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Complementary activity of trifludimoxazin and saflufenacil when used in combination for postemergence and residual weed control

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Abstract

Trifludimoxazin is a new herbicide that inhibits protoporphyrinogen oxidase (PPO) and is targeted for commercial market introduction in North America, South America, and Asia. It will be available both as a stand-alone product and in a 1:2 mixture with saflufenacil. The herbicide is intended for use in preplant burndown and preemergence applications in cereal, corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and pulse crops to control a variety of annual broadleaf and grass weed species. Additionally, it is intended to be used in tree crops, oil palm (*Elaeis guineensis* Jacq.), and non-crop areas. In this study, we meticulously evaluated the performance and effectiveness of both the stand-alone herbicide and the innovative mixture concept in combating prevalent weeds commonly encountered in corn and soybean fields. Our findings revealed that both products exhibited exceptional efficacy, significantly reducing the presence of these troublesome weeds. Furthermore, the mixture concept not only demonstrated commendable soil mobility but also showcased impressive residual activity, positioning it as a powerful tool for sustainable weed control. These promising effects are further substantiated by our comprehensive adsorption–distribution–metabolism–extraction (ADME) studies, which provide insight into the behavior and longevity of the herbicides in the agricultural ecosystem.

Introduction

The integration of soil-residual herbicides into glyphosate-resistant crops is widely recommended as a strategy to enhance the reliability of weed management systems (Bond et al. 2014; Riar et al. 2013). By employing soil-residual herbicides, growers can effectively eliminate or significantly reduce early-season weed competition, thereby optimizing crop yields. Additionally, these herbicides offer flexibility regarding the timing of postemergence applications, should they be necessary. Currently, soil-residual herbicides are employed extensively to manage glyphosate-resistant weed populations across various crops (Ellis and Griffin 2002).

One promising candidate in this category is trifludimoxazin [1,5-dimethyl-6-sulfanylidene-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione], a novel herbicide under development by BASF. This compound functions by inhibiting protoporphyrinogen oxidase (PPO) and has recently been submitted to the U.S. Environmental Protection Agency (USEPA) for registration. Trifludimoxazin provides effective preemergence and/or postemergence (burndown) control of a diverse range of problematic annual broadleaf and some annual grass weed species. Its application spans various agricultural settings, including field and row crops such as corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.], as well as bearing and nonbearing tree crops like citrus and oil palm (*Elaeis guineensis* Jacq.) plantations in Asia. Additionally, it is suitable for use in non-agricultural (non-cropland) areas.

Trifludimoxazin is particularly adept at targeting economically significant dicot weed species, including Palmer amaranth (*Amaranthus palmeri* S. Watson), waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], ragweed (*Ambrosia* spp.), common cocklebur (*Xanthium strumarium* L.), velvetleaf (*Abutilon theophrasti* Medik.), baconweed (*Chenopodium* album), kochia [*Bassia scoparia* (L.) A.J. Scott], and morningglory (*Ipomoea* spp.). It also effectively controls rigid ryegrass (*Lolium rigidum* Gaudin), a troublesome grass species in small grain cereals. Notably, trifludimoxazin operates efficiently at relatively low application rates, which is beneficial for preserving conservation tillage practices, such as no-till or reduced-till methods commonly utilized in contemporary agricultural systems.

From the perspective of weed resistance management and integrated pest management, trifludimoxazin presents a novel alternative for controlling weeds that have developed resistance to other herbicides. Its unique differential binding characteristics may enhance its efficacy against weeds resistant to other commercial PPO-inhibiting herbicides (Porri et al. 2022). Moreover, when applied at the appropriate dosage, trifludimoxazin exhibits notable soil-residual activity (Asher et al. 2020).

In this study, we evaluated the effectiveness of trifludimoxazin both as a stand-alone product and in combination with saflufenacil. Our objective was to compare its efficacy against common weeds typically found in corn and soybean fields, using established benchmark standards for reference. Additionally, we conducted adsorption–distribution–metabolism–extraction (ADME) studies to investigate the mobility of trifludimoxazin within plants. This research enabled us to understand the distribution of the active ingredient and identify strategies to maximize its effectiveness against weeds. Furthermore, we performed dedicated soil-residual activity tests to gather insights into the residuality of trifludimoxazin. By comparing its performance with that of other PPO-inhibiting herbicides, we aimed to assess its long-term impact on weed control, providing valuable data for future weed management strategies.

Materials and Methods

Postemergence Greenhouse Trials

The active ingredients selected for the postemergence trials were among the most commonly utilized PPO inhibitors in soybean fields across the United States and Brazil. These include saflufenacil (a PPO inhibitor, HRAC E, 14, belonging to the *N*-phenyl-imides chemical group, produced by BASF, Ludwigshafen, Germany), trifludimoxazin (also an *N*-phenyl-imide from BASF, Ludwigshafen, Germany), a two-to-one mixture of saflufenacil and trifludimoxazin, flumioxazin (*N*-phenyl-imides, Sumitomo, Tokyo, Japan), tiafenacil (*N*-phenyl-imides, Nufarm, Melbourne, Australia), and sulfentrazone (*N*-phenyl-triazolinones, FMC, Philadelphia, USA). Additionally, we incorporated two compounds from alternative modes of action that are widely used in soybean cultivation in both regions: dicamba (an auxin inhibitor, classified under the benzoates chemical group, produced by BASF, Ludwigshafen, Germany) and glufosinate (a glutamine synthetase inhibitor from the phosphonic acid group, now under BASF after being previously associated with Bayer).

The trials assessed key broadleaf weed species, grass species, and relevant crops, all of which are detailed in Table 1, alongside their EPPO codes (previously Bayer codes, as defined by the European and Mediterranean Plant Protection Organization). All seeds used in these trials were produced at our facility in Limburgerhof, Germany. Standard cultivation methods were employed utilizing Limburgerhof soil (slightly loamy sand soil; clay: 6.9% dm; loam: 16.6% dry matter (dm); sand: 76.5% dm; organic matter [OM]: 1.38% dm; pH 7.4). The plant pots used were 9 cm in diameter at their widest point, containing approximately 313 cm^3 of soil. Monocot weeds were sown directly into these pots, while dicot weeds were initially cultivated in propagation soil (pH 5.6; N 14%, P_2O_5 16%, K_2O 18%, Fe 0,09%) before being transplanted into pots filled with Limburgerhof soil after germination.

The plants were treated with specific formulated active ingredients at various application rates to evaluate their responses to different dosages. The application was carried out under controlled conditions to facilitate a clear distinction between the active compounds and to manage the various weed species effectively. An initial trial aimed to establish suitable application rates. Given that most of the compounds are UV dependent,

Table 1. Crops and monocot and dicot weeds investigated in the postemergence trial.

Preferred scientific name	EPPO code[a]	Common name
Crops		
Zea mays L. ‘Benedicto’	ZEAMX	Corn
Glycine max (L.) Merr., ‘Shouna’	GLXMA	Soybean
Monocot weeds		
Lolium perenne L. ssp. *multiflorum* (Lam.) Husnot	LOLMU	Annual ryegrass
Echinochloa crus-galli (L.) P. Beauv.	ECHCG	Jungle rice
Setaria faberi Herrm.	SETFA	Giant foxtail
Sorghum halepense (L.) Pers.	SORHA	Johnsongrass
Setaria viridis (L.) P. Beauv.	SETVI	Green foxtail
Digitaria sanguinalis (L.) Scop.	DIGSA	Hairy crabgrass
Dicot weeds		
Conyza canadensis (L.) Cronquist	ERICA	Canadian horseweed
Bassia scoparia (L.) A.J. Scott	KCHSC	Kochia
Chenopodium album L.	CHEAL	Baconweed
Commelina benghalensis L.	COMBE	Benghal dayflower
Abutilon theophrasti Medik.	ABUTH	Velvetleaf
Amaranthus retroflexus L.	AMARE	Redroot pigweed
Ambrosia artemisiifolia L.	AMBEL	Common ragweed
Raphanus raphanistrum L.	RAPRA	Wild radish

[a]From EPPO Global Database: https://gd.eppo.int/.

significantly lower rates were employed in greenhouse trials compared with field rates. For consistency, all PPO inhibitors were applied at a uniform rate, which was set at 2.5 times lower than the field rate (as detailed in Table 2).

The postemergence trial was replicated twice, with three replications for each rate and species, resulting in a total of six evaluations. The application volume was standardized at 200 L ha^{-1}, with 0.5% methylated seed oil used as an adjuvant. All applications were conducted using a flat spray nozzle from the XR TeeJet® 110015VS series (AGRAVIS Raiffeisen AG, Mannheim, Germany). After treatment, the solvents and water were allowed to evaporate from the plants for 30 min in a separate tunnel with an airflow of 3,000 m^3 h^{-1}. Subsequently, the plants were transferred to greenhouses tailored to the required growing conditions. The trials utilized three different greenhouses: a warm house (22 to 24 C, mean humidity 57%), a cold house (18 to 21 C, mean humidity 64%), and a cold cabin (12 to 14 C, mean humidity 83%). Each greenhouse was illuminated with photosynthetically active radiation (380 to 780 nm) from 10:00 PM to 4:00 AM, in addition to natural daylight.

Irrigation for the plants was conducted using specially prepared water that included nutrients tailored to their growth stage, biomass availability, and water needs. The irrigation water was prepared by diluting 1‰ of the liquid fertilizer Kamasol brilliant Grün 10-4-7® (Compo Expert, www.compo-expert.com) in tap water.

Plant damage was assessed at 7 and 20 d postapplication of the active ingredients. The evaluation involved a visual inspection of the aboveground parts of the plants, with damage quantified as a percentage of plant damage compared with untreated control (PDCU) using a scale ranging from 0 to 100, including increments of 2 (0%, 5%, 10%, 15%, . . . , 90%, 95%, 98%, 100%). A PDCU

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Table 2. Application conditions for the different active ingredients in the postemergence trial.

Active ingredient	Formulation[a]	Rate			
		—— g ai ha^{-1} ——			
Water	Control				
Saflufenacil	342 g L^{-1} SC	16	8	4	2
Trifludimoxazin	500 g L^{-1} SC	16	8	4	2
Saflufenacil + trifludimoxazin	375 g L^{-1} SC (250 g L^{-1} saflufenacil + 125 g L^{-1} trifludimoxazin)	16	8	4	2
Flumioxazin	51% WG	16	8	4	2
Tiafenacil	50 g L^{-1} ME	16	8	4	2
Sulfentrazone	480 g L^{-1} SC	16	8	4	2
Dicamba	480 g L^{-1} SL	200	100	50	25
Glufosinate	200 g L^{-1} SL	200	100	50	25

[a]ME, microencapsulated pesticides; SC, suspension concentrate; SL, soluble liquid concentrate; WG, water-dispersible granules.

value of 0% indicated no damage, while 100% indicated complete plant death. The statistical software R was used for the analysis of the rating data collected Scott and Knott (1974). The ANOVA technique, as outlined by Stahle and Wold (1989) was employed to identify differences in means. When significant differences were noted in the ANOVA results, the means were categorized into distinct groups following the method described by Scott and Knott (1974), using a significance level (α) of 0.05. The clustering analysis method developed by Scott and Knott (1974) was applied to group the variants into cohesive and homogeneous categories.

Residual Activity Trial

The primary objective of this trial is to gain a deeper understanding of the residual activity of various active ingredients and their biodegradation by soil-borne microorganisms. To evaluate the herbicidal effectiveness, we utilized watercress (*Nasturtium officinale* W.T. Aiton) as a bioindicator for the residual and soil mobility trials, following the methodology established by Schuchardt et al. (2019). The active ingredients tested included saflufenacil, trifludimoxazin, a combination of saflufenacil and trifludimoxazin, flumioxazin, and tiafenacil, which are detailed in Table 3. Various application rates were examined, specifically 100, 50, 25, 12.5, 6.25, and 3.125 g ai ha^{-1}. Each rate and timing was replicated three times to ensure reliability.

To initiate the trial, a tray containing 35 wells, each with a capacity of approximately 120 cm^3, was filled with active Limburgerhof soil that harbored soil microorganisms. Within each well, 2 ml of the respective herbicide was applied. After application, watercress was seeded to create a patchy lawn, and vermiculite was spread over the tray to maintain moisture and prevent rapid soil drying.

At the initial time point (T_0, or 0 d postapplication), the samples were seeded and placed in a phytotron for 7 d to allow for an initial growth (for specific growth chamber conditions, refer to Supplementary Table 1). For subsequent evaluations at 10, 20, and 30 d, the trays were incubated at a constant temperature of 26 C in a climate chamber. After the designated incubation periods, watercress was seeded onto each sample and returned to the climate chamber for another 7 d. Immediately following seeding, the samples were treated with propamocarb (Proplant®, Raiffeisen AG) to prevent soil-borne fungal infestations. Irrigation was provided using water mixed with 1‰ liquid fertilizer, tailored to the growth stage, available biomass, and specific water requirements of the plants.

After the 7-d incubation period in the climate chamber, a visual evaluation of plant damage was conducted. This damage was quantified and expressed as a percentage of PDCU, using the same statistical tools employed in the postemergence trials (R Tool and ANOVA). For additional details regarding the trial setup, please refer to Supplementary Figure 1.

Leaching Trial (Soil Mobility)

The objective of this trial was to assess and differentiate the soil mobility of various active ingredients. The active ingredients investigated, listed in Table 4, included saflufenacil, trifludimoxazin, a mixture of saflufenacil and trifludimoxazin, tiafenacil, flumioxazin, and pendimethalin, which served as a reference compound. Each PPO active ingredient was applied twice at a rate of 50 g ai ha^{-1}, while a higher rate of 2,000 g ai ha^{-1} was used for pendimethalin.

For the application, two filter papers were placed in a metal tray 1 d before treatment (for setup details, see Supplementary Figure 2). The tray was filled with 360 cm^3 of sandy soil (strong sandy loam soil; clay: 19.9% dm; loam: 18% dm; sand: 62% dm; pH 7.7; OM: 0.92%), which was leveled evenly across the entire surface. Any soil that spilled onto the filter papers was carefully removed. The tray was then elevated on a block to create a slope of 40°, and it was positioned within a seed tray under a fume hood, which was covered for safety.

Tubes were connected to a peristaltic pump (IP 16/ISM 943C, Ismatec, Wertheim, Germany) and fed through integrated holes in the hood, positioned directly over the upper filter paper. Two hours before the application, the water pump was activated to moisten the top 2 cm of the sandy soil with deionized water. For the herbicide application, 1 ml of each formulated active ingredient was evenly distributed over the moistened top layer of soil using a single-droplet technique. Subsequently, the peristaltic pump was initiated to drip deionized water onto the filter paper at a flow rate of 70.9 µl min^{-1}, ensuring the soil was consistently moistened. This process continued for approximately 27 h, allowing for the absorption of around 110 ml of deionized water.

After this period, watercress seeds were sown. The seeds were evenly distributed over the tray and gently pressed into the soil using a piece of paper and a roller. To prevent rapid soil drying, a layer of vermiculite was spread evenly over the tray and was also pressed into the soil with the roller. All samples received treatment with propamocarb (Proplant®) to inhibit the growth of soil-borne fungi.

The trays were then placed in a climate chamber for a duration of 7 d and irrigated with water mixed with 1‰ liquid fertilizer, tailored to the growth stage, available biomass, and specific water requirements of the plants.

For the evaluation of plant damage expressed as a percentage of PDCU, each tray was divided into 16 sections, each measuring 2.5 cm. Each section was individually assessed for damage to the aboveground parts of the plants. The extent of damage was quantified as a percentage of PDCU. Data from the two replications per treatment were analyzed using the R Tool to ensure statistical accuracy.

Table 3. Application conditions for the different active ingredients for residual activity trial.

Active ingredient	Formulation[a]	Rate					
		g ai ha^{-1}					
Water	Control						
Saflufenacil	342 g L^{-1} SC	100	50	25	12.5	6.25	3.125
Trifludimoxazin	500 g L^{-1} SC	100	50	25	12.5	6.25	3.125
Saflufenacil + trifludimoxazin	375 g L^{-1} SC (250 g L^{-1} saflufenacil + 125 g L^{-1} trifludimoxazin)	100	50	25	12.5	6.25	3.125
Flumioxazin	51% WG	100	50	25	12.5	6.25	3.125
Tiafenacil	50 g L^{-1} ME	100	50	25	12.5	6.25	3.125

[a]ME, microencapsulated pesticides; SC, suspension concentrate; SL, soluble liquid concentrate; WG, water-dispersible granules.

Table 4. Application conditions for the selected active ingredients for soil mobility trial.

Active ingredient	Formulation[a]	Rate
		g ai ha^{-1}
Water	Control	
Saflufenacil	342 g L^{-1} SC	50
Trifludimoxazin	500 g L^{-1} SC	50
Saflufenacil + trifludimoxazin	375 g L^{-1} SC (250 g L^{-1} saflufenacil + 125 g L^{-1} trifludimoxazin)	50
Flumioxazin	51% WG	50
Tiafenacil	50 g L^{-1} ME	50
Pendimethalin	400 g L^{-1} SC	2,000

[a]ME, microencapsulated pesticides; SC, suspension concentrate; SL, soluble liquid concentrate; WG, water-dispersible granules.

Adsorption–Distribution–Metabolism–Extraction (ADME) Trials

To investigate the uptake, stability, and translocation of various compounds, an ADME study was conducted using foliar applications on two grass species: barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] at growth stages 13/14 on the BBCH scale. The compounds evaluated in this study included a ready-mix formulation of trifludimoxazin and saflufenacil (375 g ai L^{-1}: 250 g L^{-1} saflufenacil + 125 g ai L^{-1} trifludimoxazin), as well as a tank-mix product (saflufenacil, SC, 342 g ai L^{-1} + trifludimoxazin, SC, 500 g ai L^{-1}). These were compared with the individual compounds: saflufenacil (solo, SC, 342 g ai L^{-1}) and trifludimoxazin (solo, SC, 500 g ai L^{-1}).

The application was performed at very low rates: 5.4 g ai ha^{-1} for saflufenacil (200 L ha^{-1}, 27 ppm), 2.7 g ai ha^{-1} for trifludimoxazin (200 L ha^{-1}, 13 ppm), and 8 g ai ha^{-1} for the ready-mix and tank-mix products (a 2:1 mixture of saflufenacil and trifludimoxazin, 200 L ha^{-1}, 40 ppm). A 5-μl droplet of each mixture was applied to the surface of the second leaf. To minimize phytotoxicity, the plants were incubated in a growth chamber with low light intensity, following a regimen of 18 h of light at 22 C and 6 h of darkness at 20 C, with a light intensity of approximately 3,500 LUX and 75% relative humidity.

Each treatment was replicated five times, and mean values were calculated along with standard deviations. At 24 and 72 h after application (HAA), each plant was carefully dissected into three parts: the treated leaf, the rest of the aerial plant (rest of plant [RoP]), and the root. The treated leaf was immersed in a 1:1 (v/v) acetonitrile–water solution for 20 s with gentle agitation to remove any non-absorbed deposits of the test compound from its surface (referred to as "leaf deposit"). All plant sections were then extracted using a tissue homogenizer (GentleMACS Dissociator, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) with the same acetonitrile–water solution.

Additional plant samples were treated in parallel and harvested immediately after application to assess total compound recovery at T_0. The leaf rinses and tissue extracts were analyzed using liquid chromatography–tandem mass spectrometry (LC/MS/MS; Waters ACQUITY UPLC coupled with an AB SCIEX API 4000 triple-quadrupole MS featuring an electrospray ionization interface; Waters GmbH, Eschborn, Germany). The mass spectrometer operated in multiple-reaction monitoring mode, targeting two characteristic mass transitions for each analyte, with concentrations determined through a matrix-matched standard calibration procedure.

In the context of the experimental data:

- "Leaf deposit" refers to the fraction of active ingredient present on the surface of the treated leaf, recovered through a standardized rinsing process and measured via LC/MS/MS.
- "Treated leaf" indicates the fraction of active ingredient within the leaf where the droplet was deposited, which is extracted after rinsing.
- "Rest of plant" signifies the active ingredient present in the entire plant, excluding the treated leaf, reflecting the translocation of the active ingredient out of the treated leaf, extracted without including the treated leaf.
- "Root" refers to the active ingredient within the root system, excluding both the treated leaf and the rest of the plant, indicating further translocation.
- "Total recovery" encompasses the sum of all fractions: leaf deposit, treated leaf, rest of plant, and root. Ideally, when no losses occur due to volatilization, chemical/physical degradation, or metabolism, total recovery should equal 100%.

The application onto a glass slide, labeled as "glass," was used to assess the photolytic stability of the compound. "Uptake" represents the percentage of the applied active ingredient, calculated by subtracting the leaf deposit fraction from the original amount, which is considered to be 100%. "Metabolic stability" is defined as the ratio of the active ingredient within the plant to the uptake at a specific time postapplication. In the absence of metabolism, metabolic stability would also be 100%.

Preemergence Field Trials

All preemergence field trial applications were carried out across seven different locations using a randomized block design. The first

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Table 5. Trial locations for the field preemergence trials.

U.S. state	Trial location	Farm	No. of trials	Coordinates
California	Dinuba	BASF Research Station	8	36.54476°N, 119.34511°W
Illinois	Seymour	Midwest Research Farm	4	40.04033°N, 88.40257°W
Nebraska	Seward	Beaver Crossing	3	40.72617°N, 97.28771°W
Iowa	Story City	Midwest Research Farm	2	42.16591°N, 93.64561°W
North Carolina	Pine Level	Old ICI Farm	2	35.489705°N, 78.204521°W
Georgia	Chula	Southeast Ag Research	1	31.54226°N, 83.55123°W
Tennessee	Memphis	Memphis North Agricenter International	1	35.12688°N, 89.81318°W

Table 6. Selected locations for the field trials and soil conditions in the different locations.

Location	Soil type	Sand	Silt	Clay	pH	OM[a]
		%				
Dinuba, CA, USA	Flamen Series (fine-loamy, mixed, superactive, thermic Calcic Pachic Haploxerolls)	63	22	16	7.1	1
Seward, NE, USA	Hastings (fine, smectitic, mesic Udic Argiustolls)	12	56	32	6.1	8.8
Pikeville, NC, USA	Bibb (Coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents)	62	29	9	5.6	1.1
Story City, IA, USA	Webster (Fine-loamy, mixed, superactive, mesic Typic Endoaquolls)	26	42	32	7.2	4.1
Seymour, IL, USA	Drummer (fine-silty, mixed, superactive, mesic Typic Endoaquolls)	21	46	3.3	6.1	3.2
Chula, GA, USA	Tifton (fine-loamy, kaolinitic, thermic Plinthic Kandiudults)	88	8	4	5.8	0.9
Memphis, TN, USA	Falaya (Coarse-silty, mixed, active, acid, thermic Aeric Fluvaquents)	12	80	8	5.3	1.6

[a]OM, organic matter.

Table 7. Weed spectrum in the preemergence field trials.

Preferred scientific name	EPPO code[a]	Common name
Monocot weeds		
Amaranthus palmeri S. Watson	AMAPA	Palmer amaranth
Amaranthus retroflexus L.	AMARE	Redroot pigweed
Amaranthus × *tamariscinus*	AMATA	Tall amaranth
Dicot weeds		
Senna obtusifolia (L.) Irwin & Barneby	CASOB	American sicklepod
Chenopodium album L.	CHEAL	Baconweed
Sida spinosa L.	SIDSP	Prickly fanpetals
Solanum nigrum L.	SOLNI	Black nightshade

[a]From EPPO Global Database: https://gd.eppo.int/.

replication adhered to the treatment list order rather than being randomized, which facilitated easier differentiation during site visits and evaluations. Each trial comprised three replications, and the plot sizes varied according to local conditions, ranging from 9 to 20 m^2.

For the applications, a water volume of 200 L ha^{-1} was utilized, employing either a tractor-mounted sprayer or a backpack sprayer, depending on the equipment available at each location. Detailed information regarding locations and soil conditions can be found in Tables 5 and 6. Both saflufenacil and trifludimoxazin were applied at a rate of 50 g ai ha^{-1}.

Weed control was assessed visually on a percentage scale, ranging from 0% (no efficacy) to 100% (total control) for each individual weed species compared with the untreated check (PDCU). Any herbicide-induced damage to a weed plant within a treated plot, as compared with the untreated plot, was recorded as an "effect." Evaluations were conducted at various time points after application, tailored to the specific conditions at each location. The different weed species present at each trial site are detailed in Table 7, which includes the corresponding EPPO codes for each species.

Results and Discussion

In the postemergence greenhouse trials, the efficacy of trifludimoxazin was assessed both as a stand-alone product and in combination with saflufenacil against selected weed species. The study included individual applications of several other PPO-inhibiting herbicides, such as saflufenacil, flumioxazin, tiafenacil, and sulfentrazone. Additionally, dicamba and glufosinate-ammonium were included due to their widespread use in corn and soybean crop systems. While the greenhouse trials focused on postemergence efficacy, the residual efficacy of these compounds was evaluated separately.

All active ingredients were applied to key grass and broadleaf weed species relevant to corn and soybean fields. To ensure a fair comparison, the PPO inhibitors were applied at identical rates. The results indicated that all active ingredients effectively controlled

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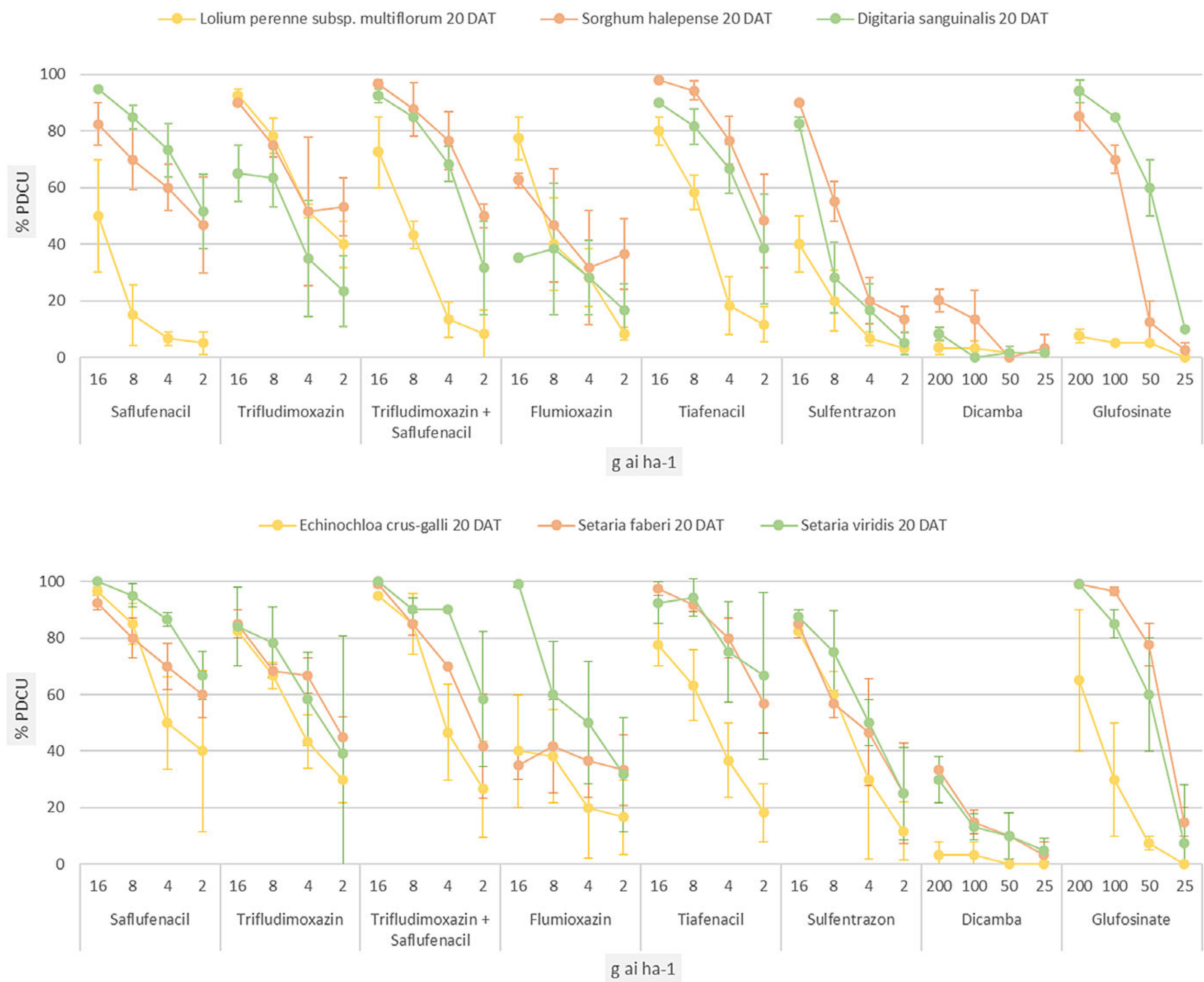

Figure 1. Results of the grass weed efficacy in the postemergence trials. Shown are the means out of the six repetitions. Activity was measured in % plant damage compared with untreated control (PDCU). Results at 20 d after treatment.

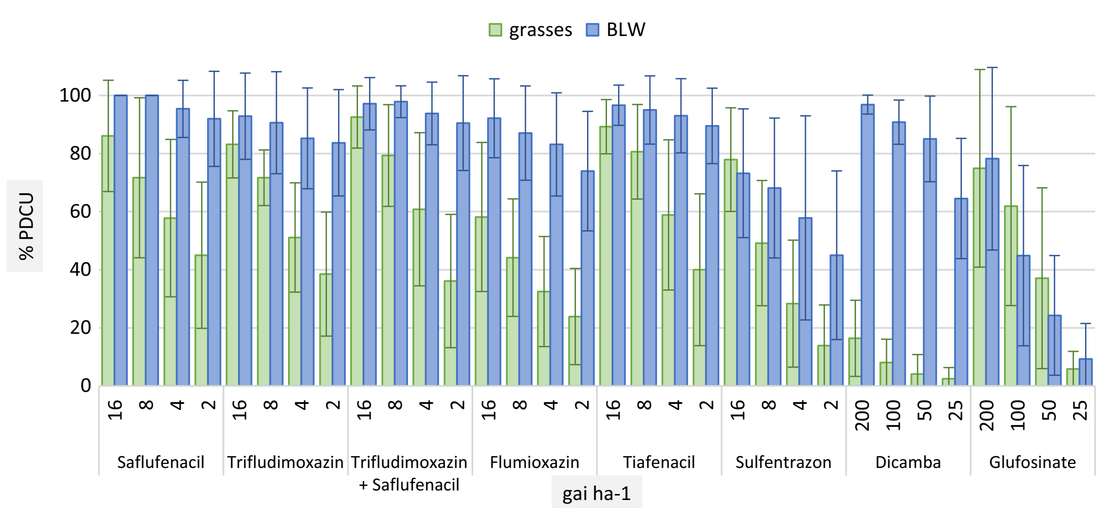

Figure 2. Results of the mean grass and dicot weeds efficacy in the postemergence trials. Shown are the means out of the six repetitions. Activity was measured in % plant damage compared with untreated control (PDCU). Results 20 d after treatment.

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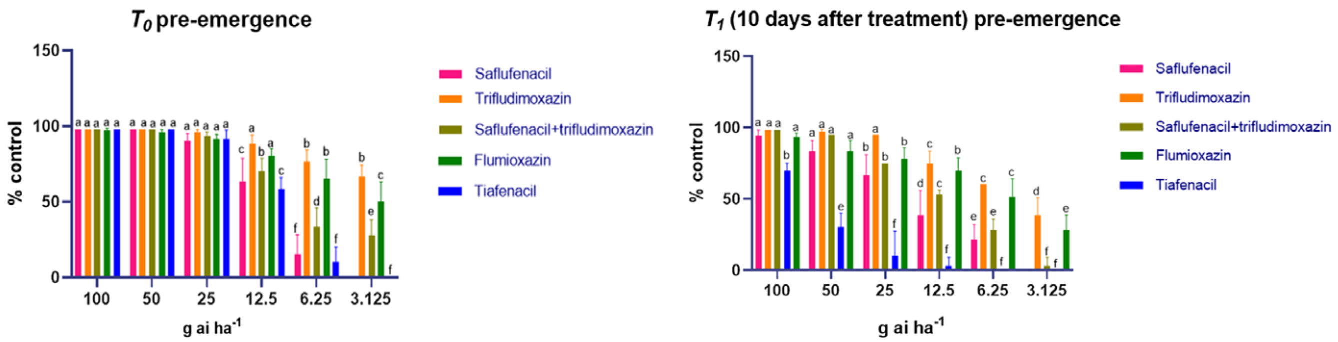

Figure 3. Residual activity at 0 and 10 d of saflufenacil, trifludimoxazin, and their mixture, as well as tiafenacil and flumioxazin. Presented are the means ($n = 3$) of the variants. Bars with no common letter are significantly different from the test group average after Scott and Knott (1974), with an $\alpha = 0.05$. g ha^{-1}, gram active ingredient per hectare.

broadleaf weeds, with minimal performance differentiation. For the purposes of discussion, we concentrate on the observed differences in grass control (see Figures 1 and 2).

For warm-season grass control, tiafenacil demonstrated high efficacy, as expected (Park et al. 2018). This was closely followed by the combination of saflufenacil and trifludimoxazin, which exhibited broader and stronger efficacy in grass control compared with either active ingredient applied individually (Duke et al. 1991; Grossmann et al. 2010; Kraehmer et al. 2014). A particularly notable finding was the excellent control of *L. perenne* ssp. *multiflorum*, a critical concern due to widespread weed resistance issues globally, especially in Australia. Among the PPO inhibitors, only tiafenacil achieved a similar level of control.

Because residual herbicides are highly effective in managing a wide range of weeds and remaining active in the soil for extended periods, they can be applied before, during, or after planting to ensure season-long weed control. Their effectiveness often requires fewer applications compared with non-residual herbicides, which helps reduce labor costs associated with weeding. Additionally, residual herbicides minimize the need for tillage, preserving soil structure and reducing erosion while facilitating incorporation into conservation tillage systems. They also provide effective control of weeds that have developed resistance to non-residual herbicides.

With these considerations in mind, we aimed to compare the residual activity levels of the same herbicides used in the postemergence trials (Table 3). The study focused on the following active ingredients: saflufenacil, trifludimoxazin, a mixture of saflufenacil and trifludimoxazin, flumioxazin, and tiafenacil using watercress as a bioindicator to measure herbicidal activity at 0-, 10-, 20-, and 30-d intervals.

At the time of application (T_0; Figure 3), all active ingredients displayed effective control at the three highest rates (100, 50, and 25 g ai ha^{-1}), with no significant differences noted (letter a; Scott and Knott [1974], $\alpha = 0.05$). At the three lower rates, trifludimoxazin exhibited significantly better control compared with all other active ingredients (letters a, b, and f at 12.5 g ai ha^{-1}, 6.25 g ai ha^{-1}, and 3.125 g ai ha^{-1}, respectively), aside from flumioxazin.

By 10 d after application (T_1; Figure 3), trifludimoxazin maintained its position as the most potent active ingredient among the highest rates, closely followed by its mixture with saflufenacil. Notably, at 25 g ai ha^{-1}, trifludimoxazin showed significant differences, indicated by letter a compared with letter b (saflufenacil, saflufenacil + trifludimoxazin, and flumioxazin) and letter f (tiafenacil). Flumioxazin demonstrated effective control at the two highest rates, similar to saflufenacil. However, tiafenacil exhibited a significant decline in activity across all rates within the 10-d period.

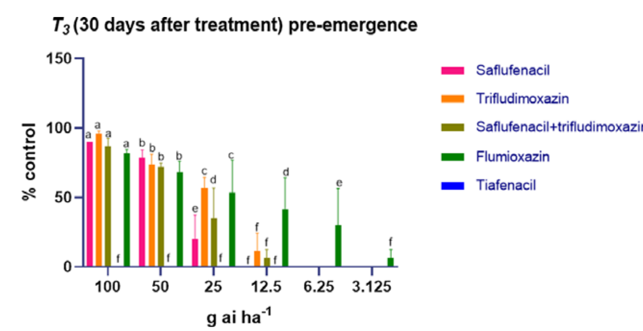

Figure 4. Residual activity at 30 d of saflufenacil, trifludimoxazin, and their mixture, as well as tiafenacil and flumioxazin. Presented are the means ($n = 3$) of the variants. Bars with no common letter are significantly different from the test group average after Scott and Knott (1974), with an $\alpha = 0.05$. g ha^{-1}, gram active ingredient per hectare.

By 30 d after application (T_3; Figure 4), both saflufenacil and trifludimoxazin showed the highest levels of activity, achieving greater than 80% control at the highest rate. The mixture of saflufenacil and trifludimoxazin displayed comparable efficacy, followed by flumioxazin. Unfortunately, there were no significant differences observed according to Scott and Knott, for instance, between 100 g ai ha^{-1} (a) and 50 g ai ha^{-1} (b). Tiafenacil showed no activity at any rate (0% control, letter f).

The lowest loss of activity was recorded for trifludimoxazin (over 95% control at 100 g ai ha^{-1}, letter a according to Scott and Knott), attributed to its DT_{50} value (dissipation time to have 50% of the original concentration) of 27.3 d (geometric mean; range: 11.8 to 87.4) (PMRA 2020). This indicates that trifludimoxazin has superior residual activity compared with the other active ingredients evaluated. Conversely, tiafenacil experienced the greatest decline in activity, with a low DT_{50} value of 0.064 d (geometric mean; range: 0.03 to 0.15 d) (USEPA 2020). For instance, at rates of 100 g ai ha^{-1} and 50 g ai ha^{-1}, tiafenacil initially achieved 98% control (a), but by 30 d later, it dropped to 0% control (0). This significantly shorter persistence in the soil compared with the other active ingredients is noteworthy.

Interestingly, the loss of activity for the mixture of saflufenacil and trifludimoxazin was similar to that of saflufenacil alone.

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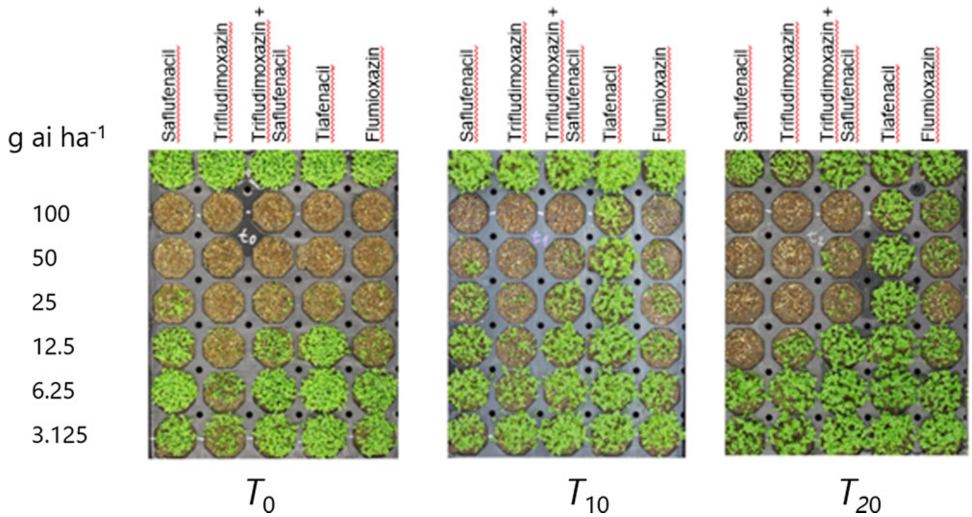

Figure 5. Residual activity after treatment of saflufenacil, trifludimoxazin, and their mixture (trifludimoxazin + saflufenacil), as well as tiafenacil and flumioxazin at 0, 10, and 20 d after treatment. g ai ha^{-1}, gram active ingredient per hectare.

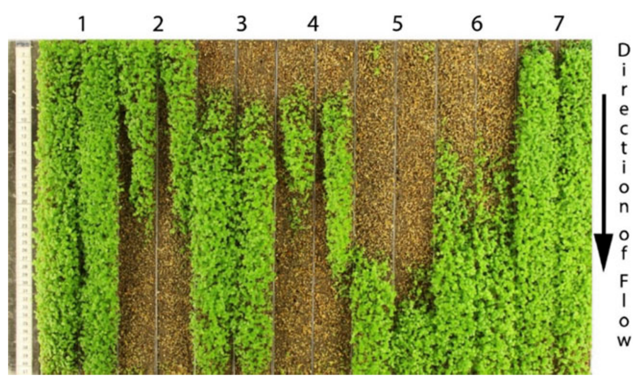

Figure 6. Image of the soil mobility trial. The trial consisted of two repetitions. Watercress was used as bioindicator. 1, control, without any active ingredients; 2, saflufenacil; 3, trifludimoxazin; 4, mixture of saflufenacil and trifludimoxazin; 5, tiafenacil; 6, flumioxazin; 7, pendimethalin.

Although both saflufenacil and trifludimoxazin, whether used individually or in their mixture, displayed no significant differences at the first two rates, they were consistent at 100 g ai ha^{-1} (a) and 50 g ai ha^{-1} (b).

The experiment (see Figure 5) aligns with the published DT_{50} data, confirming that trifludimoxazin exhibits the highest residual potential when applied at the correct rate.

In terms of soil mobility behavior, we conducted a soil mobility experiment with the same PPO inhibitors, and the experimental data are summarized in Table 4. The qualitative soil mobility of saflufenacil (Figure 6, panel 2), trifludimoxazin (3), the mixture of saflufenacil and trifludimoxazin (4), tiafenacil (5), and flumioxazin (6) was investigated, with water containing no active ingredients (1) and pendimethalin (7) used as controls. The results are illustrated in Figure 6 and Table 8 as well as in Supplementary Figure 3.

The high soil mobility of saflufenacil corresponds well with its high water solubility of 2,100 mg L^{-1} and low K_{oc} value of 6.6 ml g $^{-1}$. In contrast, the low soil mobility of trifludimoxazin can be attributed to its low water solubility of 1.78 mg L^{-1}, high logP value of 3.33, and moderately high K_{oc} value of 477.1 (APVMA 2020; PMRA 2017, 2020). This indicates that trifludimoxazin is likely to bind to the soil and not easily move with water. The ready-mix combination of saflufenacil and trifludimoxazin demonstrates excellent coverage of the soil surface, as reported by Witschel et al. (2021), indicating effective distribution of the herbicide against existing weed seeds.

Tiafenacil exhibited behavior similar to saflufenacil, while flumioxazin's behavior aligned more closely with that of trifludimoxazin (Jaremtchuk et al. 2009). The combination of trifludimoxazin and saflufenacil showcased good soil mobility and residual activity, making it a highly effective tool for efficient weed control.

Finally, to achieve effective herbicidal activity, herbicides must be absorbed by the plant, translocated to the target site, and react effectively. Trifludimoxazin is quickly absorbed by both roots and foliage, causing plant death through membrane damage after inhibiting PPO. Under optimal growing conditions, susceptible weeds show injury symptoms within hours and typically die within days. The ADME study focused on the foliar uptake of

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Table 8. Results of the soil mobility trial showing the means of the two repetitions[a].

Active ingredient	g ai ha^{-1}	— Leaching activity in separate 2.5-cm sections —															
	from	0	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30	32.5	35	37.5
	to	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30	32.5	35	37.5	40
Saflufenacil	50	0 (e)	0 (e)	12.5 (e)	25 (d)	38 (d)	43 (d)	45 (d)	55 (c)	78 (b)	93 (a)	97 (a)	98 (a)	98 (a)	98 (a)	98 (a)	93 (a)
Trifludimoxazin	50	98 (a)	98 (a)	93 (a)	58 (c)	38 (d)	13 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	
Saflufenacil + trifludimoxazin	50	98 (a)	98 (a)	75 (b)	53 (c)	38 (d)	33 (d)	40 (d)	58 (c)	65 (b)	70 (b)	79 (b)	92 (a)	97 (a)	98 (a)	98 (a)	90 (a)
Tiafenacil	50	90 (e)	93 (a)	98 (a)	98 (a)	98 (a)	98 (a)	98 (a)	98 (a)	98 (a)	97 (a)	73 (b)	50 (c)	20 (d)	0 (e)	0 (e)	0 (e)
Flumioxazin	50	98 (a)	98 (a)	98 (a)	97 (a)	93 (a)	75 (b)	68 (b)	60 (c)	50 (c)	30 (d)	5 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)
Pendimethalin	2000	55 (c)	5 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)	0 (e)

[a]Watercress was used as bioindicator. Activity was measured in % plant damage compared with untreated control (PDCU). Presented are the means ($n = 2$) of the variants. Different letters in parentheses following the means are significantly different from the test group average after Scott and Knott (1974), with an $\alpha = 0.05$.

trifludimoxazin combined with saflufenacil, comparing ready-mix and tank-mix formulations with their solo counterparts. Results indicate that saflufenacil has higher uptake (approximately 50%) but lower metabolic stability and translocation, while trifludimoxazin shows around 20% uptake with excellent metabolic stability after 3 d, although it does not translocate to the root.

For *L. perenne* ssp. *multiflorum*, similar low translocation was observed, with trifludimoxazin being less stable compared with *E. crus-galli*. Interestingly, the uptake of saflufenacil and metabolic stability of trifludimoxazin slightly increased in the ready-mix formulation. Both active ingredients exhibited photolytic stability and similar injury symptoms. Notably, the tank-mix application may reduce trifludimoxazin uptake. Autoradiographic results for *L. perenne* ssp. *multiflorum* indicated improved distribution of trifludimoxazin when combined with saflufenacil (Tables 9 and 10; Figure 7).

These findings on residual activity, soil mobility, and ADME behavior suggest that we can expect improved residual effects in field applications. Trifludimoxazin, both as a stand-alone treatment and in combination with saflufenacil, has been extensively evaluated in numerous field trials around the world, specifically for its performance in preemergence, postemergence, and preplant burndown applications. Consistent results have shown that trifludimoxazin offers longer residual activity compared with other PPO-inhibiting herbicides, such as saflufenacil, when applied before weed emergence for controlling broadleaf weeds.

Figure 8 provides an overview of broadleaf weed control based on 21 trials conducted in the United States between 2010 and 2011. The results clearly indicate that, at the same application rate, the effectiveness of saflufenacil diminishes over time, while trifludimoxazin maintains a high level of efficacy for up to 80-d posttreatment. This demonstrates that trifludimoxazin provides extended weed control, as it remains active in the soil for a longer duration. These findings align well with the residual activity experiments conducted in the greenhouse and the calculated DT_{50} data that have been reported.

In conclusion, the search for new and effective active ingredients is essential for maintaining effective weed control in integrated weed management, especially considering the presence of numerous weed resistances to current herbicides. Trifludimoxazin has shown its suitability for controlling postemergence dicot weeds and has demonstrated strong control over *L. perenne* ssp. *multiflorum*. Saflufenacil and trifludimoxazin have exhibited high metabolic stability in dicots and relatively lower metabolic stability in monocots. Field trials have further validated the efficacy of trifludimoxazin and the trifludimoxazin plus saflufenacil ready-mix in various applications. Trifludimoxazin has shown longer residual activity when used in preemergence to control broadleaf weeds compared with other PPO-inhibiting herbicides like saflufenacil. Additionally, the use of trifludimoxazin as a synergistic partner to saflufenacil could potentially enhance the control of resistant weeds (Porri et al. 2022). Trifludimoxazin has also demonstrated better inhibition of PPO2 enzymes carrying the three most widespread target-site mutations, compared with benchmarked products, even when these target mutations are combined in the same PPO2 enzyme (double mutants) (Porri et al. 2022). This has been confirmed in vivo, in *Arabidopsis* transgenics that ectopically express PPO2 carrying single and double target-site mutations (Porri et al. 2022).

Table 9. Foliar uptake, distribution, and metabolic stability of test compounds and recovery from different plant sections in *Echinochloa crus-galli* at 24 and 72 h after application (HAA)[a].

Test compound		Solo application		Ready-mix		Tank-mix	
		Saflufenacil	Trifludimoxazin	Saflufenacil	Trifludimoxazin	Saflufenacil	Trifludimoxazin
				% of applied amount			
Recovered from glass slide	24 HAA	93 (7)	99 (14)	99 (1)	100 (5)	100 (7)	100 (2)
	72 HAA	88 (2)	100 (28)	91 (9)	94 (7)	100 (3)	100 (10)
Leaf deposit	24 HAA	55 (10)	80 (4)	35 (28)	79 (10)	49 (10)	94 (12)
	72 HAA	47 (13)	71 (4)	47 (18)	57 (9)	44 (15)	75 (8)
Section	24 HAA	7 (3)	20 (2)	10 (4)	20 (4)	2 (1)	6 (1)
treated leaf	72 HAA	7 (3)	25 (5)	7 (2)	20 (1)	4 (0.5)	10 (2)
Section	24 HAA	0 (0)	0 (0)	1 (0.2)	0 (0)	0 (0)	0 (0)
RoP[b]	72 HAA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Section	24 HAA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
root	72 HAA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total recovery	24 HAA	62	100	46	99	51	100
	72 HAA	54	96	54	77	48	85
Uptake	24 HAA	45	20	65	21	51	6
	72 HAA	53	29	53	43	56	25
Metabolic	24 HAA	17	100	16	95	5	100
stability	72 HAA	14	72	14	47	7	39

[a]Data represent mean values of five plants per treatment with standard deviation in parentheses. Total recovery, uptake, and metabolic stability are calculated from measured mean values as described in "Materials and Methods."
[b]RoP, rest of plant.

Table 10. Foliar uptake, distribution, and metabolic stability of test compounds and recovery from different plant sections in *Lolium perenne* ssp. *multiflorum* at 24 and 72 h after application (HAA)[a].

Test compound		Solo application		Ready-mix		Tank-mix	
		Saflufenacil	Trifludimoxazin	Saflufenacil	Trifludimoxazin	Saflufenacil	Trifludimoxazin
				% of applied amount			
Leaf deposit	24 HAA	73 (17)	59 (12)	33 (12)	54 (10)	52 (7)	86 (7)
	72 HAA	30 (12)	10 (6)	20 (8)	53 (14)	32 (2)	87 (6)
Section	24 HAA	3 (1)	12 (2)	4 (1)	16 (5)	3 (2)	6 (1)
treated leaf	72 HAA	4 (1)	10 (2)	1 (0.5)	15 (2)	4 (1)	12 (3)
Section	24 HAA	0 (0)	0 (0)	1 (0.1)	3 (1)	0 (0)	1 (0.2)
RoP[b]	72 HAA	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)
Section	24 HAA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
root	72 HAA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total recovery	24 HAA	76	71	38	73	55	93
	72 HAA	34	20	22	68	36	99
Uptake	24 HAA	27	41	67	46	48	14
	72 HAA	70	90	80	47	68	13
Metabolic	24 HAA	11	30	6	41	7	44
stability	72 HAA	5	11	1	31	6	40

[a]Data represent mean values of five plants per treatment with standard deviation in parentheses. Total recovery, uptake, and metabolic stability are calculated from measured mean values as described in "Materials and Methods."
[b]RoP, rest of plant.

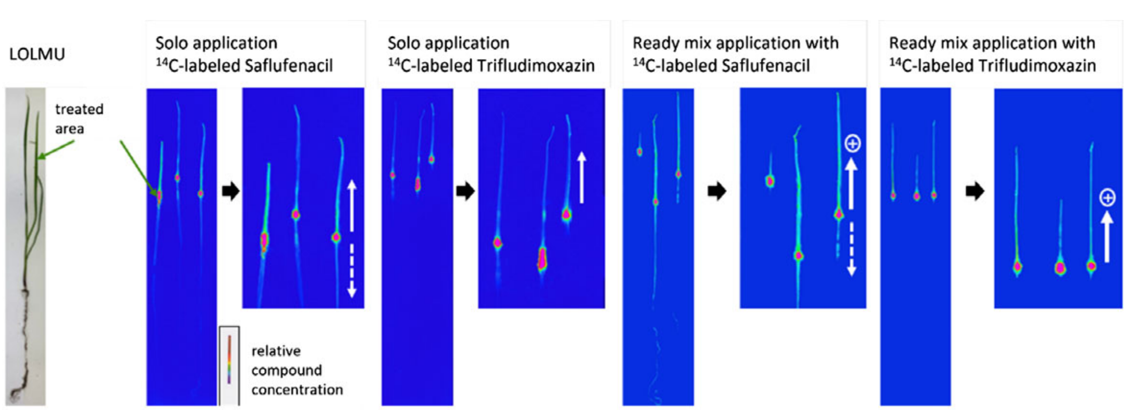

Figure 7. Autoradiography of ^{14}C-labeled saflufenacil and trifludimoxazin as solo application and as ready-mix at 24 h after treatment to demonstrate postemergence mobility. Xylem and phloem mobility indicated by arrows. LOLMU, *Lolium perenne* ssp. *multiflorum*.

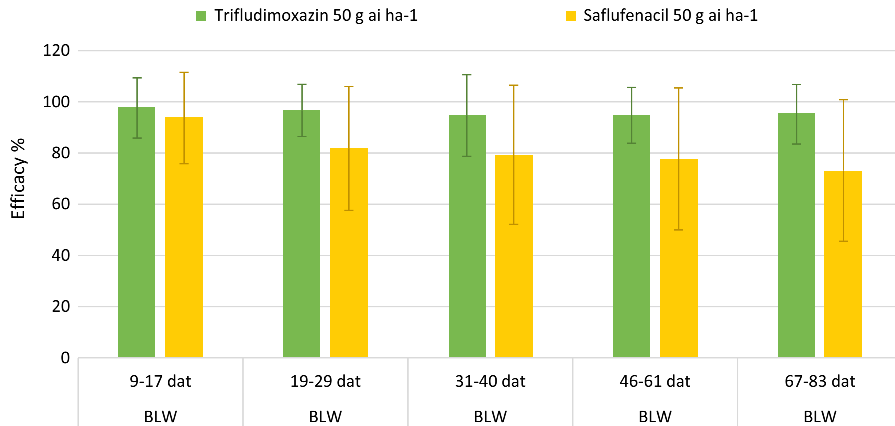

Figure 8. Overview of broadleaf weed (BLW) control in U.S. field trials. Dat, days after treatment. Efficacy from 21 trials in 7 locations in the United States during 2010–2011. Rate, 50 g ai ha^{-1}. Weeds are natural infestation.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2024.92

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