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Halosulfuron Absorption, Translocation, and Metabolism in White and Adzuki Bean

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Halosulfuron-methyl, a sulfonylurea herbicide, was registered for broadleaf weed control in dry bean. This herbicide has an adequate margin of crop safety in white bean, but causes unacceptable injury to adzuki bean. Halosulfuron-methyl absorption, translocation, and metabolism were evaluated in white and adzuki bean using radiolabeled herbicide to determine if differences in these parameters could explain the difference in crop safety between these two species. Adzuki bean had more rapid halosulfuron-methyl absorption than white bean. Adzuki bean reached 90% absorption (t_{90}) 26.2 h after treatment (HAT), whereas white bean required 40.1 HAT to reach t_{90}. The maximum halosulfuron-methyl absorption was higher in adzuki bean (75.7%) than in white bean (65.3%). More ^{14}C-halosulfuron was translocated to the apex, first trifoliate, stem above the treated leaf, and roots in aduzki bean than in white bean. The maximum radioactivity translocated out of treated leaf was higher in adzuki bean (17.7%) than in white bean (12.1%). Halosulfuron-methyl was broken down to the same metabolites in white and adzuki bean. The half-life of halosulfuron-methyl in adzuki bean was 16 HAT, compared with less than 6 HAT in white bean. More herbicide remained as the free acid in adzuki bean compared with white bean over the entire 48-h time course. The differential tolerance of white and adzuki bean to halosulfuron can be attributed to greater absorption and translocation and decreased metabolism in adzuki bean.
Nomenclature: Halosulfuron; white bean, *Phaseolus vulgaris* L.; adzuki bean, *Vigna angularis* (Willd.) Ohwi & H.Ohashi.
Key words: Halosulfuron-methyl, sulfonylurea.

White and adzuki bean are two different dry bean species. More than 23 million t of dry bean were produced worldwide on a 29-million-ha harvested area in 2013 (FAOSTAT 2014). Weed interference can result in substantial yield losses in dry bean (Blackshaw and Esau 1991; Chikoye et al. 1995, 1996; Wilson et al. 1980) and herbicides are one of the most common weed management strategies used in dry bean production. When this study was initiated, imazethapyr was the only soil-applied dry-bean herbicide for broadleaf weed control in Ontario. Although imazethapyr is very effective for broadleaf weed control in dry bean, one concern is its narrow margin of crop safety under some environmental conditions (Arnold et al. 1993; Bauer et al. 1995; Blackshaw and Saindon 1996; Renner and Powell 1992; Soltani et al. 2007; Wilson and Miller 1991). Halosulfuron-methyl was registered in Ontario for use in white bean production in 2014 (Soltani et al. 2014a). Halosulfuron-methyl is a sulfonylurea herbicide that provides broad-spectrum broadleaf weed control (Brown and Masiunas 2002; Senseman 2007; Soltani et al. 2013, 2014a, 2014b). In studies conducted in Ontario, it was determined that there is adequate crop safety in white bean when halosulfuron-methyl is applied PPI, PRE, and POST (Soltani et al. 2009, 2012), but aduzki bean can be seriously injured by halosulfuron-methyl applied POST (Stewart et al. 2010). Halosulfuron-methyl applied POST at 35 g ai ha^{-1} caused 51, 72, and 73% injury in adzuki bean 1, 2, and 4 wk after treatment, respectively and reduced adzuki bean yield 58% (Soltani et al. 2012).

In most cases, the differential physiological selectivity of acetolactate synthase (ALS)-inhibiting herbicides is due to differential absorption, translocation, and/or metabolism (Bukun et al. 2012; Dubelman et al. 1997; Gallaher et al. 1999; Ma et

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al. 1997; McElroy et al. 2004). Dubelman et al. (1997) reported that corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) metabolized ^{14}C-halosulfuron- methyl more rapidly than soybean [*Glycine max* (L.) Merr.]. The reason that broadleaf signalgrass [*Brachiaria platyphylla* (Munro ex C. Wright) R. D. Webster] is sensitive to nicosulfuron but tolerant to primisulfuron is due to more absorption and translocation of nicosulfuron (Gallaher et al. 1999). Ma et al. (1997) found less translocation and more rapid metabolism of prosulfuron in sicklepod [*Senna obtusifolia* (L.) H. S. Irwin & Barneby] than common cocklebur (*Xanthium strumarium* L.) and common lambsquarters (*Chenopodium album* L.), which explains why sicklepod is more tolerant to prosulfuron. The reason for higher injury in adzuki bean from halosulfuron-methyl relative to white bean is unknown. Therefore, the objective of this study was to compare halosulfuron-methyl absorption, translocation, and metabolism in white and adzuki bean.

Materials and Methods

Plant Material. 'T9905' white bean and 'Erimo' adzuki bean were seeded in 10-cm-diam pots filled with a professional growing mix, Sunshine LA4 (Sungro Horticulture, Agawam, MA). After seeding, all pots were placed in a growth chamber with 25/20 C day/night temperature, 16/8 h day/night length, and approximately 500 μmol m^{-2} s^{-1} light intensity at University of Guelph, Guelph, ON, Canada. Water was applied every 2 or 3 d depending on the soil moisture and no fertilizer was added in this experiment. Two plants were seeded for each pot and only the more vigorous plant in each pot was retained at the cotyledon stage for further study. Sixteen adzuki and 16 white bean plants were used for each repeated experiment. Adzuki bean was seeded 3 d earlier than white bean, since it emerges more slowly than white bean on the basis of previous experience.

Absorption and Translocation. Uniform white and adzuki bean individuals at the first trifoliate leaf stage were selected for the study. One unifoliate leaf of each plant was marked and covered with aluminum foil before spraying the beans with commercially formulated halosulfuron-methyl (Sandea, Gowan Canada, P.O. Box 248 Station T, Calgary, AB, Canada) at 35 g ai ha^{-1} with 0.25% v/v nonionic surfactant (Agral 90, Syngenta Canada Inc., Guelph, ON). Herbicide was sprayed using a single-nozzle sprayer with a Teejet 8002E nozzle in a spray chamber calibrated to deliver 206 L ha^{-1} at 275 kPa. After application, the aluminum foil was removed and the protected leaf was treated with a ^{14}C-labeled halosulfuron-methyl solution that contained commercially formulated halosulfuron, 0.25% v/v Agral 90, and 3.7 kBq ^{14}C halosulfuron-methyl (Figure 1; provided by Gowan Canada). Ten 1-μl drops of the ^{14}C treatment solution were applied per leaf. The concentration of the application solution was equivalent to 35 g ai ha^{-1} halosulfuron-methyl. After application, all plants were returned to the growth chamber until harvest.

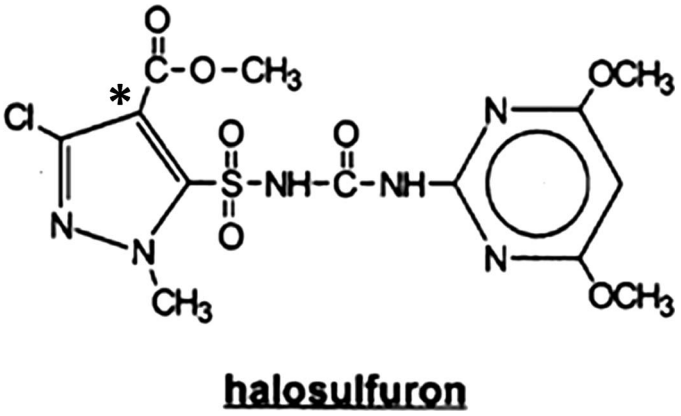


Figure 1. Structure of halosulfuron-methyl with an asterisk (*) showing the ^{14}C-labeled carbon atom.

Plants were removed from the soil 6, 12, 24, and 48 HAT and divided into the following seven parts: treated leaf, root, stem below treated leaf, opposite unifoliate leaf, stem above treated leaf, first trifoliate leaf, and plant apex. The treated leaf was washed twice with 10 ml of 9 : 1 : 0.05 distilled water, ethanol, and Tween 20 mix. Ten milliliters of Ecolite (ICN Biomedicals Inc., Irvine, CA) was added to scintillation vials and the ^{14}C halosulfuron-methyl in the leaf wash solution was quantified by liquid scintillation spectrometry (LSS) (Packard Tri-Carb, Model 2500 TR, Packard Instrument Co., Meriden, CT). All plant parts were dried in a 60 C oven for 3 d, weighed, and combusted using a biological sample oxidizer (OX500, R. J. Harvey Instrument Co., Tappan, NY). Radioactivity was quantified by LSS.

Metabolism. All plants were grown as described for the absorption and translocation experiments. Plants were grown in 22/20 day/night temperature greenhouse at Colorado State University, Fort Collins, CO. One unifoliate leaf was used from each bean plant when they were at first trifoliate leaf stage. Under degassed water, 16 unifoliate leaves of

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white and adzuki bean were cut at the petiole base and transferred to a 0.5-ml microcentrifuge tube. Approximately 0.05-μCi ^{14}C-halosulfuron-methyl was placed into each microcentrifuge tube. The amount of water that the leaf absorbed was monitored every hour and degassed water was added into the microcentrifuge tube when the water level became low. Water was added to each microcentrifuge tube multiple times to ensure that enough ^{14}C-halosulfuron-methyl would be absorbed by each leaf. Leaves were then transferred into six-well Corning Costar cell culture plates (Sigma-Aldrich, St. Louis, MO) that contained enough degassed water to ensure leaf survival for the duration of the experiment. Leaves were collected 6, 12, 24, and 48 HAT. Upon collection, liquid nitrogen was immediately applied to every leaf to stop metabolism. All samples were dried in a 60 C drier for 3 d and then ground in 50-ml glass tubes for 3 min with a pestle. Five milliliters of a solution containing 40% acetonitrile and 60% water was added to ground samples, and vortexed in a RapidVap for 45 min (Labconco, Kansas City, MO). Once the biomass settled, the supernatant was poured off into a separate 50-ml glass tube. This step was repeated two more times until there was a total of at least 15 ml of supernatant. Fifteen milliliters of the supernatant were then filtered through a 0.45-μm syringe filter using a 20-ml glass syringe. The filtrate was evaporated and centrifuged to dryness using a RapidVap at 38 C under vacuum for 5 h. Samples were resuspended in 225 μl of mobile phase A, which contained 89.95% water, 10% acetonitrile, and 0.05% phosphoric acid. Radioactivity levels were determined using reverse-phase high-performance liquid chromatography (HPLC; Hitachi Instruments, Inc., San Jose, CA) using a C18 4.6 mm by 250 mm column (Zorbax, Agilent Technologies, Santa Clara, CA). The injection volume was 200 μl. Halosulfuron-methyl was detected at 18.67 ± 0.08 min and halosulfuron-free acid was detected at 12.37 ± 0.08 min using the following gradient: 89.95% water : 10% acetonitrile : 0.05% phosphoric acid solution to 99.95% acetonitrile : 0.05% phosphoric acid solution over 25 min with a flow rate of 1 ml min^{-1}. Radioactivity was detected using HPLC coupled with an inline radioactivity detector equipped with a solid cell (Berthold LB 513 radioactivity detector, flow cell YG 400-S5D).

Statistical Analysis. Experiments were conducted as a randomized complete block design with four replications. The absorption, translocation, and metabolism experiments were repeated. The data from repeated experiments were analyzed by PROC MIXED in SAS 9.3 (SAS Institute Inc., Cary, NC) to assess if the repeated experiments could be combined for analysis. Absorption and translocation out of treated leaf regression were analyzed in R (R Core Team 2015) on the basis of Kniss et al. (2011) (Equation 1).

$$y = (A_{\max} t)/(0.11 t_{90} + t) \quad [1]$$

The metabolism regression was plotted using Sigmaplot v. 12.1 (Systat Software Inc., Chicago, IL). Halosulfuron-methyl metabolism to the free acid was described by linear regression (Equation 2) in adzuki bean, and by logistic regression (Equation 3) in white bean. Conversion of halosulfuron free acid to unknown metabolite(s) in adzuki bean and white bean was described by asymptotic regression (Equation 4).

$$y_a = b + ax \quad [2]$$

$$y_{wA} = ae^{(-bx)} \quad [3]$$

$$y_{wB} = a(1 - e^{-bx})^c \quad [4]$$

Equation 2 is a simple linear regression where y_a is halosulfuron-methyl in adzuki bean, a is the slope, b is the y intercept, and x is the time after application (h). Equation 3 is a logistic regression where y_{wA} is halosulfuron-methyl conversion to the free acid in white bean, a is the slope of the line, b is the point of inflection, and x is the time after application (h). Equation 4 is an asymptotic regression where y is the halosulfuron free acid in white bean and adzuki bean, a is the minimum percent of halosulfuron free acid, b is the slope, c is the exponent, and x is the time after application (h). Half-life was calculated as the point where y was equal to 50.

Results and Discussion

Absorption. Data from the absorption experiments were pooled because there was no significant difference between repeated experiments ($P = 0.4851$). The average recovery of applied ^{14}C-halosulfuron-methyl was 83 and 82% for white and adzuki bean, respectively. Asymptotic regression and rectangular hyperbolic models were compared using the Akaike information criterion (AIC) to determine which function best described absorption data (Kniss et al. 2011). The rectangular hyperbolic model (AIC: 988) (see Equation 1) provided a better

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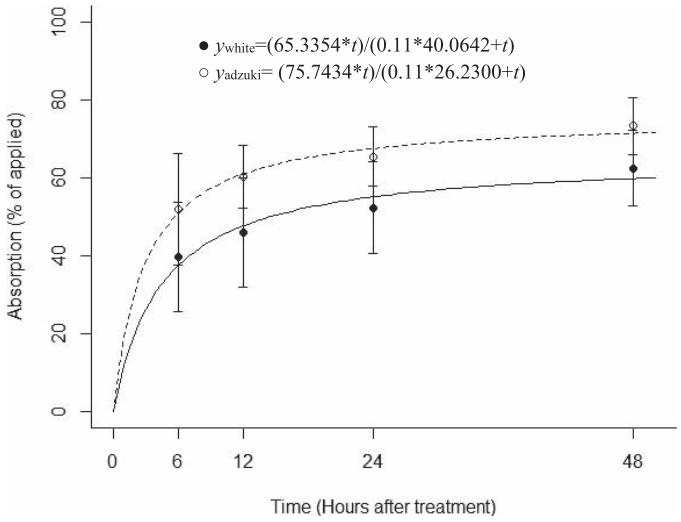


Figure 2. Rectangular hyperbolic model for ^{14}C-halosulfuron-methyl absorption, expressed as percentage of total applied ^{14}C-halosulfuron-methyl absorbed by white (•, —) and adzuki (○, —) bean plants.

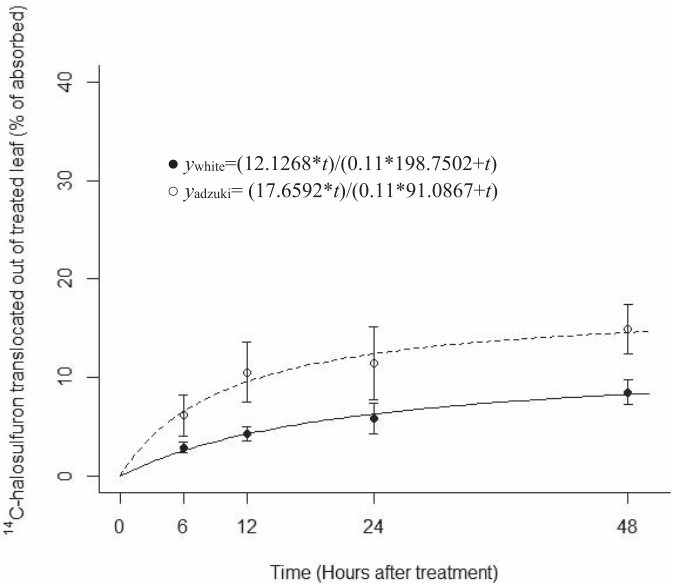


Figure 3. Rectangular hyperbolic model for total translocation of ^{14}C-halosulfuron out of the treated leaf (expressed as percentage of total absorbed) in white (•, —) and adzuki bean (○, —).

fit compared with the asymptotic regression (AIC: 998), so the rectangular hyperbolic model was used to describe ^{14}C-halosulfuron-methyl absorption by white and adzuki bean (Figure 2). Adzuki bean absorbed approximately 10% more halosulfuron-methyl than white bean over the 48-h time period (Figure 2). Using the rectangular hyperbolic model shown in Equation 1 (Kniss et al. 2011), the predicted total absorption (A_{max}) was significantly lower ($P < 0.001$) in white (A_{max} =65.3%) compared with adzuki bean (A_{max} =75.7%) (Figure 2). The time required to reach t_{90} was also significantly different. Adzuki bean reached t_{90} in 26.2 h compared with 40.1 h for white bean.

^{14}C-Halosulfuron-methyl absorption by white and adzuki bean was similar to the absorption measured in *Kyllinga* spp. (McElroy et al. 2004). Approximately 48% of applied ^{14}C halosulfuron-methyl was absorbed by *Kyllinga* spp. 4 HAT and reached 63% at 96 HAT. It was also similar to the absorption rates seen for other ALS-inhibiting group 2 herbicides, where most of the herbicide was absorbed within 24 h. Imazamox absorption was 45 and 57% at 6 and 12 HAT, respectively, and reached 60% at 96 HAT in Othello pinto bean (*Phaseolus vulgaris* L.) (Bukun et al. 2012). Nicosulfuron absorption averaged 40, 57, and 65% in annual bluegrass (*Poa annua* L.) and four other turfgrass species at 4, 24, and 96 HAT, respectively (Sidhu et al. 2014). In corn and broadleaf signalgrass, Gallaher et al. (1999) found that most applied primisulfuron and nicosulfuron was absorbed within 24 HAT. Similarly, Frazier et al. (1993) found that primisulfuron absorption in corn reached 76% at 24 HAT, with little additional absorption occurring 48 and 96 HAT.

Translocation. Data from the two translocation experiments were combined, since they were not significantly different ($P = 0.6997$). Adzuki bean translocated more halosulfuron than white bean over the 48-h time period (Figure 3). The rectangular hyperbolic model (AIC: 288) and the asymptotic regression (AIC: 290) provided similar fit for the translocation data. The rectangular hyperbolic model was chosen to describe ^{14}C-radioacitivity translocated out of the treated leaf in white and adzuki bean (Figure 3). Using the rectangular hyperbolic model shown in Equation 1 and redefining A_{max} as T_{max} (predicted total translocation) (Kniss et al. 2011), T_{max} out of the treated leaf was significantly lower ($P < 0.0001$) in white bean (T_{max} =12.1%) compared with adzuki bean (T_{max} =17.7%) (Figure 3). The time predicted to reach 90% of maximum translocation (t_{90}) for adzuki bean was 91.1 h, which was more rapid than white bean (t_{90} = 198.8 h). White and adzuki bean retained 91 and 82% of absorbed radioactivity, respectively, at 48 HAT, further indicating increased translocation out of the treated leaf in adzuki bean. Previous studies also found that most of the applied ^{14}C-sulfonylurea herbicide remained in the treated leaf. Lycan and Hart (1999) reported that 71 to 97% of absorbed ^{14}C-thifensulfuron

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remained in the treated leaf of soybean, velvetleaf (*Abutilon theophrasti* Medik.), and common lambsquarters. Three times more radioactivity accumulated in adzuki bean root than in white bean root over the 48-h time course. The total amount of radioactivity in the root was low, around 0.4% of total applied radioactivity (data not shown).

Lycan and Hart (1999) reported that more thifensulfuron translocated above the treated leaf than below the treated leaf (to the roots) in velvetleaf and common lambsquarters. Frazier et al. (1993) reported that 97% of applied ^{14}C-primisulfuron remained in the treated leaf and less than 1% of absorbed primisulfuron translocated to the shoot and roots. Bukun et al. (2012) reported that only 0.16% of total ^{14}C-imazamox absorbed was translocated out of treated leaves in pinto bean and only 0.1% of total translocated ^{14}C-imazamox was translocated to shoots and roots.

Metabolism. White and adzuki bean produced very similar metabolite profiles (Figure 4). Halosulfuron-methyl was metabolized more slowly and to a lesser extent in adzuki bean (Figure 5A). Adzuki bean metabolism resulted in a halosulfuron-methyl half-life of 16 h, compared with a half-life in white bean of less than 6 h (Figure 5A). A consistently higher level of halosulfuron free acid (approx. 10% more) remained in adzuki bean compared with white bean over 48 h (Figure 5B).

To our knowledge, there are no previous reports describing halosulfuron-methyl metabolism in white and adzuki bean; however, halosulfuron-methyl metabolism has been studied in several other species. In *Kyllinga* spp. and soybean cell culture, very little metabolism was reported (Dubelman et al. 1997; McElroy et al. 2004), but in corn and wheat cell cultures halosulfuron-methyl was quickly degraded. In soybean cell culture, approximately 86 to 96% of the halosulfuron-methyl was unchanged 168 HAT, whereas almost 100% was metabolized by corn and wheat 6 HAT. Around 92% of radioactivity was converted to an unknown metabolite (Figure 4B) within 12 h in white bean, which had similar levels of metabolism compared with corn and wheat (Figure 5A). However, much lower levels of metabolism occurred in adzuki bean compared with corn and wheat (Figure 5). The most common metabolic transformation in plants for most sulfonylurea herbicides is glucose conjugation (Rao 2000). Hydroxylation of the pyrimidine ring followed by a rapid conjugation with glucose is the reason, for example, that corn rapidly inactivates halosulfuron-methyl (Dubelman et al. 1997).

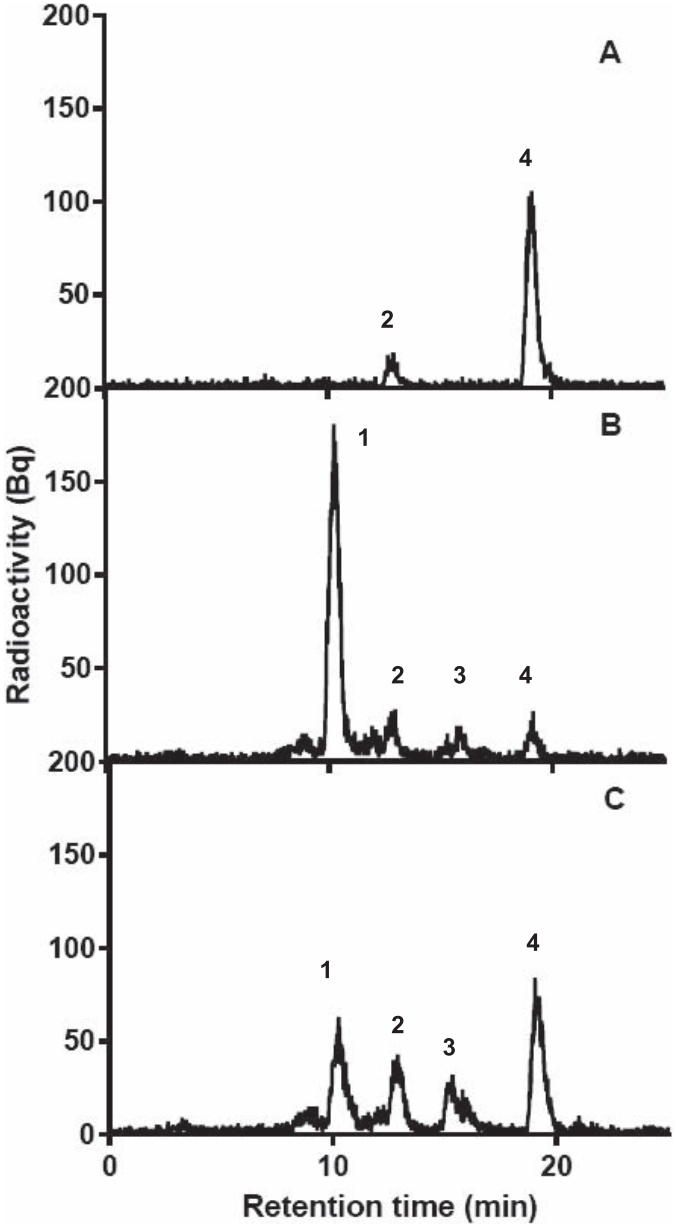


Figure 4. High-performance liquid chromatography chromatograms of ^{14}C-halosulfuron standard (A), and from excised unifoliate leaf of white (B) and adzuki bean (C) treated with ^{14}C-halosulfuron at 48 h after treatment. Peaks are labeled as (1) unknown metabolite, (2) halosulfuron free acid, (3) unknown metabolite, and (4) halosulfuron-methyl.

There appear to be several factors contributing to the differential sensitivity of white and adzuki bean to halosulfuron-methyl. Halosulfuron-methyl was absorbed more rapidly and there was significantly more halosulfuron translocation out of the treated leaf in adzuki bean compared with white bean. Metabolism studies confirmed that the half-life of halosulfuron was shorter in white bean compared with adzuki bean. Greater halosulfuron absorption, increased translocation, and reduced metabolism in

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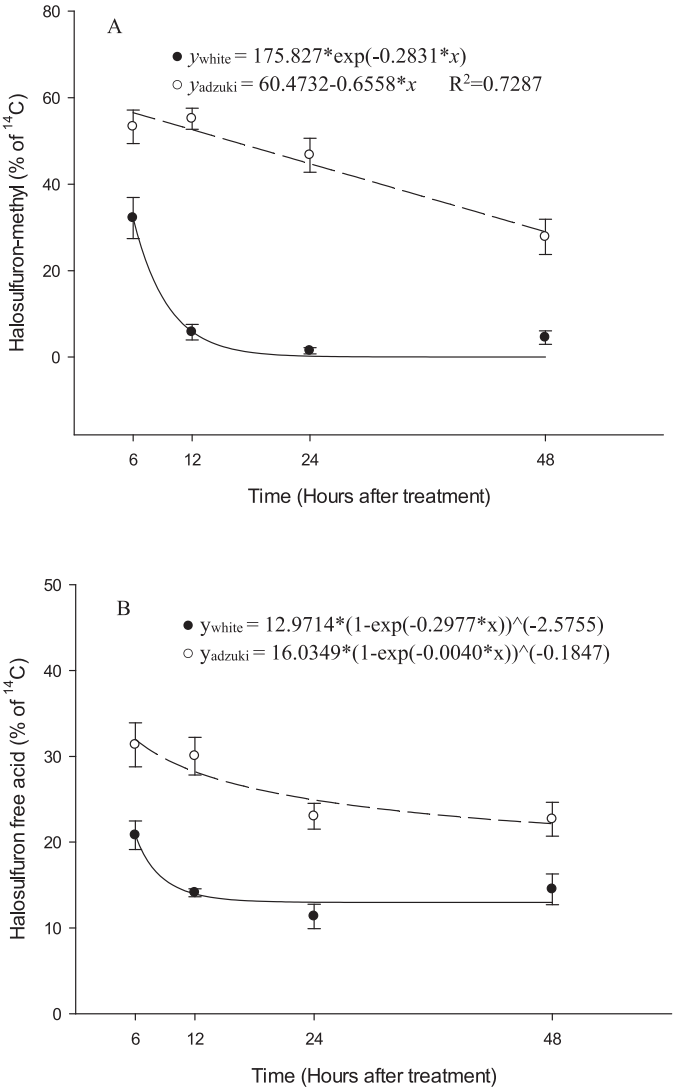


Figure 5. Metabolism of ^{14}C-halosulfuron in white (•, —) and adzuki (○, —) bean during 48-h time course. (A) Percentage of radioactivity remaining as ^{14}C-halosulfuron-methyl (peak 4 in Figure 3); (B) Percentage of radioactivity as halosulfuron free acid (peak 2 in Figure 3).

adzuki bean vs. white bean are the primary factors contributing to increased halosulfuron phytotoxicity on adzuki bean observed under field conditions. Although this work has produced a greater understanding of the mechanism responsible for differential sensitivity to halosulfuron-methyl between white bean and adzuki bean, further studies to identify the secondary halosulfuron metabolites in adzuki and white bean would be useful.

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