF									
DREB26	At1g21910	−0.35	0.06	0.31	0.69	**3.34***	**0.91****	**1.35***	**1.84***
ERF1	At4g17500	−0.25	0.43	0.24	0.31	0.40	**1.51****	0.18	**0.48***
ABR1	At5g64750	n	**5.48***	**2.27***	0.12	0.73	0.14	−0.43	0.18

Impact of Glyphosate on GA Biosynthesis and Cell Cycle. Among the transcripts monitored, the most striking change in abundance was observed for a homologue of *CPS1*/*GA1* (Table 4). The product of this transcript catalyzes the conversion of geranylgeranyl pyrophosphate to copalyl pyrophosphate, which provides substrates for the gibberellin biosynthetic pathway. However, only slight changes were observed in transcript abundance involved in biosynthesis of bioactive GA. The biosynthesis of GA can be divided into three stages: (1) cyclization of geranylgeranyl diphosphate by copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS) to produce ent-kaurene in the plastids; (2) conversion of ent-kaurene into GA12 via cytochrome P450 mono-oxygenases, such as, ent-kaurene oxidase (KO) and ent-kaurenoic acid oxidase (KAO) in the endoplasmic reticulum; and (3) conversion of GA12 to bioactive forms of GA in the cytosol. GA12 is a branch-point gibberellin undergoing various oxidation and cyclization processes to form the whole spectrum of bioactive gibberellins catalyzed by 2-oxoglutarate–dependent dioxygenases (e.g., GA20OX and GA3OX). KAO

Table 4. Changes in abundance of transcripts involved in gibberellic acid (GA) biosynthesis and signaling in crown buds (CB) of leafy spurge after foliar glyphosate treatment (surfactant + 2.24 kg ha^{-1}) compared with untreated controls (surfactant only). Paradormant CB were collected from intact plants at various time points after glyphosate treatment. Growth-induced CB were collected at various time points from treated plants that were decapitated 7 d after glyphosate treatment. The *Arabidopsis* information resource (TAIR) identifications (IDs) represent *Arabidopsis* genes used to annotate homologues of leafy spurge transcripts. Values represent fold changes; bold values represent $P < 0.05$ (*) or $P < 0.1$ (**); n, no data.

		Paradormant CB				Growth-induced CB			
Gene ID & category	TAIR ID	3 h	1 d	3 d	7 d	3 h	1 d	3 d	7 d
GA biosynthesis									
CPS1/*GA1*	At4g02780	−0.02	0.25	**14.56***	**28.77***	**10.54***	**12.90***	**11.73***	**0.58***
GA20OX1	At4g25420	0.35	−0.46	−0.62	−0.27	−0.53	−0.26	0.44	−0.07
GA2OX8	At4g21200	n	n	n	n	−0.13	0.54	−0.13	0.40
GA response - signaling									
GASA2	At4g09610	−0.34	−0.44	**−0.98***	**−2.66***	−0.44	−0.86	−0.14	−0.49
RGA1	At2g01570	−0.20	**−0.52***	**−0.30****	**−0.60***	−0.07	−0.09	−0.04	−0.05
RGA2	At1g14920	−0.03	−0.48	**−0.46****	**−0.91***	0.12	0.03	0.02	0.04
RGL2	At5g66770	−0.35	**−0.81***	−0.11	0.17	−0.10	**−1.83***	0.04	−0.28
SPY	At3g11540	0.06	−0.16	−0.17	−0.24	−0.09	−0.17	0.005	−0.06
SLY1	At2g17980	−0.06	−0.65	**−0.36****	**−0.50****	−0.25	0.55	−0.34	0.05

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

Table 5. Changes in abundance of transcripts involved in auxin biosynthesis and transport in crown buds (CB) of leafy spurge after foliar glyphosate treatment (surfactant + 2.24 kg ha^{-1}) compared with untreated controls (surfactant only). Paradormant CB were collected from intact plants at various time points after glyphosate treatment. Growth-induced CB were collected at various time points from treated plants that were decapitated 7 d after glyphosate treatment. The *Arabidopsis* information resource (TAIR) identifications (IDs) represent *Arabidopsis* genes used to annotate homologues of leafy spurge transcripts. Values represent fold changes; bold values represent $P < 0.05$ (*) or $P < 0.1$ (**).

		Paradormant CB				Growth-induced CB			
Gene ID & category	TAIR ID	3 h	1 d	3 d	7 d	3 h	1 d	3 d	7 d
Auxin transport									
PILS2	At1g71090	0.02	−0.16	0.25	0.04	−0.13	−0.03	−0.02	−0.04
PILS3	At1g76520	−0.17	−0.34	**0.88****	0.25	0.65	0.68	0.21	0.19
PILS6	At5g01990	−0.04	0.07	**0.49***	**0.27***	0.00	−0.03	−0.12	−0.25
PILS7	At5g65980	−0.16	0.67	**7.36***	**12.13***	5.24	**3.11***	**1.16****	0.01
PIN3	At1g70940	−0.08	−0.47	**−0.56***	**−0.37***	**−0.76***	−0.36	**−1.72***	**−0.97***
PIN8	At5g15100	0.05	−1.18	**−1.58***	**−0.88***	**−0.42***	−0.26	−0.53	**−1.25****
ABC transporter family									
ABCB1	At2g36910	0.03	−0.06	**−0.44****	0.05	−0.13	0.47	0.39	0.60
ABCB2	At4g25960	0.09	−0.13	**−0.60***	0.03	−0.21	0.33	−0.16	0.27
ABCB19	At3g28860	0.04	**−0.72***	**−1.10***	**−0.44***	**−1.65****	−0.06	−0.39	0.28
ABCG40	At1g15520	0.07	−0.43	**−0.58****	−0.06	−0.23	−0.14	−0.09	0.13

is the rate-limiting step for production of GA intermediates and GA dioxygenases are involved in fine-tuning GA biosynthesis (Fleet et al. 2003; Hedden and Phillips 2000; Sponsel and Hedden 2004). There were no significant changes in transcript abundance of GA dioxygenases in paradormant and growth-induced crown buds of foliar glyphosate-treated leafy spurge (Table 4). Thus, the increased abundance for the transcript of *CPS1* is noteworthy. If ent-copalyl diphosphate is produced and converted to ent-kaurene, metabolite flux through subcellular compartments could be affected because glyphosate rapidly disrupts proplastids, chloroplasts, and the endoplasmic reticulum, likely due to accumulation of shikimate (Campbell et al. 1976; Mollenhauer et al. 1987).

A GA-responsive transcript *GASA2* had decreased abundance in paradormant crown buds 3 and 7 d after glyphosate treatment of leafy spurge plants (Table 4). Cumulatively the results suggest that paradormant crown buds from foliar glyphosate-treated plants likely have decreased levels of GA. *DELLA* transcripts (i.e., *RGA1*, *RGA2*, *RGL2*), which function as negative regulators of GA-mediated signaling, had slightly decreased transcript abundance in paradormant crown buds in response to foliar glyphosate treatment. In the presence of GA, DELLA proteins are generally inhibited through targeted ubiquitination and proteolysis; consequently, GA inhibits the function of these growth-inhibiting DELLAs. If GA levels are actually low in the paradormant buds, one would expect DELLA proteins to be stabilized and possibly more abundant, and thus the decreased abundance of *DELLA* transcripts (Table 4) might be expected. However, functional regulation of DELLAs is complex and there are alternative GA-independent routes that can also affect DELLAs (Dai and Xuea 2010; Harberd et al. 2009).

Overall, these results indicate that downstream GA biosynthesis and signaling are negatively affected, which may account for the stunted vegetative growth pattern obtained from crown buds of foliar glyphosate-treated leafy spurge plants. This hypothesis is supported by the fact that certain commercial chemicals (e.g., phosphon D, cycocel, ancymidol, and paclobutrazol) used to stunt growth do so, in part, by blocking the synthesis of gibberellins (Sponsel and Hedden 2004). Additionally, loss of function of *CPS* and *KS* has been shown to cause a dwarfed phenotype in rice (*Oryza sativa* L.) (Sakamoto et al. 2004), and loss of *KO* or *KAO* gene function causes dwarfed phenotypes in pea (*Pisum sativum* L.) (Davidson et al. 2003). It should not be surprising that the transcripts involved in cell cycle (e.g., *CDC2A*, *CDC2B*, *CYCD3;1*, *H3*, *H4*, *RB*) have decreased abundance (SM-1), considering the slow and stunted vegetative growth from crown buds of glyphosate-treated leafy spurge plants compared with the nontreated controls.

Impact of Glyphosate on Auxin and Auxin Transporters. Auxin plays a critical role in nearly every aspect of plant growth and development (Zhao 2010), and is derived in part from the shikimate to chorismate to tryptophan-dependent

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

pathway. Therefore, we monitored transcript abundance of numerous genes involved in auxin biosynthesis, perception, and signaling in paradormant and growth-induced crown buds after foliar glyphosate treatment. Changes in abundance of transcripts involved in tryptophan and auxin biosynthesis were not significant, whereas changes in abundance of transcripts involved in perception and signaling (Aux/IAAs, ARFs, SCF complex) were statistically significant, but the amplitude of changes were minor both in paradormant and growth-induced crown buds (SM-1). Some transcripts involved in auxin transport changed in abundance 3–7 d posttreatment in paradormant and 3 h–7 d in growth-induced crown buds. For example, *PIN3* and *PIN8* had decreased transcript abundance both in paradormant and growth-induced crown buds. *PIN*s encode auxin efflux carriers, and these carriers mediate tissue-specific cell-to-cell polar auxin transport (Peer 2013), which is critical for developmental patterning and differential growth responses (Blilou et al. 2005). Interestingly, although strigolactones have been implicated in regulating auxin signaling by affecting *PIN*s (Shinohara et al. 2013), in this study, transcripts involved in strigolactone signaling such as *MAX1* and *MAX2* were not significantly changed in response to glyphosate treatment (SM-1).

PIN-LIKES (*PILS*) are a family of auxin transport facilitator genes that are required for auxin-dependent regulation of plant growth and organ formation by determining the cellular sensitivity to auxin (Barbez et al. 2012; Feraru et al. 2012). PILS proteins facilitate intracellular auxin accumulation at the endoplasmic reticulum membrane, which regulates intracellular auxin accumulation presumably through conjugation-based auxin metabolism, and ultimately decreases nuclear auxin signaling (Barbez et al. 2012; Peer 2013). Generally, transcripts of *PILS* reflected increased abundance in crown buds, with *PILS7* showing a remarkable increase in abundance 3–7 d posttreatment in paradormant as well as in growth-induced crown buds 1–3 d after decapitation (Table 5). Interestingly, Barbez et al. (2012) demonstrated that ectopic expression of some *PILS* resulted in various abnormalities, including dwarfed and bushy *Arabidopsis* plants. Glyphosate's effect on transcript abundance of *PILS* observed in this study may, in part, be in response to induced ethylene signaling pathways as previously indicated above, since plants previously treated with the ethylene precursor ACC also had a significant increase in transcript abundance of *PILS3* and *7* (SM-1). These results would be consistent with the known synergistic associations that occur between auxin and ethylene (Peer 2013).

ABC transporter family members contribute to adenosine triphosphate (ATP)-dependent auxin transport. They are responsive to abiotic stresses and have numerous other functions in plants, including detoxification processes and sequestration of secondary metabolites (Kang et al. 2011). Cellular uptake of glyphosate is suggested to involve a transporter (Denis and Delrot 1993; Riechers et al. 1994; Shaner 2009) and studies focusing on nontarget-site resistance also suggest that sequestration of glyphosate into vacuoles occurs by an active but yet unidentified transporter (Ge et al. 2010; Staub et al. 2012). Several ABC transporters have increased transcript abundance in response to glyphosate treatment and they were hypothesized to contribute to nontarget-site glyphosate resistance in horseweed (Peng et al. 2010; Yuan et al. 2007, 2010). Therefore, we monitored transcript abundance of several ABC transporters in crown buds of foliar glyphosate-treated plants. Transcript abundance of four homologues of ABC transporters (*ABCB1*, *ABCB2*, *ABCB19*, *ABCB40*) were decreased in paradormant crown buds 1–7 d posttreatment, and *ABCB19* had decreased abundance 3 h postdecapitation in growth-induced crown buds (Table 5). In *Arabidopsis*, an *abcb19* mutant exhibits reduced growth, decreased apical dominance, and impaired polar auxin transport (Kang et al. 2011). Thus, these results would be consistent with glyphosate affecting auxin signaling and perception in crown buds, which could be affecting altered vegetative growth from crown buds observed in this study (Figure 4).

Overall, our results suggest that foliar glyphosate treatment has an impact on stress responses and several phytohormone biosynthesis and signaling pathways, particularly ethylene, auxin, and GA, which is consistent with altered cell cycle processes involved in plant growth and development. The increase in transcript abundance of *PILS7* was one of the major outcomes of this study, suggesting that glyphosate's effect on *PILS* could affect intracellular auxin accumulation and signaling, leading to altered vegetative growth patterns. Although further studies will be needed to confirm this hypothesis, these results indicate that *PILS* are responsive to foliar glyphosate treatment. Likewise, we further hypothesize that decreased transcript abundance of *PIN*s and ABC transporter family members in crown buds of foliar glyphosate-treated leafy spurge alters auxin homeostasis, and thus vegetative growth. Overall, most of the significant processes identified in this

https://doi.org/10.1614/WS-D-13-00156.1 Published online by Cambridge University Press

study are associated with the endoplasmic reticulum, suggesting that this organelle plays some role in cellular physiology in response to glyphosate treatment, particularly since glyphosate has been reported to affect the endoplasmic reticulum (Campbell et al. 1976). Further studies utilizing next-generation sequencing and metabolite profiling, similar to those described for Canada thistle (Anderson et al. 2012), should provide a deeper understanding of molecular mechanisms affected by glyphosate and their action on vegetative growth from UABs of perennial weeds.

Acknowledgments

The authors thank Brant B. Bigger, Cheryl A. Huckle, and Wayne A. Sargent for their technical assistance.

Literature Cited

Amrhein N, Deus B, Gehrke P, Steinrucken HC (1980) The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation in vivo and in vitro. Plant Physiol 66:830–834

Anderson JV, Davis DG (2004) Abiotic stress alters transcript profiles and activity of glutathione S-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula*. Physiol Plant 120:421–433

Anderson JV, Dogramaci M, Horvath DP, Foley ME, Chao WS, Suttle JC, Thimmapuram J, Hernandez AG, Ali S, Mikel MA (2012) Auxin and ABA act as central regulators of developmental networks associated with paradormancy in Canada thistle (*Cirsium arvense*). Funct Integr Genomics 12:515–531

Anderson JV, Gesch RW, Jia Y, Chao WS, Horvath DP (2005) Seasonal shifts in dormancy status carbohydrate metabolism and related gene expression in crown buds of leafy spurge. Plant Cell Environ 28:1567–1578

Anderson JV, Horvath DP, Chao WS, Foley ME, Hernandez A, Thimmapuram J, Liu L, Gong GL, Band M, Kim R, Mikel MA (2007) Characterization of an EST database for the perennial weed leafy spurge: an important resource for weed biology research. Weed Sci 55:193–203

Barbez E, Kubeš M, Rolčík J, Béziat C, Pěnčík A, Wang B, Rosquete MR, Zhu J, Dobrev PI, Lee Y, Zažímalová E, Petrášek J, Geisler M, Friml J, Kleine-Vehn J (2012) A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature 485:119–122

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433:39–44

Boocock MR, Coggins JR (1983) Kinetics of 5-enolpyruvylshikimate-3 phosphate synthase inhibition by glyphosate. FEBS Lett 154:127–133

Campbell WF, Evans JO, Reed SC (1976) Effect of glyphosate on chloroplast ultrastructure of quackgrass mesophyll cells. Weed Sci 24:22–25

Cebeci O, Budak H (2009) Global expression patterns of three Festuca species exposed to different doses of glyphosate using the Affymetrix GeneChip wheat genome array. Comp Funct Genomics 2009:1–10

Chao WS, Doğramacı M, Foley ME, Horvath DP, Anderson JV (2012) Selection and validation of endogenous reference genes for qRT-PCR analysis in leafy spurge (*Euphorbia esula*). PLoS One 7:e42839

Dai C, Xuea HW (2010) Rice *early flowering1*, a CKI, phosphorylates DELLA protein SLR1 to negatively regulate gibberellin signaling. EMBO J 29:1916–1927

Davidson SE, Elliott RC, Helliwell CA, Poole AT, Reid JB (2003) The pea gene NA encodes ent-kaurenoic acid oxidase. Plant Physiol 131:335–344

Della-Cioppa G, Bauer SC, Klein BK, Shah DM, Fraley RT, Kishore G (1986) Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants in vitro. Proc Natl Acad Sci U S A 83:6873–6877

Denis MH, Delrot S (1993) Carrier-mediated uptake of glyphosate in broad bean (*Vicia faba*) via a phosphate transporter. Physiol Planta 87:569–575

Doğramacı M, Foley ME, Chao WS, Christoffers MJ, Anderson JV (2013) Induction of endodormancy in crown buds of leafy spurge (*Euphorbia esula* L.) implicates a role for ethylene and cross-talk between photoperiod and temperature. Plant Mol Biol 81:577–593

Doğramacı M, Horvath DP, Chao WS, Foley ME, Christoffers MJ, Anderson JV (2010) Low temperatures impact dormancy status, flowering competence, and transcript profiles in crown buds of leafy spurge. Plant Mol Biol 73:207–226

Doğramacı M, Horvath DP, Christoffers MJ, Anderson JV (2011) Dehydration and vernalization treatments identify overlapping molecular networks impacting endodormancy maintenance in leafy spurge crown buds. Funct Integr Genomics 11:611–626

Duke SO, Powels SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

Feraru E, Vosolsobě S, Feraru MI, Petrášek J, Kleine-Vehn J (2012) Evolution and structural diversification of PILS putative auxin carriers in plants. Front Plant Sci 3:227

Fleet CM, Yamaguchi S, Hanada A, Kawaide H, David CJ, Kamiya Y, Sun TP (2003) Overexpression of AtCPS and AtKS in *Arabidopsis* confers increased ent-kaurene production but no increase in bioactive gibberellins. Plant Physiol 132:830–839

Foley ME, Anderson JV, Horvath DP (2009) The effects of temperature photoperiod and vernalization on regrowth and flowering competence in *Euphorbia esula* (Euphorbiaceae) crown buds. Botany 87:986–992

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide. Am Chem Soc Monogr 189:521–615

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proc Natl Acad Sci U S A 107:1029–1034

Ge X, d'Avignon DA, Ackerman JJH, Sammons RD (2010) Rapid vacuolar sequestration: the horseweed glyphosate resistance mechanism. Pest Manag Sci 66:345–348

Gottrup O, O'Sullivan PA, Schraa RJ, Vanden Bobn WH (1976) Uptake, translocation, metabolism and selectivity of glyphosate in Canada thistle and leafy spurge. Weed Res 16:197–201

Gruys KJ, Walker MC, Sikorski JA (1992) Substrate synergism and the steady-state kinetic reaction mechanism for EPSP synthase from *Escherichia coli*. Biochemistry 31:5534–5544
Harberd NP, Belfield E, Yasumura Y (2009) The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an ''inhibitor of an inhibitor'' enables flexible response to fluctuating environments. Plant Cell 21:1328–1339
Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci 5:523–530
Herrmann KM, Weaver LM (1999) The shikimate pathway. Annu Rev Plant Physiol Plant Mol Biol 50:473–503
Kang J, Park J, Choi H, Burla B, Kretzschmar T, Lee Y, Martinoia E (2011) Plant ABC transporters. *Arabidopsis* Book 9:e0153
Krishnaswamy S, Verma S, Rahman MH, Kav NN (2011) Functional characterization of four APETALA2-family genes (*RAP26*, *RAP2.6L*, *DREB19* and *DREB26*) in *Arabidopsis*. Plant Mol Biol 75:107–127
Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo-, para-, and eco-dormancy: physiological terminology and classification for dormancy research. Hort Sci 22:371–377
Lym RG (2000) Leafy spurge (*Euphorbia esula*) control with glyphosate plus 2,4-D. J Range Manage 53:68–72
Lym RG, Messersmith CG (2013) Leafy spurge identification and chemical control. NDSU Extension Service, http://www.ag.ndsu.edu/pubs/plantsci/weeds/w765.pdf
Lym RG, JA Nelson (2002) Integration of *Aphthona* spp. flea beetles and herbicides for leafy spurge (*Euphorbia esula*) control. Weed Sci 50:812–819
Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K (2008) The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in *Arabidopsis*. Plant J 56:613–626
Maxwell BD, Foley ME, Fay PK (1987) The influence of glyphosate on bud dormancy in leafy spurge (*Euphorbia esula*). Weed Sci 35:6–10
Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819:86–96
Mollenhauer C, Smart CC, Amrhein N (1987) Glyphosate toxicity in the shoot apical region of the tomato plant. I. Plastid swelling is the initial ultrastructural feature following in vivo inhibition of 5-enolpyruvylshikimic acid 3-phosphate synthase. Pestic Biochem Physiol 29:55–65
Peer WA (2013) From perception to attenuation: auxin signaling and responses. Curr Opin Plant Biol 23:1–8
Peng Y, Abercrombie LL, Yuan JS, Riggins CW, Sammons RD, Tranel PJ, Stewart CN, Jr (2010) Characterization of the horseweed (*Conyza canadensis*) transcriptome using GS-FLX 454 pyrosequencing and its application for expression analysis of candidate non-target herbicide resistance genes. Pest Manag Sci 66:1053–1062
Plant Metabolic Network (2008) http://pmn.plantcyc.org/PLANT/class-tree?object=Pathways
Raju MVS, Steeves TA, Coupland RT (1964) On the regeneration of root fragments of leafy spurge (*Euphorbia esula* L.). Weed Res 4:2–11
Riechers DE, Wax LM, Liebl RA, Bush DA (1994) Surfactant-increased glyphosate uptake into plasma membrane vesicles isolated from common lambsquarters leaves. Plant Physiol 105:1419–1425
Riggins CW, Peng Y, Stewart CN Jr, Tranel PJ (2010) Characterization of de novo transcriptome for waterhemp (*Amaranthus tuberculatus*) using GS-FLX 454 pyrosequencing and its application for studies of herbicide target-site genes. Pest Manag Sci 66:1042–1052
Sakamoto T, Miura K, Itoh H, Tatsumi T, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Agrawal GK, Takeda S, Abe K, Miyao A, Hirochika H, Kitano H, Ashikari M, Matsuoka M (2004) An overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol 134:1642–1653
Shaner DL (2009) The role of translocation as a mechanism of resistance to glyphosate. Weed Sci 57:118–123
Shinohara N, Taylor C, Leyser O (2013) Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein PIN1 from the plasma membrane. PLoS Biol 11(1):e1001474
Sponsel VM, Hedden P (2004) Gibberellin biosynthesis and inactivation. Pages 63–94 *in* Davies PJ, ed. Plant Hormones: Biosynthesis, Signal Transduction, Action! Dordrecht, The Netherlands: Kluwer Academic Publishers
Staub JM, Brand L, Tran M, Kong Y, Rogers SG (2012) Bacterial glyphosate resistance conferred by overexpression of an *Escherichia coli* membrane efflux transporter. J Ind Microbiol Biotechnol 39:641–647
Steinrücken HC, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 94:1207–1212
Tong Z, Hong B, Yang Y, Li Q, Ma N, Ma C, Gao J (2009) Overexpression of two chrysanthemum DgDREB1 group genes causing delayed flowering or dwarfism in *Arabidopsis*. Plant Mol Biol 71:115–129
Wyrill JB III, Burnside OC (1976) Absorption, translocation, and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. Weed Sci 24:557–566
Yuan JS, Abercrombie LLG, Cao Y, Halfhill MD, Zhou X, Peng Y, Hu J, Rao MR, Heck GR, Larosa TJ, Sammons RD, Wang X, Ranjan P, Johnson DH, Wadl PA, ScheZer BE, Rinehart TA, Trigiano RN, Stewart CN Jr (2010) Functional genomics analysis of horseweed (*Conyza canadensis*) with special reference to the evolution of non-target-site glyphosate resistance. Weed Sci 58:109–117
Yuan JS, Tranel PJ, Stewart CN Jr (2007) Non-target-site herbicide resistance: a family business. Trends Plant Sci 12:6–13
Zhao Y (2010) Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol 61:49–64
Zhu J, Patzoldt WL, Shealy RT, Vodkin LO, Clough SJ, Tranel PJ (2008) Transcriptome response to glyphosate in sensitive and resistant soybean. J Agric Food Chem 56:6355–6363

Received October 23, 2013, and approved January 10, 2014.