Weed Science 2015 63:229–234

Aryloxyalkanoate Dioxygenase-12 Soybean Protein Expression

Andrew P. Robinson, D. M. Simpson, Kerrm Yau, Sarah Canada, and William G. Johnson*

New trait technology incorporating 2,4-D resistance in soybean is dependent upon the ability of the plant to metabolize 2,4-D by the aryloxyalkanoate dioxygenase-12 protein (AAD-12). Our objectives were to determine AAD-12 expression during the daytime, throughout the leaf canopy, and before and after 2,4-D treatment for the events DAS-68416-4 and DAS-21606-3. Field experiments were conducted near Wanatah, IN in 2009 and Fowler, IN in 2009, 2010, and 2011. During the daytime, total AAD-12 expression was lowest between 12:30 and 15:30, averaging 161 ng cm^{-2}, as compared to an average of 245 ng cm^{-2} in the morning and 243 ng cm^{-2} in the evening. The youngest fully emerged trifoliate in the DAS-68416-4 event had the highest AAD-12 expression, with means ranging from 369 to 390 ng cm^{-2}, while the older leaves maintained a lower level of expression, 171 to 211 ng cm^{-2}. The youngest leaves of event DAS-21606-3 had the highest level of AAD-12 expression (205 to 225 ng cm^{-2}), while the level of AAD-12 was lower in older leaves (71 to 149 ng cm^{-2}). In general, 2,4-D treatments did not reduce AAD-12 expression at 3, 7, 14, and 21 days after treatment; however, in a few instances AAD-12 expression was increased or decreased by 8 to 11% after 2,4-D treatment. Expression of AAD-12 was between 152 to 390 ng cm^{-2} for DAS-68416-4 and from 71 to 244 ng cm^{-2} for DAS-21606-3.
Nomenclature: 2,4-D; *Glycine max* (L.) Merr., soybean.
Key words: 2,4-dichlorophenoxyacetic acid, 2,4-D-tolerant soybean, transgenic soybean.

New trait technology incorporating 2,4-D resistance in soybean is dependent upon the ability of the plant to metabolize 2,4-D by the aryloxyalkanoate dioxygenase-12 (AAD-12) protein. The *aad*-12 gene was isolated from the soil bacteria *Delftia acidovorans* and when inserted into soybean plants, the AAD-12 metabolizes 2,4-D, triclopyr, and fluroxypyr applied preemergence or postemergence (Wright et al., 2010). The mechanism for 2,4-D resistance was shown to be a rapid single step metabolic detoxification mediated by an Fe(II)/α-ketoglutarate-dependent dioxygenase (Wright et al., 2010). Soybean has been reported to have resistance of up to 4,480 g ae ha^{-1} 2,4-D, a 4X rate of 1,120 g ha^{-1}, the likely field use rate (Robinson, et al., 2010; Wright et al., 2010).

The most successful herbicide resistant trait utilized recently has been glyphosate-resistance. Resistance to glyphosate in soybean was developed by inserting the glyphosate-tolerant *CP4 EPSPS* gene into soybean DNA (Padgette et al., 1995). The CP4 EPSPS is a target site resistance mechanism, while the AAD-12 codes for detoxification of the 2,4-D molecule. The presence of the glyphosate-tolerant CP4 EPSPS conveys resistance to postemergence treatments of glyphosate with 1,680 g ae ha^{-1} causing no sustained injury. Taylor et al. (1999) compared the expression of CP4 EPSPS protein in soybean treated with glyphosate at 840 g ha^{-1} at early postemergence and late postemergence (prior to the R1 soybean growth stage) and transformed plants with no glyphosate treatments. Expression of CP4 EPSPS protein in soybean leaves expressed 0.489 and 0.415 µg of protein mg^{-1} of fresh weight for treated and untreated plants, respectively (Taylor et al., 1999). This report indicated that CP4 EPSPS protein levels were not changed by glyphosate treatments.

The expression of transgenic insect traits throughout the plant has been studied in greater detail than herbicide traits. Expression of the *Bacillus thuringiensis* (Bt) Cry1Ab protein in corn (*Zea mays* L.) was measured in each leaf at the R3 growth stage. It was found that the amount of Cry1Ab protein ranged from 3.9 to 11.6 µg g^{-1} leaf, with the oldest leaf having the lowest level of Cry1Ab while the other leaves averaged 8.0 µg Cry1Ab g^{-1} leaf (Székács et al., 2010b). Expression of Cry1Ab in corn leaves changed throughout the plant's life cycle, reaching 17.2 µg Cry1Ab g^{-1} leaf at V5 corn and 9.6 µg Cry1Ab g^{-1} leaf at R4 corn (Székács et al., 2010a). In cotton (*Gossypium hirsutum* L.) the Bt protein expression was lowest in the youngest and oldest leaves, while the other leaves along the main stem had the highest expression (Chen et al., 2000).

DOI: 10.1614/WS-D-13-00035.1

* First and last authors: Graduate Research Assistant and Professor, Department of Botany and Plant Pathology, 915 W. State Street, Purdue University, West Lafayette, IN 47907; second, third, and fourth authors: Field Scientist, Research Biochemist, and Biologist, Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268. Corresponding author's E-mail: wgj@purdue.edu

https://doi.org/10.1614/WS-D-13-00035.1 Published online by Cambridge University Press

Table 1. Sources of material for herbicides applied to experiments.

Common name	Trade name	Rate in active ingredient	Manufacturer
2,4-D	Weedar 64	456 g L^{-1}	Nufarm, Inc. Burr Ridge, IL, www.nufarm.com/US
Imazethapyr	Pursuit	240 g L^{-1}	BASF Corporation Agricultural Products, Research Triangle Park, NC, www.agro.basf.com
Metribuzin plus Chlorimuron	Canopy DF	0.643 g g^{-1} 0.107 g g^{-1}	DuPont Crop Protection, Wilmington, DE www.dupont.com
Trifluralin	Treflan HFP	480 g L^{-1}	Dow AgroSciences, Indianapolis, IN, www.dowagro.com
Sulfentrazone plus Cloransulam-methyl	Sonic	0.62 g g^{-1} 0.08 g g^{-1}	Authority Assist, FMC Corporation, Philadelphia, PA, www.fmc.com

The variable expression of transgenic proteins have been associated with the environment surrounding the plants which include various biotic and abiotic stresses (water stress, nitrogen deficiency, anaerobic conditions, or salinity stress) (Bruns and Craig, 2003; Luo et al., 2008), sample timing (Greenplate, 1999; Székács et al., 2010a), reduced activity of transgenic gene promoters (Dong and Li, 2007; Finnegan et al., 1998), and location of sample (Szekacs et al., 2010b). The Bt δ-endotoxin in maize increased from 350 μg kg^{-1} with no nitrogen to 534 μg kg^{-1} when 336 kg ha^{-1} was applied (Bruns and Craig, 2003). Cotton leaves expressed a lower level of Bt protein, 38 to 50%, when grown in waterlogged soils (Luo et al., 2008). Cry1Ac levels decreased as cotton plants aged, from 110.3 μg g^{-1} at 53 days after planting to 20.6 μg g^{-1} at 116 days after planting (Greenplate, 1999). Among six locations, the Cry1Ac expression ranged from 17.7 to 86.4 μg g^{-1} (Greenplate, 1999). Maize containing the Cry1Ab toxin changed throughout the season with the lowest Cry1Ab toxin measured at the V1 growth stage (8 μg g^{-1}), increasing to the highest level at V5 growth stage (17 μg g^{-1}), then decreasing down to 10 μg g^{-1} at the R4 growth stage, and again increasing to 13 μg g^{-1} at the R5 growth stage (Székács et al., 2010a).

Sampling time can be critical when trying to measure plant proteins. Because plants have a circadian rhythm, they are constantly adjusting to events such as sunrise and sunset. This photosensitive nature enables the plant to prepare for future environmental changes and ensures plant survival by oscillating proteins, enzymes, and other chemicals (Dunlap, 1999; Kay and Millar, 1995; McClung, 2006).

It is unknown how the AAD-12 expression is affected by time of day, age of leaf, or treatment of 2,4-D. A series of experiments were conducted to characterize AAD-12 expression in transformed soybeans. The first experiment was designed to determine what effect time of day has on level of AAD-12 expression. The second experiment was designed to determine what effect the age of the leaf (position in canopy) has on AAD-12 expression. The third experiment evaluated the effect of 2,4-D treatment on AAD-12 expression.

Materials and Methods

Experiments were conducted at the Dow AgroSciences Midwest Research Center located near Fowler, IN (40.63°N, 87.10°W) and/or at the Pinney Purdue Agriculture Research Center located near Wanatah, IN (41.44°N, 86.93°W). Soil type at Fowler, IN was a Drummer silty clay loam (Fine-silty mixed, superactive, mesic Typic Endoaquolls) and at Wanatah was a Bourbon sandy loam (coarse-loamy, mixed, mesic, Aquollic Hapludalf). Plots were kept weed free by the application of preemergence herbicides and by hand weeding. At Wanatah, metribuzin (158 g ai ha^{-1}) plus chlorimuron (26 g ai ha^{-1}) were applied prior to soybean emergence. At Fowler in 2009 and 2010, trifluralin (1,400 g ai ha^{-1}) plus imazethapyr (70 g ae ha^{-1}) were incorporated twice prior to planting, and in 2011 sulfentrazone (46 g ai g^{-1}) and cloransulam-methyl (5.9 g ai g^{-1}) plus trifluralin (1,400 g ai ha^{-1}) were incorporated twice prior to planting. Detailed information of herbicides utilized can be found in Table 1. Soybeans were planted in 79-cm rows at 296,000 seeds ha^{-1}.

Daytime Expression of AAD-12. Three field experiments were planted with the event DAS-68416-4 at Fowler, IN in 2009. The experimental design was a randomized complete block with four replications. Experiments were conducted on soybean plants at the V6, R2, and R4 growth stages, because the growth stages occur prior to reproduction or during reproduction when plant needs would be focused on reproduction and AAD-12 synthesis. Six plants were arbitrarily selected in each plot to sample. The third expanded trifoliate from the top of the plant was sampled at 6:30, 9:30,

https://doi.org/10.1614/WS-D-13-00035.1 Published online by Cambridge University Press

12:30, 15:30, 18:30, and 21:30. At each sampling time, half of a leaflet was punched four times near the center of the leaflet with a 0.29 cm^2 circular area using a paper single-hole punch. Immediately after disks were punched, samples were placed on dry ice for up to 24 h then stored in a freezer at −80 C until extraction occurred. The amount of AAD-12 was quantified by using enzyme-linked immunosorbent assay (ELISA) as described by Wright et al. (2010).

Data were analyzed using SAS PROC MIXED (Statistical Analysis Systems®, version 9.2, SAS Institute Inc., Cary, NC) with the repeated measures statement to test for significant effects of time of day on AAD-12 expression. The V6, R2, and R4 timings were pooled. The experiment was treated as a random variable. Tukey's pairwise comparison was used to determine if time of day had a significant effect ($P \leq 0.05$) on AAD-12 expression.

Expression of AAD-12 in Soybean Leaf Canopy. Field experiments were planted with the events DAS-68416-4 and DAS-21606-3 at Wanatah, IN in 2009 and at Fowler, IN in 2009, 2010, and 2011. Event DAS-68416-4 has higher expression and is the lead event.

The experimental design was a randomized complete block. Three replications were implemented with six plants being sampled in each plot. Sampling was done at the V8 soybean growth stage (8 trifoliates on the main stem) and at the R4 soybean growth stage (12 trifoliates on the main stem). At the V8 growth stage each trifoliate on the main stem was sampled; whereas, at the R4 growth stage every other trifoliate was sampled. Each trifoliate was punched four times near the center of the leaf. Disks measured 0.29 cm^2 each. Samples were stored and analyzed as previously explained in the daytime expression of AAD-12 section.

Data were subjected to ANOVA by SAS PROC MIXED to test for significant effects ($P \leq 0.05$) of AAD-12 expression at each trifoliate position on the main stem. Experiment was treated as a random variable and each event and sampling time (V8 or R4 soybean growth stage) were analyzed separately because there was an interaction.

Effect of 2,4-D on AAD-12 Expression. Field experiments were planted with the events DAS-68416-4 and DAS-21606-3 at Wanatah, IN in 2009 and at Fowler, IN in 2009, 2010, and 2011. The experimental design was a randomized complete block. Experiments were replicated three times and in each plot six plants were sampled. Treatments were 2,4-D (dimethylamine salt) at rates of 0 and 2,240 g ha^{-1} applied during vegetative growth (V6 or V7 growth stage) and reproductive growth (R3 or R4 soybean growth stage). All 2,4-D treatments were applied in 140 L ha^{-1} carrier volume with a CO_2 pressurized backpack sprayer at 138 kPa with a 2.3-m-wide boom and XR11002 flat fan nozzles (TeeJet Spraying Systems Company, Wheaton, IL 60189).

Expression of AAD-12 was quantified at 0 (before 2,4-D treatment), 3, 7, 14, and 21 days after treatment (DAT). Soybean plants were sampled from the same leaf node at each sampling interval on different plants. The leaf node sampled was the uppermost open trifoliate at the time 2,4-D treatments were applied. Plants at the same growth stage were marked at the time of treatment.

Data were subjected to ANOVA using the SAS PROC MIXED to test for significant effects of 2,4-D rate at each sampling time. Experiment was treated as a random variable and each event and sample timing were analyzed separately. Tukey pairwise comparison was used to determine if 2,4-D had a significant effect ($P \leq 0.05$) on AAD-12 expression.

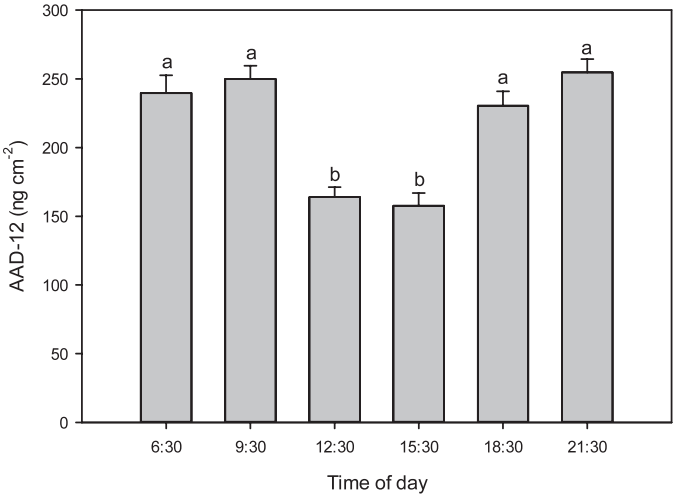


Figure 1. Aryloxyalkanoate dioxygenase-12 protein (AAD-12) expression from sunrise to sunset for the event DAS-68416-4 grown near Fowler, IN in 2009. Means of each sampling time of day with a different letter are significantly different according to Tukey pairwise comparison ($P \leq 0.05$). Bars represent the standard error.

Results and Discussion

Daytime Expression of AAD-12. Expression of AAD-12 differed ($P < 0.0001$) according to time of day ($P < 0.0001$) (Figure 1). There were no differences in the amount of AAD-12 at 6:30, 9:30,

https://doi.org/10.1614/WS-D-13-00035.1 Published online by Cambridge University Press

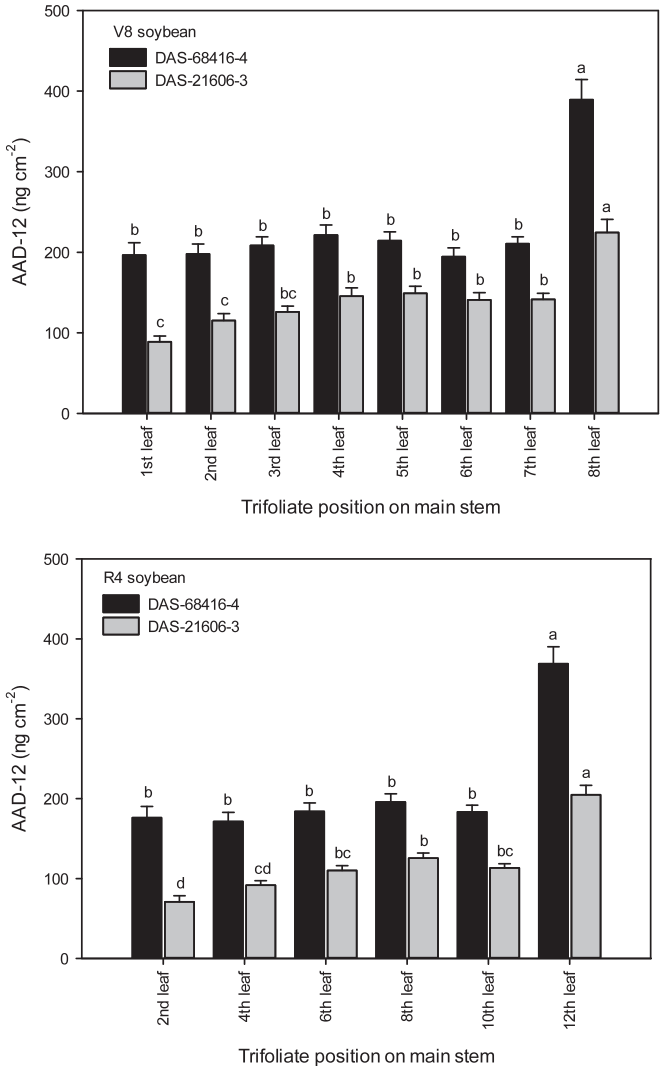


Figure 2. Expression of aryloxyalkanoate dioxygenase-12 protein (AAD-12) in leaves along the main stem at V8 and R4 soybean growth stages for the events DAS-68416-4 and DAS-21606-3. Studies were conducted near Fowler, IN in 2009, 2010, and 2011 and near Wanatah, IN in 2009. Means of each trifoliate with a different letter(s) are significantly different according to Tukey pairwise comparison ($P \leq 0.05$) within event. Bars represent the standard error.

18:30, and 21:30 (230 to 255 ng cm^{-2}). However, at 12:30 and 15:30 AAD-12 levels were reduced, to 164 and 158 ng cm^{-2}, respectively.

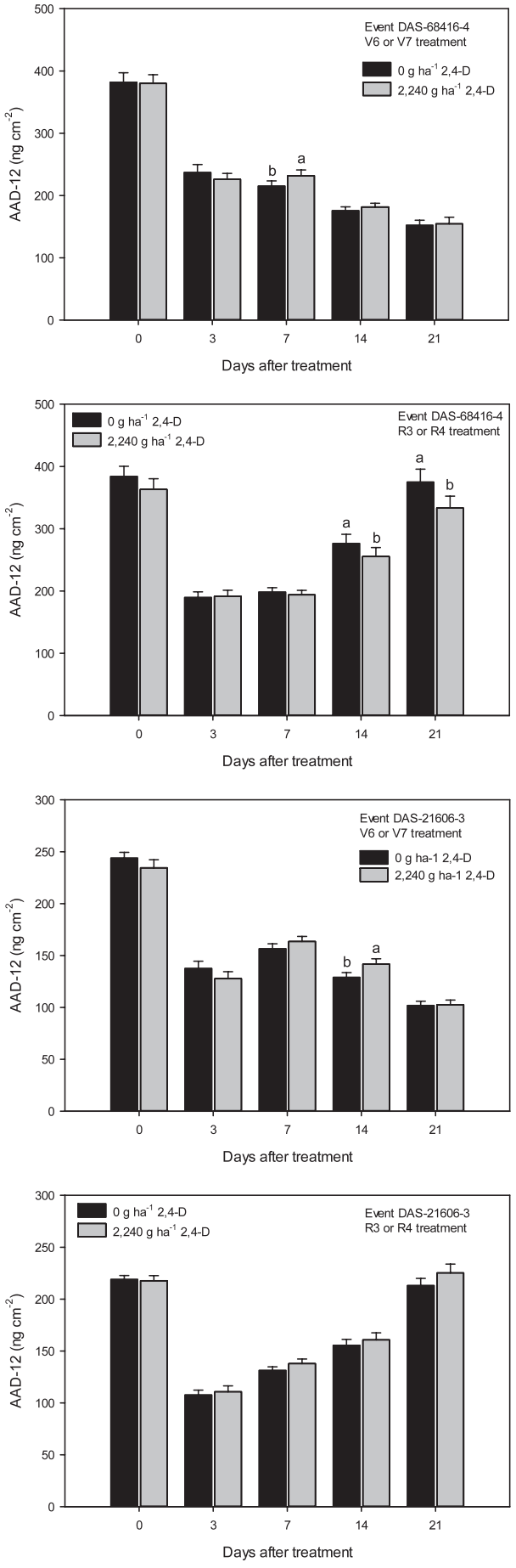
The reduction in the AAD-12 expression during that time period could be due to the degradation of proteins because of high temperatures and solar intensity. Protein degradation naturally occurs in plants, but can be accelerated by stressful conditions such as high temperature stress or nutrient deprivation (Dong and Li, 2007; Vierstra, 1993).

Expression of AAD-12 in Soybean Leaf Canopy. Leaf age had a significant effect ($P < 0.0001$, V8 growth stage; $P < 0.0001$, R4 growth stage) on the amount of AAD-12 expressed at both the vegetative and reproductive growth stages for the event DAS-68416-4 (Figure 2). At the V8 growth stage, the youngest trifoliate (8th leaf) had the highest AAD-12 expression (390 ng cm^{-2}) while older trifoliates, the first through seventh, maintained a lower level of expression (194 to 221 ng AAD-12 cm^{-2}). Similar to the vegetative sampling, at the reproductive sampling (R4) the youngest trifoliate had a higher expression of AAD-12 (369 ng cm^{-2}) than the older trifoliates, which all had a similar amount of AAD-12 (171 to 196 ng cm^{-2}). DAS-68416-4 soybean plants maintained a similar level of AAD-12 expression on leaves below the top trifoliate.

For event DAS-21606-3, leaf age was significant ($P < 0.0001$) during the vegetative sampling and during the reproductive sampling ($P < 0.0001$) (Figure 2). The youngest trifoliate (V8) had 225 ng cm^{-2} of AAD-12 which was greater than the older trifoliates. Trifoliates positioned on the V3 through V7 trifoliates had a similar amount of AAD-12 (126 to 149 ng cm^{-2}), while the trifoliates at V1 through V3 had a similar expression of AAD-12 (89 to 126 ng cm^{-2}) (Figure 2). The effect of leaf age at the R4 sampling time was similar to the V8 sampling time in trifoliates of DAS-21606-3 (Figure 2). The youngest trifoliate (12th trifoliate) had the highest amount of AAD-12 expressed (205 ng cm^{-2}), while older trifoliates expressed 71 to 126 ng cm^{-2} AAD-12. The oldest leaf sampled (2nd trifoliate) had the lowest amount of AAD-12.

The youngest trifoliate sampled consistently had the highest level of AAD-12 cm^{-2} when compared to older trifoliates regardless of the event or soybean growth stage. This is likely because in rapidly growing new leaves amino acids, proteins, and metabolites are being produced at higher rates to support new growth, and likewise transgenic proteins will also be produced in higher concentrations. In *aad*-12 transformed soybean it is likely that as leaves expand the transgenic proteins become less concentrated, thus a sampling of the same amount of leaf area in a young leaf (after leaf margins have unfolded) compared to an old leaf (when the leaf is expanded) will yield different levels of expression per leaf area sampled. However, in one study the youngest leaf in cotton, near the terminal growing point, had a lower level of expression of the *Bt* transgene compared to the fully expanded leaves on the main stem or mature basal leaves (Chen et al., 2000). The difference could be attributed to the area sampled on the leaf. In corn, it was shown that samples taken near the main vein had higher levels

https://doi.org/10.1614/WS-D-13-00035.1 Published online by Cambridge University Press



of transgenic protein expression, while sampling in older tissues especially in regions of chlorosis reduced amounts of transgenic proteins (Szekacs et al., 2010). For the *aad*-12 transgene, a higher expression in the meristem of the leaf buds could protect the plant from injury typical of 2,4-D that do not show until the new leaf buds emerge 7 to 14 days after treatment.

Effect of 2,4-D on AAD-12 Expression. The main effect of 2,4-D treatment was significant for AAD-12 expression at the V6 or V7 growth stage for the event DAS-68416-4 at 7 DAT ($P = 0.0222$) (Figure 3). At 7 DAT, the treatment of 2,240 g ha^{-1} 2,4-D averaged 232 ng cm^{-2} AAD-12, whereas 0 g ha^{-1} 2,4-D averaged 215 ng cm^{-2} AAD-12, which likely has little biological significance. At 0, 3, 14, and 21 DAT no differences of AAD-12 expression were observed with or without 2,4-D treatment. Treatment of 2,4-D at R3 or R4 soybean growth stage decreased AAD-12 expression in DAS-68416-4 at 14 DAT ($P = 0.0096$) and 21 DAT ($P = 0.0210$). At 14 DAT the untreated plants averaged 277 ng cm^{-2} AAD-12 while the plants receiving 2,240 g ha^{-1} averaged 255 ng cm^{-2}. At 21 DAT, the untreated planted averaged 375 ng cm^{-2} while the planted treated with 2,240 g ha^{-1} 2,4-D averaged 333 ng cm^{-2} of AAD-12. There was no difference in AAD-12 expression at 0, 3, and 7 DAT.

For event DAS-21606-3 the treatment of 2,240 g ha^{-1} 2,4-D on AAD-12 soybean at 14 DAT increased AAD-12 expression ($P = 0.0043$) at the V6 or V7 treatment timing. At 14 DAT, 0 g ha^{-1} 2,4-D resulted in 129 ng cm^{-2} AAD-12 while 2,240 g ha^{-1} 2,4-D averaged 142 ng cm^{-2} AAD-12 (Figure 3). At 0, 3, 7, and 21 DAT there was no difference in AAD-12 expression. AAD-12 expression was not significantly affected by 2,4-D treatment at the R3 or R4 treatment timing when applied on DAS-21606-3 at 0, 3, 7, 14, or 21 DAT (Figure 3).

Except for a few instances, there was no difference in AAD-12 expression when 2,4-D treatments were applied. The expression of AAD-12 was not consistent with respect to soybean growth stage, event, and days after 2,4-D treatment. Differences were likely a result

←

Figure 3. Effect of 2,4-D (0 and 2,240 g ha^{-1}) treatment on aryloxyalkanoate dioxygenase-12 protein (AAD-12) expression at V6 or V7 and R3 or R4 soybean growth stages each in the event DAS-68416-4 and DAS 1606-3 AAD-12 (ng cm^{-2}). Studies were conducted near Fowler, IN in 2009, 2010, and 2011 and near Wanatah, IN in 2009. Means of each day after treatment with a different letter are significantly different according to Tukey pairwise comparison ($P \leq 0.05$) within event and treatment timing. Bars represent the standard error.

https://doi.org/10.1614/WS-D-13-00035.1 Published online by Cambridge University Press

of variation in protein expression, which could be attributed to plant-to-plant variability. Expression of AAD-12 was likely high at 0 DAT because the trifoliate had not expanded completely, thus concentration of AAD-12 was greater. For the R3 or R4 treatments, the trend of AAD-12 increasing may be related to cooler temperatures late in the growing season slowing down protein degradation. The Cry1Ab toxin in corn was reported to have a 22% plant-to-plant variation in the leaves (Székács et al., 2010b). These data indicate that soybean plants were able to metabolize treatments of 2,4-D up to 2,240 g ha^{-1} (two to four times the likely field use rate) on *aad*-12 transformed soybean plants and the amount of AAD-12 was not affected.

Expression of AAD-12 throughout the plant and after 2,4-D treatment indicates the amount of AAD-12 generally remains consistent. The presence of the AAD-12 is essential for the metabolism of 2,4-D in the plant to allow the use of 2,4-D to control broadleaf weeds after soybean plants have emerged. Additionally, the presence of AAD-12 throughout the plant as leaves age is important because as spray coverage penetrates through the canopy, AAD-12s are present. Hanna et al. (2008) reported that fungicide spray coverage was 43% or greater at the middle canopy depth (30 cm) of R1 soybean canopy. Because a reduction of AAD-12 was observed during the middle of the day, further research needs to address the amount of AAD-12 needed to metabolize 1,120 g ha^{-1} 2,4-D. This will be important to ensure that 2,4-D treatments do not injure *aad*-12 transformed soybean plants.

Literature Cited

Bruns H, Craig A (2003) Nitrogen fertility effects on Bt δ-endotoxin and nitrogen concentrations of maize during early growth. Agron J 95:207

Chen S, Wu J, Zhou B, Huang J, Zhang R (2000) On the temporal and spatial expression of Bt toxin protein in Bt transgenic cotton. Acta Gossypii Sinica 12:189–193

Dong H, Li W (2007) Variability of endotoxin expression in Bt transgenic cotton. J Agron Crop Sci 193:21–29

Dunlap JC (1999) Molecular bases for circadian clocks. Cell 96:271–290

Finnegan EJ, Llewellyn DJ, Fitt GP (1998) What's happening to the expression of the insect protection in field-grown Ingard cotton. Pp 291–297 *in* Proceedings of the 9th Australian Cotton Conference. Broadbeach, Queensland: Australian Cotton Growers' Research Association

Greenplate JT (1999) Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J Econ Entomol 92:1377–1383

Hanna SO, Conley SP, Shaner GE, Santini JB (2008) Fungicide application timing and row spacing effect on soybean canopy penetration and grain yield. Agron J 100:1488–1492

Kay SA, Millar AJ (1995) New models in vogue for circadian clocks. Cell 83:361–364

Luo Z, Dong H, Li W, Ming Z, Zhu Y (2008) Individual and combined effects of salinity and waterlogging on Cry1Ac expression and insecticidal efficacy of Bt cotton. Crop Prot 27:1485–1490

McClung CR 920060 Plant circadian rhythms. Plant Cell 18:792–803

Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, Rhodes WK, Otero YI, Barry GF, Eichholtz DA (1995) Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Sci 35: 1451–1460

Robinson AP, Johnson WG, Simpson DM (2010) Effect of postemergence applications of 2,4-D on the yield components of DHT soybean. Proceedings North Central Weed Science Society 65:80

Székács A, Lauber É, Juracsek J, Darvas B (2010a) Cry1Ab toxin production of MON 810 transgenic maize. Environ Toxicol Chem 29:182–190

Székács A, Lauber É, Takács E, Darvas B (2010b) Detection of Cry1Ab toxin in the leaves of MON 810 transgenic maize. Anal Bioanalyt Chem 396:2203–2211

Taylor NB, Fuchs RL, MacDonald J, Shariff AR, Padgette SR (1999) Compositional analysis of glyphosate-tolerant soybeans treated with glyphosate. J Agric Food Chem 47: 4469–4473

Vierstra RD (1993) Protein degradation in plants. Ann Rev Plant Biol 44:385–410

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proc Natl Acad Sci USA 107:20240–20245

Received March 13, 2013, and approved July 25, 2014.

https://doi.org/10.1614/WS-D-13-00035.1 Published online by Cambridge University Press