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Bases for Interactions between Saflufenacil and Glyphosate in Plants

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Buckwheat (*Fagropyrum esculentum* Moench.), cabbage (*Brassica oleracea* L), and conventional and glyphosate-resistant varieties of canola (*Brassica napus* L.) were used to study the bases of saflufenacil and glyphosate interactions. Compared to the addition of Merge (surfactant), the addition of both Transorb (i.e., commercial product, Transorb formulation with glyphosate) and Merge increased the cuticular absorption of [^{14}C] saflufenacil in cabbage plants with thick epicuticular wax layers. However, in all cases, the addition of glyphosate reduced the translocation of [^{14}C]saflufenacil in glyphosate-susceptible plants, while translocation was not affected in glyphosate-resistant canola. Moreover, the phytotoxicity of saflufenacil reduced the activity of glyphosate, possibly by reducing its translocation in all plant species studied. Increased absorption of saflufenacil by the addition of Transorb (i.e., Transorb formulation with glyphosate) plus Merge appears to increase its contact activity, thus the interaction of saflufenacil and glyphosate involves two separate processes, absorption and translocation.

KEYWORDS: Saflufenacil; glyphosate; buckwheat (*Fagropyrum esculentum* Moench.); cabbage (*Brassica oleracea* L.); canola (*Brassica napus* L.); glyphosate-resistance; herbicide antagonism; radiolabeled herbicide; uptake; translocation

INTRODUCTION

Saflufenacil (BAS 800H; *N*′-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4(trifluoromethyl)-3,6-dihydro-1(2*H*)-pyrimidinyl)-benzoyl]-*N*-isopropyl-*N*-methylsulfamide) is a uracil-based herbicide manufactured by BASF. This herbicide is a potent inhibitor of protoporphyrinogen oxidase (PPO, EC 1.3.3.4, also known as Protox) and is used for pre-emergence and postemergence control of major broadleaf weeds and has pre-emergence selectivity in crops such as corn (*Zea mays* L.) and soybean (*Glycine max* L.) (unpublished data, BASF). PPO catalyzes the last common reaction in the tetrapyrrole biosynthetic pathway, leading to formation of both chlorophyll and heme. While the biosynthesis of chlorophyll takes place exclusively in plastids, heme is catalyzed in both plastids and mitochondria of plants (*1*, *2*). In both organelles, PPO catalyzes the oxidation of protoporphyrinogen IX (Protogen IX) to highly conjugated (i.e., double bonds) protoporphyrin IX (Proto IX) (*3*).

PPO is the target site of several photodynamically active porphyric herbicides of diphenyl ether, phenyl heterocycle, and heterocyclic carboxamide chemical families (*4*, *5*). In susceptible species, PPO-inhibiting herbicides competitively inhibit PPO by occupying the binding site for Protogen IX (*6*). As a result, PPO substrate (i.e., Protogen IX) accumulates and diffuses from the organelles into the cytoplasm (*7*, *8*). Once in the cytoplasm, Protogen IX is metabolized to Proto IX through either a nonenzymatic oxidative process (*7*) or oxidation by a herbicide-insensitive peroxidase-like enzyme in the plasma membrane (*8*, *9*). Thus, Proto IX accumulates in the cytoplasm and, in the presence of light, induces the formation of singlet oxygen that damages the cell membranes (*9*). Most PPO-inhibitors exhibit little or no phloem movement; however, saflufenacil appears to be different, having physical/chemical properties (intermediate logP and acidic functionality) consistent with phloem mobility as well as demonstrated biological activity that is suggestive of phloem mobility (unpublished data, BASF).

Historically, PPO-inhibitors have not been used extensively due to their narrow range of tolerant crops. The primary use of commercialized PPO-inhibitors has been limited to postemergence applications in soybeans and minor crops (e.g., ornamentals and peanuts). Pre-emergence use of PPO inhibiting herbicides has been largely unsuccessful because of poor weed control unless applied at high doses (*10*). Consequently, it has been suggested that the addition of complementary herbicides (e.g., imazamox) may improve the postemergence effectiveness of PPO inhibiting herbicides in soybeans (*11*, *12*). However, in most cases, the combinations were antagonistic and resulted in reduced efficacy of both herbicides (*11*, *12*).

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After the introduction of glyphosate-resistant soybean, it was thought that PPO-inhibitors could complement glyphosate for the control of weed species that are sensitive to PPO-inhibitors but exhibit natural tolerance to glyphosate (e.g., ragweed (*Ambrosia artemisiifolia* L.), velvetleaf (*Abutilon theophrasti* Medikus.), and pitted morning-glory (*Ipomoea lacunose* L.)) (*13*). However, in many cases, combinations of glyphosate and a PPO-inhibitor, such as fomesafen or sulfentrazone, caused reduced efficacy of both herbicides (*13*). Further investigation showed a decrease in absorption and translocation of [^{14}C]glyphosate with the addition fomesafen (*13*). However, because the absorption and translocation of either fomesafen or sulfentrazone was not evaluated, the cause of decreased efficacy of these two products is unknown. To further expand the use of this group of herbicides with tank mixes, the compatibility of products added together must be thoroughly investigated because several researchers have shown antagonistic effects, and in some cases synergistic effects, between glyphosate and different PPO-inhibiting herbicides (*14*−*16*).

Glyphosate is the world's most widely used herbicide because of its broad spectrum, cost-effective, and environmentally sound weed control. Since its initial nonselective use in orchards, vineyards, and industrial situations, glyphosate has found a range of selective uses in agriculture, predominantly as a result of the introduction of genetically modified glyphosate-resistant crop varieties and the widespread adoption of minimum and no-tillage conservation practices (*17*). While there are many benefits associated with transgenic, glyphosate-resistant crops, over-reliance on glyphosate has increased the risk of weed species evolving resistance to glyphosate (*18*). Currently, 14 prominent weed species have developed resistance to glyphosate worldwide (*19*). Thus, the proactive adoption of resistance management practices (e.g., herbicide mixtures) is required to maintain the benefits of glyphosate technology for future generations (*20*).

Because phloem mobility is a valuable trait and saflufenacil appears to be unique among PPO-inhibitors by possessing this property, quantification of the phloem mobility of this herbicide may be important for differentiating it from other PPO inhibiting herbicides. Furthermore, combination of saflufenacil with the commercial formulation of glyphosate, under field conditions, has shown weed control that is better than expected based on the individual performance of the two herbicides (unpublished data, BASF). Determination of the basis of interaction between glyphosate and saflufenacil may help identify more efficient, environmentally friendly, and economically efficient weed management systems by optimizing the application of these products. The hypotheses for this research were (i) formulated glyphosate will increase the uptake of saflufenacil when the two herbicides are applied in a mixture with a surfactant, and (ii) saflufenacil will reduce the herbicidal activity of glyphosate. Therefore, the goals of this research were to: (i) quantify the absorption and phloem mobility of saflufenacil, using [^{14}C]saflufenacil, (ii) determine the synergistic effect of glyphosate and thus understand the mechanism(s) by which glyphosate improves the efficacy of saflufenacil, and (iii) study the influence of saflufenacil on the herbicidal activity of glyphosate.

MATERIALS AND METHODS

Plant Materials, Growth Conditions. Buckwheat (*Fagropyrum esculentum* Moench.) and cabbage (*Brassica oleracea* L. var. Capitata) plants were used for initial absorption and translocation studies. These species were selected based on the thickness of their epicuticular wax layers, with cabbage plants having a thicker epicuticular wax layer than buckwheat plants. Further studies were conducted using conventional (CL) and glyphosate-resistant (Roundup Ready, RR) varieties of canola (*Brassica napus* L.), i.e. 45H73 (CL) and 45H21 (RR) (Pioneer Hi-Bred Limited, Chatham, ON, Canada). In all cases, seeds were planted in 450-mL pots containing a commercial potting mix, Promix BX (Premier Brands, Brampton, ON, Canada). Plants were grown in a growth room at 25 ± 5 °C and 75% (±5%) relative humidity, with light being supplied by a mixture of incandescent bulbs and fluorescent tubes, which provided a constant light intensity between 200 and 500 μEinstein m^{-2} s^{-1}. Light and dark periods were 16 and 8 h, respectively. Water-soluble fertilizer (20% N, 20% P_2O_5, 20% K_2O) was applied twice weekly to promote optimal growth.

Radiolabeled Saflufenacil. [Phenyl-U-^{14}C]saflufenacil (BASF), with a specific activity of 5.54 MBq/mg, was dissolved in acetone (pesticide grade) and kept at 4 °C. [^{14}C]saflufenacil, formulation blank, and formulated saflufenacil were combined with water to create a herbicide concentration of 4.99 mM, which is equivalent to 25 g of ai ha^{-1} (recommended field rate) applied at 100 L ha^{-1}. The final working stock solution contained approximately 200 Bq of [^{14}C]saflufenacil in 1 μL (0.122 mM).

Herbicide Absorption Using Isolated Cuticles. Leaf cuticles from the second leaf of 3- to 4-leaf buckwheat and cabbage seedlings were isolated using the ZnCl/HCl technique described by Kloppenburg and Hall (*21*, *22*) and Ramsey et al. (*23*). Using a small wire spatula, isolated leaf cuticles were transferred to 2.5 cm deep Petri plates filled with water. Cuticle holders made of plexiglass and nylon mesh (developed by Ramsey et al. (*23*)) were submerged and moved underneath a floating isolated cuticle. When raised, the isolated cuticle rested on the nylon membrane of the cuticle holder; the cuticle holders were moved to a paper towel prior to application of the herbicide to the cuticle. Herbicide treatments were: (i) [^{14}C]saflufenacil plus formulated saflufenacil (i.e., saflufenacil), (ii) [^{14}C]-saflufenacil plus formulated saflufenacil + Merge (M; 50% surfactant blend + 50% solvent (petroleum hydrocarbons), BASF Canada Inc.), (i.e., saflufenacil+M), (iii) [^{14}C]saflufenacil plus formulated saflufenacil plus Transorb (T, Roundup Transorb, isopropylamine salt of glyphosate with transorb formulation, Monsanto Canada, Winnipeg, MB, Canada), (i.e., saflufenacil+T), and (iv) [^{14}C]saflufenacil plus formulated saflufenacil plus T and M (i.e., saflufenacil+M+T). Concentration of saflufenacil and glyphosate, were 4.99 and 39 mM, respectively, which is equivalent to 25 and 900 g ai ha^{-1} applied at 100 L ha^{-1}. Merge was applied at 0.5% v/v. Cuticles were treated with 2 1 μL drops of herbicide solution thus delivering 400 Bq of [^{14}C]saflufenacil, using a Wiretrol II 10 μL micropipet that delivered 1.0 μL droplets.

Immediately after herbicide application, the cuticle holders were moved to their respective plexiglass bases inside a plexiglass chamber containing the finite-dose diffusion half-cell apparatus previously described by Ramsey et al. (*23*). This apparatus consists of a plexiglass chamber inside which four cuticles can be placed in individual holders. Beneath each cuticle holder is a plexiglass base connected to two plastic tubes, one leading to a 60-mL syringe filled with distilled water and the other to a clamped rubber hose where samples were collected. When a cuticle was in place, water from the 60-mL syringe was injected into the plexiglass base up through the cuticle holder such that the cuticles were floating on the water. Thus, when the cuticle holders are placed on the plexiglass bases, they form a cuvette from which sampling solution can be pumped underneath the floating cuticle from the 60-mL syringes and collected in scintillation vials from the clamped rubber hose. To prevent leaks between the cuticle holder and base, silicone grease was applied to the surface of the plexiglass bases upon which the cuticle holders were placed. Samples containing [^{14}C]-saflufenacil that penetrated the cuticles were taken by injecting 1 mL of water into the cuvette and then allowing 1 mL of water already in the cuvette to drain into a scintillation vial. This procedure was repeated three times at each sampling time. The relative humidity inside the chamber was measured by thermohygrometers (Fisher Scientific, Nepean, ON, Canada) and controlled at 40% by regulating the flow of dry or moist air into the chamber. Humid air (95% + RH at 23 °C, VPD 0.12 kPa) was supplied from cooled air that had been bubbled through heated water. Air circulation inside the chamber was provided by two 12 V, 0.08 A brushless fans (Radio Shack, Ft. Worth, TX) connected to a 6 V power supply.

Samples from the solutions underlying each cuticle were taken 0, 0.5, 1, 2, 4, 6, and 12 h after treatment (HAT). After the last samples were taken, the cuticles were removed from the holders using forceps, rinsed in 5 mL of leaf-wash solution (aqueous 20% ethanol, 0.5% Tween 20), and placed in a scintillation vial prior to standard liquid scintillation spectrometry (LSS) using a Beckman LS6K-SC scintillation counter (Beckman Instruments Inc., Fullerton, CA).

Absorption and Translocation Studies. In all studies, plants were grown as described previously and were treated at the 3- to 4-leaf stage of development. Treatment combinations are described above and were applied as 10 1 μL droplets, delivering 2000 Bq of [^{14}C]saflufenacil to the center of the adaxial side of the second leaf of each plant. Furthermore, to determine whether absorption and translocation of saflufenacil were influenced by active ingredient or formulation of T, CL, and RR, canola varieties were subjected to a fifth treatment, i.e., [^{14}C]saflufenacil+formulated saflufenacil + glyphosate (nonadjuvant loaded glyphosate (Accord Concentrate, isopropylamine salt; G))+M (i.e., saflufenacil+G+M). The herbicide concentrations in this treatment were the same as those used in the previously described treatments. One hour after the application, the treated plants were returned to the greenhouse. Plants were harvested 6, 24, and 48 HAT. Each treated leaf (TL) was divided into three sections: the treated area (TA), the leaf above (leaf tip) and below (petiole end) the treated area, while the rest of plant was dissected into plant tissues above and below (including root) the treated leaf. To measure the amount of unabsorbed [^{14}C]saflufenacil, the treated area of each leaf was rinsed three times, each time with 5 mL of 20% ethanol and 0.5% Tween 20 (enzyme grade) in water (v/v/v). Each rinsate was dissolved in 15 mL of Ecolite (+) (MP Biomedicals Inc., Irvine, CA), and radioactivity was quantified by LSS. Harvested plant parts were immediately wrapped in tissue paper and kept in a drying oven at 50−60 °C until combusted. Combustions were carried out with a biological oxidizer (model OX-500, R. J. Harvey Instruments Corp., Hillsdale, NJ) set at a 3 min combustion cycle with a flow rate of 350 mL min^{-1} of N_2 and O_2. The resulting $^{14}CO_2$ was trapped in 15 mL of ^{14}C cocktail (R. J. Harvey instrument Corp.), and radioactivity quantified by LSS. Recovery of radioactivity after combustion in the oxidizer was 87.1% (with a standard deviation of 3.5%), as determined by combustion of a known amount of D-manitol-1-^{14}C. Foliar uptake was determined by addition of ^{14}C recovered in all plant tissues. Total ^{14}C recovered outside the TA was determined by addition of ^{14}C recovered in all plant tissues other than TA, accounting for both active and passive (e.g., diffused) translocation.

Bioassay Study. Bioassay experiments were conducted to determine the influence of saflufenacil on the herbicidal activity of glyphosate in CL canola seedlings. Herbicide treatments were applied as described in Absorption and Translocation Studies, i.e., to the second leaf of 3- to 4-leaf stage CL canola seedlings. In these experiments, 22 nonradiolabeled treatments were used (**Table 1**). Live plants were harvested by cutting them at the soil level 10 days after treatment, followed by drying at 70 °C for 72 h prior to recording their dry weight (DW). The biomass of the dead plants at the time of harvest was considered to be zero and scored as complete control.

Experimental Design and Statistical Analysis. *Herbicide Absorption Studies* (*Isolated Cuticle Studies*). The experimental design in these experiments was randomized complete block with six repetitions, with each cuticle being an experimental unit. Data were subjected to analysis of variance (ANOVA) and residual analysis using SAS 9.2 (SAS Institute Inc., Cary, NC) that employed PROC GLM, PROC UNIVARIATE. Type I error was set at 0.05. These analyses revealed whether the distribution of the residuals (the experimental error component of the general linear model) met the criteria for ANOVA, what data could be eliminated as outliers, and the sources of variance (*24*).

Absorption and Translocation Study. The absorption and translocation studies were organized as a factorial design with herbicide treatments, time after treatment, and plant part as the factors. The experimental units were individual plants. Treatments were replicated three times, and the experiments were conducted twice. Data were combined because there were no time by treatment interactions ($\alpha = 0.05$). Total foliar uptake of ^{14}C, total unabsorbed ^{14}C, and total ^{14}C recovered outside the TA were expressed as percentage of total ^{14}C recovered, whereas the data from translocated ^{14}C, including total translocated ^{14}C (recovered ^{14}C in plant tissues above and below TL) and translocated ^{14}C in plant tissues above TL, were expressed as percentage of the ^{14}C recovered in planta. Data were subjected to ANOVA, residuals analyses, and Duncan's multiple-range tests (used to separate treatment means within harvest times) using SAS 9.2 that employed PROC GLM and PROC UNIVARIATE.

Bioassay Studies. All of the experiments were organized as randomized complete blocks, with individual plants as the experimental unit. Treatments were replicated three times ($n = 3$), and the experiments were conducted twice. Data from experiments were combined because no time by treatment interactions were detected ($\alpha = 0.05$). DW data from surviving plants were subjected to analysis of variance (ANOVA) and residual analysis using SAS 9.2 that employed PROC GLM and PROC UNIVARIATE.

Table 1. Doses of Formulated Saflufenacil, Transorb (T), Unformulated Glyphosate (G), and Merge (M) in Different Treatments Used in the Bioassay Study[a]

		g ai ha^{-1}			
no.	treatment	saflufenacil	T	G	M % v/v
1	saflufenacil (12.5)	12.5			
2	suflufenacil (25)	25			
3	suflufenacil (12.5)+M	12.5			0.5
4	saflufenacil (25)+M	25			0.5
5	T(225)		225		
6	T(450)		450		
7	T(900)		900		
8	T(225)+M		225		0.5
9	T(450)+M		450		0.5
10	T(900) +M		900		0.5
11	saflufenacil (12.5)+T (225)+M	12.5	225		0.5
12	saflufenacil (12.5)+T(450)+M	12.5	450		0.5
13	saflufenacil (12.5)+T(900)+M	12.5	900		0.5
14	saflufenacil (25)+T(225)+M	25	225		0.5
15	saflufenacil (25)+T(450)+M	25	450		0.5
16	saflufenacil (25)+T(900)+M	25	900		0.5
17	saflufenacil (12.5)+G(225)+M	12.5		225	0.5
18	saflufenacil (12.5)+G(450)+M	12.5		450	0.5
19	saflufenacil (12.5)+G(900)+M	12.5		900	0.5
20	saflufenacil (25)+G(225)+M	25		225	0.5
21	saflufenacil (25)+G(450)+M	25		450	0.5
22	saflufenacil (25)+G(900)+M	25		900	0.5

[a] Herbicide doses were: formulated saflufenacil at saflufenacil concentrations of 2.495 and 4.99 mM in water, which is equivalent to 12.5 and 25 g of ai ha^{-1}, respectively; T and G at 9.75, 19.5, and 39 mM, which is equivalent to 225, 450, and 900 g ai ha^{-1} of glyphosate, respectively, applied at 100 L ha^{-1}. M was added at 0.5% v/v.

RESULTS AND DISCUSSION

Herbicide Absorption Using Isolated Cuticles. The isolated cuticle research was conducted only as a model to compare the hypothetical difference in uptake by, and diffusion through, the cuticle of intact plants treated with various formulants plus saflufenacil. Isolated cuticle studies indicated that the addition of both M and T increased the absorption of [^{14}C]saflufenacil in buckwheat and cabbage cuticles. However, M had a greater effect on the absorption of [^{14}C]saflufenacil than T (**Figure 1A**,**B**). The addition of T to saflufenacil+M (i.e., saflufenacil+M+T) did not influence the cuticular absorption of [^{14}C]saflufenacil in buckwheat cuticles (**Figure 1A**); however, it significantly increased the rate and total cuticular absorption of [^{14}C]saflufenacil in cabbage ($p = 0.0001$) (**Figure 1B**).

The amount of unabsorbed (recovered in washing solutions) and adsorbed (retained in cuticles) [^{14}C]saflufenacil was consistently lower for buckwheat and cabbage cuticles receiving herbicide treatments with M compared to those without M (**Figure 2A**,**B**), indicating M increased absorption of saflufenacil. However, when T was added to saflufenacil plus M, the uptake of [^{14}C]saflufenacil was improved in cabbage but not in buckwheat ($p = 0.0017$) (**Figure 2A**,**B**). Conversely, when these two treatments (S+M vs S+M+T) were compared in terms of adsorbed (retained in cuticles) [^{14}C]saflufenacil, there was no difference between the treatments in cabbage and buckwheat ($p = 0.23$) (**Figure 2B**). It is important to note that the data in **Figure 2** does not add to 100% (i.e., unabsorbed + adsorbed $\neq$ 100% at

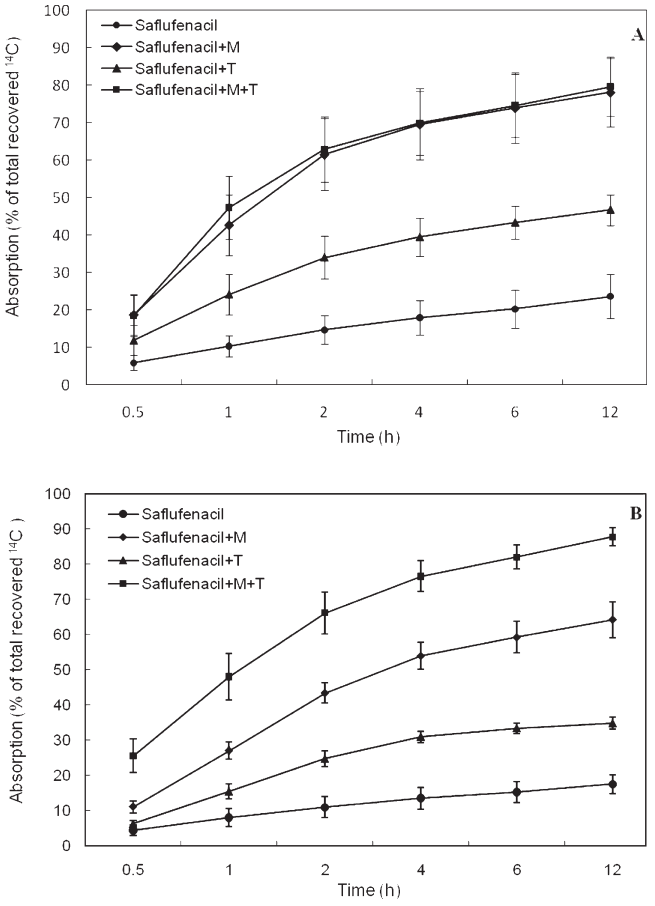

Figure 1. Isolated cuticles: Absorption of [^{14}C]saflufenacil by isolated cuticles of buckwheat (**A**) and cabbage (**B**), expressed as a percent of total recovered radioactivity when applied alone or in combination with Merge (M), Transorb (T), or both M and T. Bars represent standard error of the mean (α = 0.05). [^{14}C]saflufenacil was dissolved in formulated saflufenacil and mixed with water to yield 4.99 mM saflufenacil, which is equivalent to 25 g of ai ha^{-1}; Transorb (T) was mixed with water to yield 39 mM glyphosate, which is equivalent to 900 g ai ha^{-1}. All herbicides were applied in a volume equivalent to 100 L ha^{-1}. In treatments with M, M was added at 0.5% (v/v). Treatments were applied as two 1 μL droplets delivering a total of 400 Bq of [^{14}C]saflufenacil to the center of isolated cuticles.

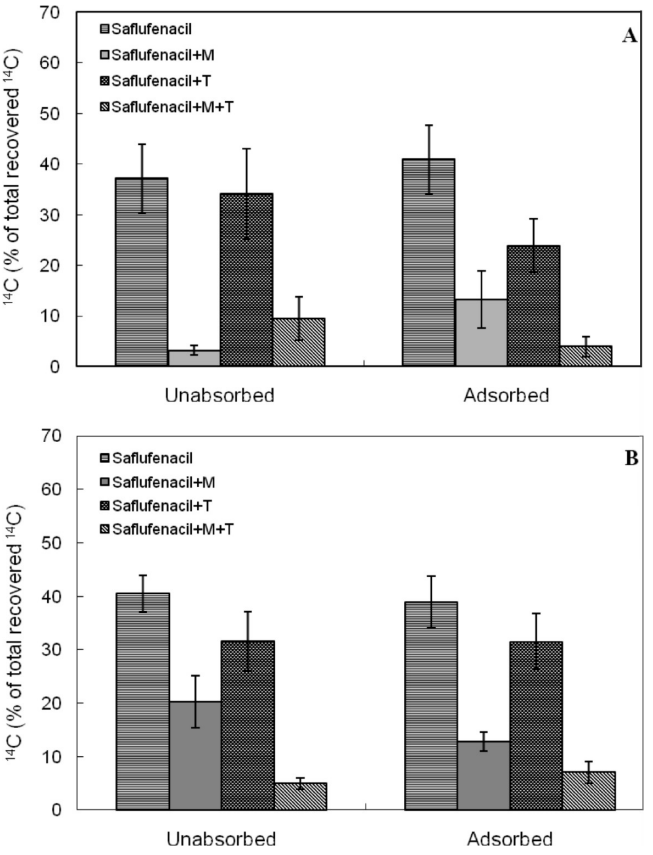

Figure 2. Isoated cuticles: Unabsorbed and adsorbed (retained in cuticles) [^{14}C]saflufenacil when applied alone or in combination with Merge (M), Transorb (T), or both M and T to isolated cuticles from buckwheat (**A**) and cabbage (**B**). Bars represent standard error of the mean (α = 0.05). [^{14}C]saflufenacil and formulated saflufenacil were mixed with water to yield 4.99 mM saflufenacil, which is equivalent to 25 g of ai ha^{-1}; Transorb (T) was mixed with water to yield 39 mM glyphosate, which is equivalent to 900 g ai ha^{-1}. All herbicides were applied in a volume equivalent to 100 L ha^{-1}. In treatments with M, M was added at 0.5% (v/v). Treatments were applied as two 1 μL droplets delivering a total of 400 Bq of [^{14}C]saflufenacil to the center of isolated cuticles. Note: data from ^{14}C in leaf wash plus that trapped in the cuticle (adsorbed), i.e., not translocated beyond treated area, does not add to 100% because the data of ^{14}C translocated out of treated area are not presented.

each time) because only data of ^{14}C in leaf washes and that trapped in the cuticles (adsorbed), i.e., not translocated beyond treated area, are presented. The unaccounted ^{14}C represents what was translocated out of the treated area of each leaf.

The results from **Figures 1** and **2** suggest that absorption of [^{14}C]saflufenacil is improved in both species with the addition of M and further enhanced, only in cabbage, with the addition of T (i.e., M+T), thereby improving the phototoxicity of saflufenacil in plants with thick cuticles. Similar observations have been made under field conditions when cotton (*Gossypium* spp.), which has thick cuticles, was treated with the formulated saflufenacil+M+T as opposed to saflufenacil+M (BASF, personal communication). Furthermore, the faster rate of [^{14}C]saflufenacil absorption in cabbage, when combined with M and T as compared to other treatments, may indicate that this combination could improve the rainfastness of saflufenacil in plants with thick epicuticular wax layers (**Figure 1B**).

Differential foliar absorption plays a major role in the selectivity and efficacy of herbicides (*25*). For instance, an inverse relationship was found between the foliar absorption of the PPO inhibitor sulfentrazone (no addition of surfactants) and the thickness of cuticular wax on the leaves of several weed species. However, the addition of surfactant increased the absorption of herbicide as indicated by enhanced phytotoxicity of sulfentrazone (*25*). Limited foliar absorption in hemp dogbane (*Apocynum cannabinum* L.) was considered the primary factor for its resistance to glyphosate; lack of glyphosate absorption was attributed to a thicker epicuticular wax layer, thicker cuticle, and lack of stomata and trichomes on the adaxial leaf surface (*26*). Moreover, the mechanism of intraspecific sensitivity of cabbage varieties to nitrofen (a PPO-inhibitor) has been shown to be dependent on the amount of cuticular wax on the leaves at the time of herbicide application (*27*).

Absorption and Translocation Study. Regardless of the treatment or species, foliar uptake of ^{14}C, six HAT, was the greatest and reached a maximum when M was added (i.e., both saflufenacil and saflufenacil+T; **Table 2**). However, the foliar uptake of ^{14}C in all treatments without M did not reach a maximum until 24 or 48 HAT (**Table 2**). When compared to suflufenacil alone, addition of T (i.e., no M) 6 HAT increased the foliar uptake of ^{14}C

Table 2. Mean Percent Foliar Uptake of ^{14}C, Unabsorbed ^{14}C, and Total ^{14}C Recovered Outside the Treated Area (TA) of Buckwheat, Cabbage, as well as Conventional (CL) and Glyphosate-Resistant (RR) Canola Plants Treated with [^{14}C]Saflufenacil plus Formulated Saflufenacil in Combination with Merge (M), Unformulated Glyphosate (G), and/or Transorb (T)[a]

	foliar uptake of ^{14}C (% of total recovered ^{14}C)			unabsorbed ^{14}C (% of total recovered ^{14}C)			total ^{14}C translocated beyond the TA (% of total recovered ^{14}C)		
treatment[b]	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
				Buckwheat					
saflufenacil	47.2	80.0	83.6	52.8	20.0	16.3	7.1	5.8	5.3
saflufenacil+M	83.2	89.2	89.3	16.8	10.8	10.7	7.6	6.0	6.3
saflufenacil+T	49.2	72.5	74.8	50.8	27.5	25.2	3.8	3.8	3.9
saflufenacil+M+T	74.5	85.8	83.2	25.5	14.2	16.8	3.0	3.4	3.1
LSD[c]	14.01	9.19	9.41	14.01	9.20	9.41	1.65	1.96	1.76
				Cabbage					
saflufenacil	35.2	58.9	59.7	64.8	41.1	44.1	2.1	6.1	4.6
saflufenacil+M	89.3	89.7	91.3	10.7	10.3	8.7	5.4	9.3	7.7
saflufenacil+T	53.5	67.5	68.8	46.5	32.4	30.9	2.1	4.3	4.5
saflufenacil+M+T	86.9	92.6	92.5	13.1	7.4	7.5	3.2	5.5	5.6
LSD	8.34	4.25	5.95	8.34	4.25	8.10	2.52	1.53	1.59
				Canola (CL)					
saflufenacil	70.0	82.1	79.8	30.0	17.9	20.2	3.0	8.7	6.1
saflufenacil+M	92.8	94.3	95.9	7.2	8.4	4.1	9.0	17.4	18.8
saflufenacil+T	70.1	76.2	83.1	29.9	23.8	16.9	4.4	7.9	10.0
saflufenacil+M+T	93.9	93.6	95.0	6.1	6.4	5.0	6.4	11.6	13.5
saflufenacil+M+G	90.5	89.4	94.7	9.5	10.6	5.3	6.3	12.7	14.3
LSD	5.52	6.08	4.08	5.51	6.24	4.08	1.23	3.36	2.44
				Canola (RR)					
saflufenacil	72.5	83.8	80.9	27.5	16.1	19.1	3.9	6.0	6.9
saflufenacil+M	94.6	92.7	94.9	5.3	7.3	5.1	8.2	15.8	20.4
saflufenacil+T	70.0	78.9	85.3	30.0	21.1	14.7	4.8	9.8	13.7
saflufenacil+M+T	93.1	92.4	93.6	6.9	7.6	6.4	7.5	12.7	18.3
saflufenacil+M+G	93.6	91.0	93.7	6.4	9.0	6.3	7.8	15.5	15.3
LSD	7.19	4.37	5.75	7.18	4.36	5.75	1.30	2.75	4.50

[a] Data were taken 6, 24, and 48 h after treatments. [b] Approximately 2000 Bq of [^{14}C]saflufenacil was applied to the center of adaxial side of second leaf of each plant. The concentrations of saflufenacil, glyphosate and Merge were 4.99 mM, 39 mM and 0.5%, respectively. [c] Least significant difference value was generated by Duncan's multiple range test ($\alpha = 0.05$).

in cabbage, but not in buckwheat and canola (**Table 2**). With regard to unabsorbed ^{14}C, less radioactivity was present in leaf washes in treatments with M (**Table 2**). In addition, more ^{14}C was recovered outside the TA of cabbage and CL canola plants treated with saflufenacil+M compared to the rest of the treatments, while in RR canola, total ^{14}C recovered outside the TA was the same in all treatments with M and greater than in treatments without M (**Table 2**). Finally, 6 and 24 HAT of buckwheat, total ^{14}C recovered outside the TA was greater in plants treated with saflufenacil alone or in combination with M than in plants treated with T (**Table 2**).

In general, regardless of the plant species or harvest time, there was little movement of saflufenacil in the phloem ($\leq$5%; **Table 3**). Six HAT of buckwheat, both ^{14}C in plant tissues above the TL and the total amount of traslocated ^{14}C, were greater in plants treated with saflufenacil+M when compared to all other treatments (**Table 3**). However, by 24 and 48 HAT, there was no difference in total translocated ^{14}C between saflufenacil and saflufenacil+M treated plants (**Table 3**). Total translocated ^{14}C in buckwheat treated with saflufenacil+T was generally lower than that in plants treated with saflufenacil (**Table 3**). These results may explain why buckwheat treated with either saflufenacil or saflufenacil+M did not survive 48 HAT, whereas buckwheat treated with saflufenacil+T showed necrosis in tissues above the treated leaf and survived (**Figure 3A**). In buckwheat plants treated with saflufenacil+M+T, the total translocated ^{14}C was lower than in plants treated with saflufenacil and saflufenacil+M, regardless of the sampling times (**Table 3**). Furthermore, buckwheat treated with saflufenacil+M+T had necrosis confined to the treated leaf, i.e., there was no injury observed in other above ground plant tissues (**Figure 3A**). Although the addition of T had a positive influence on cuticular absorption of [^{14}C]saflufenacil in buckwheat (**Figure 1A**), there was less translocation of ^{14}C compared to saflufenacil alone (**Tables 2** and **3**).

In cabbage plants, T had a positive effect on foliar uptake of ^{14}C at all times (**Table 2**). However, despite its positive effect on the uptake, T negatively influenced ^{14}C translocation (**Table 3**). While foliar uptake of ^{14}C was the lowest in cabbage plants treated with saflufenacil alone (**Table 2**), both the ^{14}C translocated above the TL and to the entire plant was greater at all times in plants treated with saflufenacil and saflufenacil+M than with treatments containing T (**Table 3**). This negative effect of T on the translocation of saflufenacil can be seen on buckwheat when T was added (**Figure 3**). In buckwheat and cabbage, the reduced translocation of ^{14}C in treatments containing T may result from the effects of glyphosate on general plant metabolism. For example, the translocation of glyphosate has been shown to be reduced because of glyphosate phytotoxicity on chloroplast carbon metabolism, thus inhibiting photosynthesis and carbon assimilation, which consequently reduced both photoassimilate and glyphosate translocation in glyphosate-susceptible plants (*28*, *29*). However, in glyphosate-resistant sugar beet (*Beta vulgaris* L.),

Table 3. Mean Percent Translocated ^{14}C in Plant Tissues Above the Treated Leaves (TL) and Total Translocated ^{14}C (Recovered in Plant Tissues Above and Below (Including Roots)) out of the TL of Buckwheat, Cabbage, Conventional (CL) Canola, and Glyphosate-Resistant (RR) Canola Plants Treated with [^{14}C]Saflufenacil plus Formulated Saflufenacil in Combination with Merge (M), Unformulated Glyphosate (G), and/or Transorb (T)[a]

	translocated ^{14}C (% of total in planta)					
	above the TL			total		
treatment[b]	6 h	24 h	48 h	6 h	24 h	48 h
Buckwheat						
saflufenacil	1.2	1.8	1.9	2.7	3.3	2.5
saflufenacil+M	2.1	1.6	1.6	4.5	2.9	3.3
saflufenacil+T	1.2	0.6	0.4	2.4	1.4	1.0
saflufenacil+M+T	0.3	0.3	0.5	1.0	0.8	0.9
LSD[c]	0.51	0.47	0.65	0.83	0.91	1.07
Cabbage						
saflufenacil	2.0	2.6	1.7	3.6	3.9	2.7
saflufenacil+M	2.0	3.1	1.8	3.3	4.5	3.2
saflufenacil+T	0.6	0.5	0.8	1.4	1.1	1.4
saflufenacil+M+T	0.4	0.5	0.9	1.0	1.1	1.5
LSD	0.37	0.52	0.39	0.55	0.57	0.74
Canola (CL)						
saflufenacil	0.4	0.7	0.8	1.8	2.4	1.9
saflufenacil+M	1.4	1.9	2.3	3.8	4.6	4.2
saflufenacil+T	0.7	1.0	1.3	2.6	2.7	2.7
saflufenacil+M+T	0.9	1.1	1.5	2.8	2.8	3.2
saflufenacil+M+G	0.9	1.2	1.7	3.1	3.0	3.7
LSD	0.31	0.55	0.62	0.63	0.89	0.95
Canola (RR)						
saflufenacil	0.4	0.7	0.7	1.6	1.8	1.4
saflufenacil+M	1.3	1.9	1.8	3.2	3.5	3.2
saflufenacil+T	0.8	1.0	1.1	2.2	2.4	2.0
saflufenacil+M+T	1.4	1.5	1.3	3.3	3.1	2.5
saflufenacil+M+G	0.9	1.3	1.3	3.3	2.9	2.6
LSD	0.36	0.51	0.50	0.63	0.79	0.78

[a] Data were taken 6, 24, and 48 h after treatments. [b] Approximately 2000 Bq of [^{14}C]saflufenacil was applied to the center of adaxial side of second leaf of each plant. The concentrations of saflufenacil, glyphosate, and Merge were 4.99 mM, 39 mM and 0.5%, respectively. [c] Least significant difference value was generated by Duncan's multiple range test ($\alpha = 0.05$).

glyphosate application did not inhibit carbon or glyphosate translocation (*28*). Therefore, to further understand the role of glyphosate on saflufenacil absorption and translocation studies were conducted using CL and RR canola varieties.

In general, when compared to saflufenacil+M, addition of T or unformulated glyphosate (G) negatively influenced the translocation of ^{14}C in CL canola plants treated with saflufenacil+M+T and saflufenacil+M+G; however, T or G did not limit the translocation of ^{14}C in RR canola plants receiving these treatments (**Table 3**). With regard to CL canola, the translocation of ^{14}C was greater in plants treated with saflufenacil+M than with other treatments (**Table 3**). Although G reduced the translocation of ^{14}C in CL canola plants 6 and 24 HAT, there was no difference in total translocated ^{14}C 48 HAT in plants treated with saflufenacil+M+G and saflufenacil+M (**Table 3**). The lower translocation of ^{14}C in CL canola plants treated with saflufenacil+M+T versus saflufenacil+M+G may be due to the reduced uptake of G in these plants, which may consequently reduce the potential negative effects of glyphosate. With regard to RR canola, total translocated ^{14}C was greater in treatments that had M as part of the mixture, 6 HAT, compared to plants treated with saflufenacil+T and saflufenacil (**Table 3**). Translocated ^{14}C in plant tissues above the TL, 6HAT, was greater in RR canola plants treated with saflufenacil+M and saflufenacil+M+T, followed by saflufenacil+M+G and saflufenacil+T. Furthermore, RR canola treated with saflufenacil had the lowest translocated ^{14}C in tissues above the TL (**Table 3**). Twenty-four and 48 HAT, total translocated ^{14}C and translocated ^{14}C in tissues above the TL were greater in RR canola where M was added to the mixture (**Table 3**). On the basis of these results, the negative effect of glyphosate on the translocation of [^{14}C]saflufenacil in CL but not RR canola support the hypothesis that the physiological effects of glyphosate may reduce translocation of saflufenacil.

Necrosis of plant tissues above the treated leaf was observed only in CL canola treated with saflufenacil+M, whereas in RR canola, necrosis was seen not only with this treatment but also with saflufenacil+M+T and saflufenacil+M+G (data not shown). Unlike buckwheat and cabbage, none of the canola varieties treated only with saflufenacil showed necrosis of tissues above the treated leaf. The higher relative percent translocation of saflufenacil when combined with T in buckwheat compared with cabbage and CL canola plants may be explained by lower susceptibility of buckwheat to glyphosate. For example, when T treated buckwheat were allowed to grow for 72 HAT, no glyphosate injury was observed, while in cabbage and CL canola, glyphosate injury (as determined by chlorosis and tissue necrosis) was clearly observed 72 HAT (data not shown).

The fact that translocation of ^{14}C in canola treated with saflufenacil was very limited compared to that in cabbage and buckwheat may be due to differences among these species in susceptibility to saflufenacil. Consequently, in buckwheat and cabbage, the amount of absorbed saflufenacil, when applied alone, may have not caused rapid tissue necrosis (i.e., cell death), hence the translocation of the herbicide was not reduced and resulted in tissue damage outside the treated leaf. Conversely, in canola plants, only the treated areas of the treated leaf became necrotic, thus limiting the translocation of saflufenacil.

Bioassay Study. All doses of T (225, 450, and 900 g ai ha^{-1}) applied with or without M completely controlled CL canola (**Figure 4**), whereas saflufenacil with or without M resulted in less than 20% suppression of growth of CL canola (**Figure 4**). Conversely, when the lowest dose of T or G (225 g ai ha^{-1}) was mixed with saflufenacil, less than 80% suppression of CL canola was achieved (**Figure 4**), indicating that the herbicidal activity of glyphosate was negatively affected by saflufenacil. This reduction in the efficacy of glyphosate by saflufenacil may result from the rapid contact activity of saflufenacil, thus reducing glyphosates uptake and translocation. The negative effects of saflufenacil on the activity of glyphosate appear to be ameliorated as the dose of glyphosate is increased (**Figure 4**). Our results agree with those of Jordan et al. (*30*), who reported that the PPO-inhibitor acifluorofen antagonized the activity of glyphosate in barnyard grass; however, the antagonistic activity of acifluorofen was eliminated by increasing the dose of glyphosate. Furthermore, several authors (*13*,*31*) have associated the reduced activity of glyphosate when mixed with PPO-inhibitor herbicides with the reduced translocation of glyphosate . For example, the addition of fomesafen reduced the absorption and translocation of glyphosate and, therefore, antagonized its activity in barnyard grass (*Echinochloa crus-galli* L.) velvet leaf (*Abutilon theophrasti* Medikus.), and pitted morning glory (*Ipomoea lacunose* L.) (*13*). The efficacy of glyphosate for grass control was also reduced by oxyfluorofen (*32*). These results are also consistent with interactions observed between sethoxydim and acifluorfen, i.e., the efficacy of the graminicide was reduced by the PPO inhibitor (*33*).

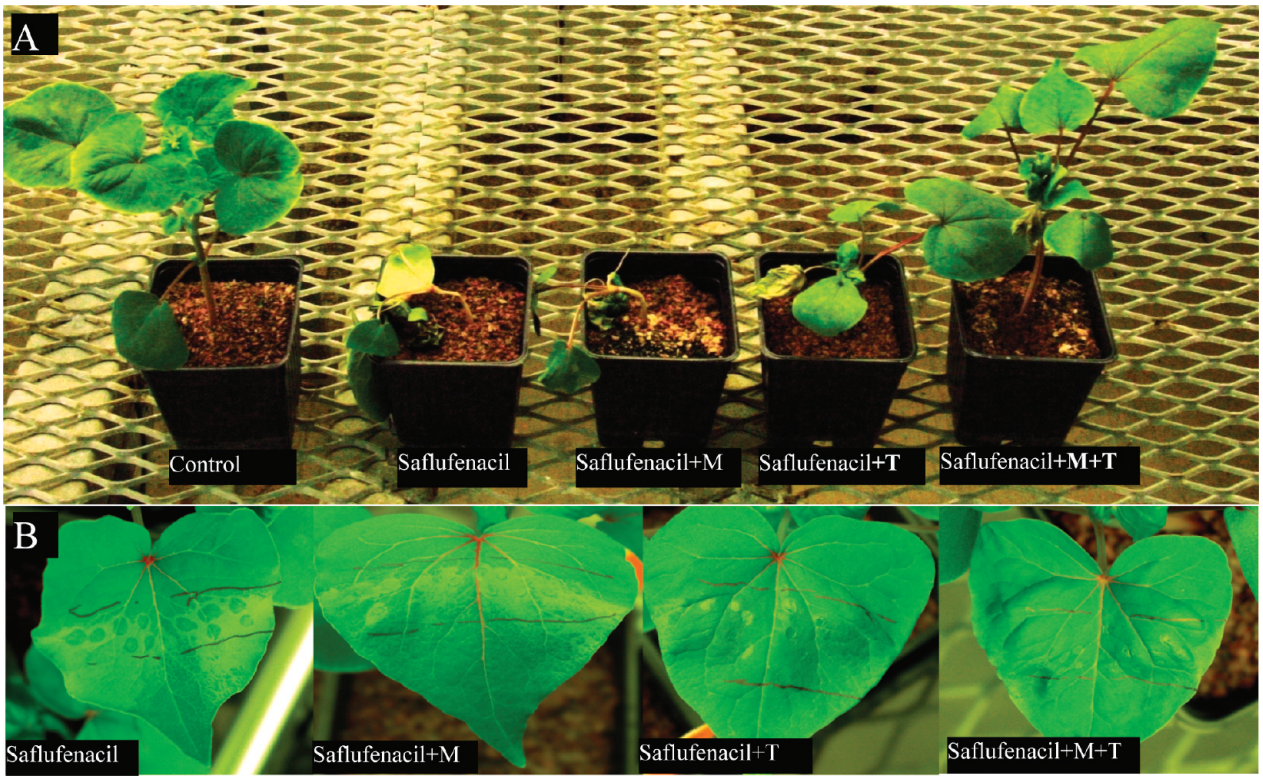

Figure 3. Effect of [^{14}C]saflufenacil applied alone or in combination with Merge (M), Transorb (T), or both M and T on buckwheat treated at the 3- to 4-leaf stage of development. [^{14}C]saflufenacil and formulated saflufenacil were mixed with water to yield 4.99 mM saflufenacil, which is equivalent to 25 g of ai ha^{-1}; Transorb (T) was mixed with water to yield 39 mM glyphosate, which is equivalent to 900 g ai ha^{-1}. All herbicides were applied in a volume equivalent to 100 L ha^{-1}. In treatments with M, M was added at 0.5% (v/v). Treatments were applied as 10 1 μL droplets, delivering a total of 2000 Bq of [^{14}C]saflufenacil to the center of adaxial side of second leaf of each plant: (**A**) 48 HAT; (**B**) 6 HAT.

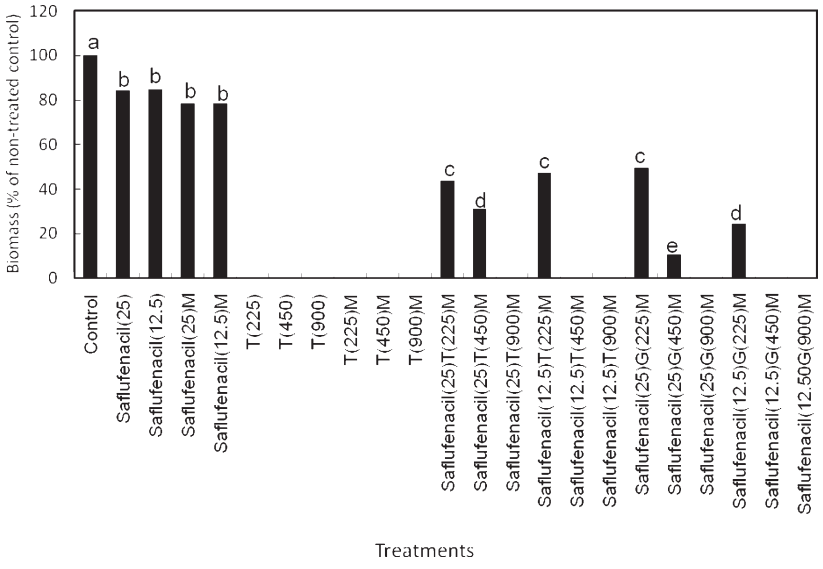

Figure 4. Effect of various doses of saflufenacil, Transorb (T), and unformulated glyphosate (G) in different combinations, with and without Merge (0.5% v/v, M) (**Table 1**), on the biomass of conventional canola. Data are expressed as a percent of untreated controls. Plants were treated at the 3- to 4-leaf stage of development. Formulated saflufenacil was mixed with water to yield 2.495 and 4.99 mM saflufenacil, which is equivalent to 12.5 and 25 g of ai ha^{-1}, respectively; T and G were mixed with water to yield 9.75, 19.5, and 39 mM glyphosate, which is equivalent to 225, 450, and 900 g ai ha^{-1}, respectively. All herbicides were applied at 100 L ha^{-1}. Treatments were applied as 10 1 μL droplets to the center of adaxial side of second leaf of each plant. Live plants were harvested 10 DAT and dry weight were measured. The biomass of the dead plants was considered to be zero. Least significant difference (LSD) values were generated by Duncan's multiple range test. Bars with the same letters are statistically the same (LSD = 7.79, α = 0.05). Treatments with no bar provided 100% control of canola (i.e., zero biomass).

Conclusions. Increased absorption of saflufenacil with the addition of M and T enhanced the contact activity of saflufenacil in plants with thick cuticle (i.e., cabbage) and thus may explain its enhanced activity when used as a defoliating agent in cotton (BASF, personal communications). However, the increased absorption and contact activity of saflufenacil in the presence of M

and/or T may negatively influence its translocation and efficacy in highly susceptible species (e.g., volunteer canola seedling at early stages of development), especially if good spray coverage is not achieved, thereby making good translocation a requirement to move the saflufenacil throughout the plant. Furthermore, reduced glyphosate activity when combined with saflufenacil+M may also be the result of rapid contact activity of saflufenacil causing cell death, thereby limiting glyphosate translocation. As an illustration, when glyphosate was applied 3 days after acifluorfen or as a mixture with acifluorfen for barnyard grass control, the efficacy of glyphosate was reduced compared to when glyphosate was applied alone (*30*). However, application of glyphosate 3 days prior to acifluorfen application did not reduce its efficacy (*30*). The results of Jordan et al. (*30*) corroborate our results. Therefore, the negative effect of saflufenacil+M on glyphosate activity could limit the application of this herbicide combination; however, depending on the type of weed species and by adjusting the application doses of the herbicides in the mixture, these effects may be minimized (**Figure 4**). The practical implications of the results of this study are yet to be evaluated under the field conditions.

Translocation of saflufenacil was negatively affected by glyphosate in glyphosate-susceptible canola varieties, which could reduce the effectiveness of saflufenacil for controlling certain weed species. However, translocation of saflufenacil was not affected by glyphosate in glyphosate-resistant canola. With this in mind, this combination (i.e., saflufenacil+T or saflufenacil+M+T) could be effective in controlling glyphosate resistant weeds and glyphosate-resistant crop volunteers. More studies are needed to clarify the effectiveness of saflufenacil and glyphosate combination in glyphosate-resistance prevention/management.

ABBREVIATIONS USED

PPO, protoporphyrinogen oxidase; ANOVA, analysis of variance; DAT, days after treatment; HAT, hours after treatments; RCBD, randomized complete block design; CL canola, conventional canola; RR canola, glyphosate-resistant canola.

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