

Research Article

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Corresponding author:

Joshua W. A. Miranda;
Email: josh.miranda@oregonstate.edu

Improved management of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) with mixtures of tiafenacil and ACCase or glutamine synthetase inhibitors

Joshua W.A. Miranda¹  and Marcelo L. Moretti² 

¹Graduate Student, Department of Horticulture, Oregon State University, Corvallis, OR, USA and ²Associate Professor, Department of Horticulture, Oregon State University, Corvallis, OR, USA

Abstract

Herbicide-resistant Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is a significant problem in multiple cropping systems because of its rapid growth, open pollination, prolific seed production, and multiple cases of resistance worldwide, except to protoporphyrinogen oxidase (PPO) inhibitors. This research evaluated tiafenacil, a new PPO inhibitor, in mixtures with glutamine synthetase- or acetyl-CoA carboxylase (ACCase)-inhibiting herbicides to manage resistant *L. perenne* ssp. *multiflorum* populations. Tiafenacil efficacy against *L. perenne* ssp. *multiflorum* was growth stage dependent, with increased efficacy at earlier stages in greenhouse studies. The LD₉₀ was 41.06 g ai ha⁻¹ at BBCH 23 and increased to 9.0-fold at BBCH 33. Field studies indicated that changes in carrier volume did not affect tiafenacil's efficacy; the highest tested rate of tiafenacil (75 g ai ha⁻¹) reduced *L. perenne* ssp. *multiflorum* inflorescence weight by 50% to 90%. Mixtures of tiafenacil and glufosinate (1,150 g ai ha⁻¹) improved *L. perenne* ssp. *multiflorum* control (+24% to 43%) and reduced inflorescence weight (+15% to 34%), particularly at the highest tested rates (50 and 75 g ai ha⁻¹), suggesting synergistic effects based on Colby's test. Tiafenacil with ACCase inhibitors improved *L. perenne* ssp. *multiflorum* control (+19% to 49%) and inflorescence weight reduction (+8% to 13%). These mixtures exhibited an additive effect when combined with fluzafop and a synergistic effect with clethodim. Herbicide mixtures and application strategies are critical to effective *L. perenne* ssp. *multiflorum* management. Tiafenacil, especially when used with glufosinate or ACCase inhibitors, offers an effective solution to *L. perenne* ssp. *multiflorum* management and is a strategic tool against herbicide resistance, as resistance to PPO inhibitors has not evolved. Further research should assess practices to ensure the long-term viability of these mixtures for resistance management.

Introduction

Lolium includes several species of economic significance used as cover crops, turf, and pasture due to their robust adaptability and distribution. Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is an important weed species in this genus because of its remarkable plasticity and high fecundity (Preston et al. 2009). This diploid ($2n = 2x = 14$) monocotyledonous winter annual obligately outcrosses via wind pollination (Beckie and Jasieniuk 2021; Karn et al. 2018; Karn and Jasieniuk 2017) and has a genetic structure that predisposes it to rapidly evolve herbicide resistance (Matzrafi et al. 2021), with 75 unique cases of herbicide and multiple-herbicide resistance reported globally as of 2024 (Heap 2024).

Lolium perenne ssp. *multiflorum* is a weed management challenge today because of the many cases of herbicide resistance in Europe (Kaya Altop et al. 2022; Scarabel et al. 2020), Asia (Kurata et al. 2018; Zhu et al. 2023), Oceania (Ghanizadeh et al. 2020), South America (Marques Hill et al. 2022; Vázquez-García et al. 2020; Vila-Aiub et al. 2009), and North America (Brunharo and Tranel 2023; Jasieniuk et al. 2008; Nandula et al. 2020). In Oregon, USA, there are confirmed cases of multiple herbicide sites of action resistance, including those targeting acetyl-CoA carboxylase (ACCase), very-long-chain fatty acids, 5-enolpyruvylshikimate-3-phosphate synthase, and glutamine synthetase (Bobadilla et al. 2021; Moretti 2021). These reduce the efficacy of single mode of action herbicide-based strategies.

Herbicide resistance threatens agricultural productivity and sustainability, increases costs, diminishes the effectiveness of traditional weed management methods, and substantially reduces yields (Peterson et al. 2018). Researchers and farmers are intensifying their efforts to counteract the escalating challenge of herbicide resistance. Herbicide rotations or mixtures are an increasingly studied approach (Beckie and Harker 2017). This approach lessens selection pressure from any one mode of action and reduces the chance of emergence and proliferation of

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resistant biotypes (Beckie and Harker 2017; Busi and Beckie 2021; Busi et al. 2020; Norsworthy et al. 2012). Herbicide mixtures can provide broader-spectrum control and target a wider range of weed species and growth stages (Rigon et al. 2023). They can enhance weed control efficacy, particularly where herbicide-resistant populations prevail (Beckie and Reboud 2009; Soltani et al. 2021; Westerveld et al. 2021). This proactive and adaptive response offers farmers resilient and sustainable weed control strategies and supports long-term productivity and environmental stewardship.

Not all herbicide mixtures are equal, and understanding the interactions is essential for optimizing herbicide use (Barbieri et al. 2022). Synergism leads to enhanced weed control; antagonism reduces efficacy compared with individual herbicide applications (Rigon et al. 2023; Ritz et al. 2021). Additivity is a neutral interaction; the combined effect equals the sum of individual effects (Ndikuryayo and Yang 2023). By exploring the interactions between herbicides with contrasting modes of action, this research aims to develop more effective weed management strategies for herbicide-resistant *L. perenne* ssp. *multiflorum*.

Tiafenacil is a new protoporphyrinogen oxidase (PPO)-inhibiting herbicide with postemergence activity on monocots (Alnafta et al. 2023; Jeschke 2024; Mattison et al. 2023). The inhibition results in accumulation of protoporphyrinogen IX, a precursor in the chlorophyll biosynthetic pathway, leading to cell membrane disruption and plant death (Cha et al. 2022; Park et al. 2018; Traxler et al. 2023). Tiafenacil is registered for use in such crops as soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium* spp.), corn (*Zea mays* L.), peanut (*Arachis hypogaea* L.), rice (*Oryza sativa* L.), sugar beet (*Beta vulgaris* L.), hazelnut (*Corylus avellana* L.), and wheat (*Triticum aestivum* L.) (Miranda and Moretti 2023). Tiafenacil is selective, with reduced impact on non-target plant species and reduced disruption to the balance within agricultural ecosystems (Cha et al. 2022; Hu et al. 2020; Park et al. 2018). However, the effectiveness of tiafenacil against *L. perenne* ssp. *multiflorum* has not yet been determined. This research is structured around two primary objectives: first, to evaluate the effectiveness of tiafenacil against *L. perenne* ssp. *multiflorum*, comparing its performance against established benchmarks; and second, to explore the interactions between tiafenacil, glufosinate, and ACCase-inhibiting herbicides to manage *L. perenne* ssp. *multiflorum*. We hypothesized that tiafenacil in mixtures will enhance the management of resistant *L. perenne* ssp. *multiflorum* biotypes, as resistance to PPO inhibitors has not been documented globally in this species.

Materials and Methods

Greenhouse Studies

We evaluated the response of *L. perenne* ssp. *multiflorum* to tiafenacil at three growth stages—2 to 3 leaves (BBCH 13), 2 to 3 tillers (BBCH 23), and 2 to 3 nodes elongated (BBCH 33)—in greenhouse studies in 2022. Seeds of *L. perenne* ssp. *multiflorum* were germinated in acrylic boxes (22 by 11 by 4.5 cm; Hoffman Manufacturing, Corvallis, OR), each containing two blotting papers and 50 ml of distilled water. These were incubated at 22 C under a 12/12-h light/dark cycle with a photon flux density of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 7 d. *Lolium perenne* ssp. *multiflorum* seedlings were transferred to individual 1-L pots filled with commercial potting mix (SS#4 PC RSi, Sun Gro® Horticulture, Agawam, MA) and held in a greenhouse at Oregon State University, Corvallis, OR (44.57°N, 123.29°W). The greenhouse environment was regulated to a day/

night temperature of 22/18 C and a 12-h photoperiod supplemented with artificial lighting. The study was a two by two factorial layout, with growth stages and tiafenacil rates as the factors, arranged in a randomized complete block design (RCBD) with four replicates and conducted twice. Each experimental unit was one seedling per pot. The growth stages were BBCH 13, BBCH 23, and BBCH 33. Tiafenacil rates ranged from 0 to 200 g ai ha⁻¹ (reference field rates range from 25 to 75 g ai ha⁻¹). Upon reaching specific growth stages, treatments were imposed with a research sprayer (DeVries Manufacturing, Generation III, Hollandale, MN) equipped with a single TP8003E nozzle (TeeJet® Technologies, Glendale Heights, IL), placed 45 cm above the canopy and calibrated to deliver 187 L ha⁻¹ of spray solution. At 35 d after treatment (DAT), the aboveground biomass of *L. perenne* ssp. *multiflorum* was harvested, dried, and weighed. Plant regrowth was assessed at 28 d post-biomass collection. Survival was quantitatively recorded, with a score of 1 indicating survival and 0 indicating mortality.

Field Studies

Field studies were conducted in eight commercial hazelnut orchards from 2020 to 2023 across the Willamette Valley, OR (Figure 1). In these orchards, *L. perenne* ssp. *multiflorum* populations were either known or suspected to have evolved resistance to glyphosate, paraquat, acetolactate synthase (ALS)-inhibiting herbicides, and ACCase-inhibiting herbicides (Table 1). These studies were divided into four separate protocols to evaluate the efficacy of tiafenacil in controlling *L. perenne* ssp. *multiflorum*, evaluate the impact of carrier volume on its performance, and explore its interaction with glufosinate and ACCase-inhibiting herbicides. Details about the *L. perenne* ssp. *multiflorum* population can be found in Table 1, including suspected herbicide resistance, growth stage, and height at the time of application, along with the experimental site, date, and environmental conditions during herbicide application. Treatments covered a 1.5-m-wide and 12-m-long swath in each side of the tree row. Herbicides were applied using a CO₂-pressurized backpack sprayer equipped with a spray boom containing three AI-11002 nozzles (TeeJet® Technologies) spaced at 50 cm and calibrated to deliver 187 L ha⁻¹ at 275 kPa at 4.8 km h⁻¹ for each study, except for the carrier volume study. For the carrier volume study, application speeds were adjusted to achieve delivery rates of 187 L ha⁻¹ at 4.8 km h⁻¹, 374 L ha⁻¹ at 2.4 km h⁻¹, and 561 L ha⁻¹ at 1.64 km h⁻¹, all at 275 kPa. Each plot measured 12 by 3 m.

Efficacy of Tiafenacil Studies

The first field study aimed to evaluate the efficacy of tiafenacil in managing *L. perenne* ssp. *multiflorum* populations compared with other postemergence herbicides (Table 2). Tiafenacil was applied at 25, 50, 75, and 150 g ai ha⁻¹. These rates were compared with clethodim at 150 g ai ha⁻¹ and glufosinate at 1,150 g ai ha⁻¹, along with tiafenacil at 50 g ai ha⁻¹ in mixture with glufosinate and clethodim. A nontreated control was included. The study resulted in nine treatments and was set as an RCBD with four replicates. The study was conducted at three sites, efficacy.1 (Canby), efficacy.2 (Canby), and efficacy.3 (Canby) (Table 1).

Impact of Carrier Volume on Tiafenacil Performance Studies

The effect of carrier volume on the efficacy of tiafenacil to control *L. perenne* ssp. *multiflorum* is an important factor to consider, because tiafenacil is a postemergence contact herbicide (Westerveld et al. 2021). Contact herbicides require direct contact with the plant's foliage to exert their effects (Creech et al. 2015). Consequently, the

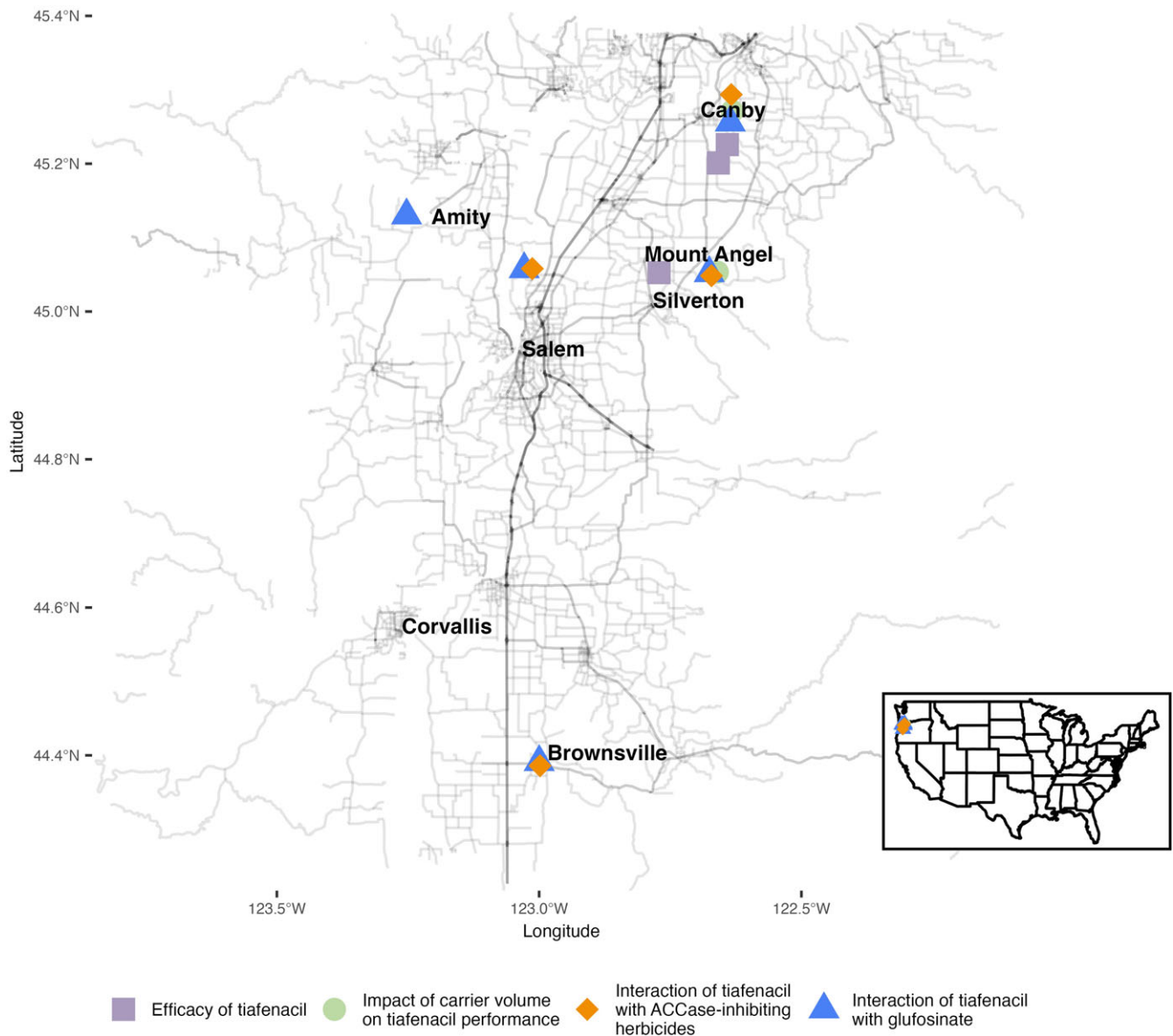


Figure 1. Geospatial distribution of experimental sites in the Willamette Valley, Oregon: This map illustrates locations of field studies evaluating tiafenacil and its interaction with current benchmarks, carrier volume, glufosinate, and acetyl-CoA carboxylase (ACCase)-inhibiting herbicides for *Lolium perenne* ssp. *multiflorum* management in hazelnut orchards. Symbols represent different studies: purple squares for the field study assessing tiafenacil efficacy, green circles for the field study examining the impact of carrier volume on tiafenacil performance, orange diamonds for the field study exploring tiafenacil interaction with glufosinate, and blue triangles for the field study investigating tiafenacil interaction with ACCase-inhibiting herbicides.

volume of the application carrier is crucial for achieving sufficient coverage of the target weeds. This study was designed as a two by two factorial, with tiafenacil rates and carrier volume as the factors, arranged in an RCBD. Tiafenacil was applied at rates of 25 (low), 50 (medium), and 75 (high) g ai ha⁻¹, and three carrier volumes of 187 (standard volume in orchard crops), 374, and 561 L ha⁻¹ (Table 2). A nontreated control was included for comparison with herbicide treatments. The study resulted in 10 treatments, each replicated 4 times. The study was conducted at three sites, carrier.volume.1 (Silverton), carrier.volume.2 (Canby), and carrier.volume.3 (Salem) (Table 1).

Interaction of Tiafenacil with Glufosinate Studies

Takano et al. (2020) proposed a synergistic interaction between PPO inhibitors, such as saflufenacil, and glufosinate herbicides in a

PPO-resistant Palmer amaranth (*Amaranthus palmeri* S. Watson) and in a glufosinate-resistant soybean. The synergistic effect enhances *A. palmeri* control by increasing reactive oxygen species (ROS) production, driven by glufosinate-induced glutamate accumulation and PPO inhibition, which together elevate protoporphyrin and ROS levels. The synergistic interaction observed between these herbicide modes of action, however, was not evaluated in monocots. This synergy has implications for delaying herbicide-resistance selection and optimizing weed control strategies.

Building on this knowledge, the third field study investigated the interaction between tiafenacil and glufosinate in controlling *L. perenne* ssp. *multiflorum* populations (Table 2). The study was a two-factor factorial RCBD study (Ritz et al. 2021), with PPO-inhibiting herbicides and glufosinate herbicides as the variables.

Table 1. Descriptions of *Lolium perenne* ssp. *multiflorum* biotypes, including suspected herbicide resistance, growth stage and height at application timing, as well as experimental site, date, and environmental conditions at the application timing in this research.

Study ID ^a	Location	Putative herbicide resistance WSSA group ^b	Growth stage	Average height	Environmental conditions at application timing		
					Temp C	HR %	Cloud cover %
				cm			
Efficacy.1	Canby	1 and 10	BBCH 39	48	29	32	25
Efficacy.2	Canby	9	BBCH 39	48	17	60	20
Efficacy.3	Mount Angel	1 and 9	BBCH 36	42	16	45	20
Carrier.volume.1	Silverton	1, 9, and 22	BBCH 39	45	22	51	0
Carrier.volume.2	Canby	2 and 9	BBCH 39	30	19	42	10
Carrier.volume.3	Salem	9 and 10	BBCH 39	30	19	42	10
Interaction. Glufosinate.1	Silverton	1, 9, 22	BBCH 39	45	22	51	0
Interaction. Glufosinate.2	Canby	2 and 9	BBCH 39	30	19	42	10
Interaction. Glufosinate.3	Salem	9 and 10	BBCH 39	30	15	55	15
Interaction. Glufosinate.4	Amity	10	BBCH 39	35	12	57	10
Interaction. Glufosinate.5	Brownsville	9	BBCH 33	30	24	42	0
Tia.ACCase.1	Silverton	1, 9, 22	BBCH 39	45	22	51	0
Tia.ACCase.2	Canby	2 and 9	BBCH 39	30	19	42	10
Tia.ACCase.3	Salem	9 and 10	BBCH 39	30	15	55	15
Tia.ACCase.4	Brownsville	9	BBCH 33	30	24	42	0

^aStudy ID abbreviations: Efficacy.X, efficacy of tiafenacil; Carrier.volume.X, impact of carrier volume on tiafenacil performance; Interaction. Glufosinate.X, interaction of tiafenacil with glufosinate; Tia.ACCase.X, interaction of tiafenacil with acetyl-CoA carboxylase (ACCCase)-inhibiting herbicides.

^bWSSA group of action abbreviations: 1, inhibitor of acetyl-CoA carboxylase; 2, inhibitor of acetolactate synthase; 9, inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase; 10, inhibitor of glutamine synthetase; 22, inhibitor of photosystem I-electron diverters.

Table 2. Herbicides, their respective application rates, and adjuvants used in studies assessing *Lolium perenne* ssp. *multiflorum* control.^a

Herbicide	Trade name	Rate	Adjuvant ^b	Manufacturer and address
		g ai ha ⁻¹		
Efficacy of tiafenacil				
Tiafenacil	Gamma™	25, 50, 75, and 150	AMS + MSO	ISK® Biosciences, Concord, OH 44077
Clethodim	Select Max®	150	AMS + MSO	Valent U.S.A. Corp., Suite 200 Walnut Creek, CA 94596
Glufosinate	Rely 280®	1,150	AMS + MSO	BASF Corp., Research Triangle Park, NC 27709
Impact of carrier volume on tiafenacil performance				
Tiafenacil	Gamma™	25, 50, and 75	AMS + MSO	ISK® Biosciences
Interaction of tiafenacil with glufosinate				
Tiafenacil	Gamma™	25, 50, and 75	AMS + MSO	ISK® Biosciences
Glufosinate	Rely 280®	1,150	AMS + MSO	BASF Corp.
Carfentrazone	Aim® EC	35	AMS + MSO	FMC Corp., Philadelphia, PA 19104
Interaction of tiafenacil with acetyl-CoA carboxylase (ACCCase)-inhibiting herbicides				
Tiafenacil	Gamma™	25, 50, and 75	AMS + MSO	ISK® Biosciences
Clethodim	Select Max®	150	AMS + MSO	Valent U.S.A. Corp.
Fluazifop-P-butyl	Fusilade® DX	420	AMS + MSO	Syngenta Crop Protection, Greensboro, NC 27419
Carfentrazone	Aim® EC	35	AMS + MSO	FMC Corp.

^aThese studies evaluate tiafenacil against established benchmarks, the influence of carrier volume on tiafenacil efficacy, and the interaction effects of tiafenacil with glufosinate or ACCCase-inhibiting herbicides.

^bAmmonium sulfate (AMS) (**Amsol**™, WinField United, Arden Hills, MN) was included at 10 g L⁻¹; methylated seed oil (MSO) (**HASTEN-EA**®, Wilbur-Ellis, Aurora, CO) was included at 8.9 g L⁻¹.

Glufosinate was assessed at a rate of 0 or 1,150 g ai ha⁻¹, PPO inhibitors included tiafenacil at 25, 50, and 75 g ai ha⁻¹ and carfentrazone at 35 g ai ha⁻¹. A nontreated control was included. Carfentrazone at 35 g ai ha⁻¹ was a reference for PPO-inhibiting herbicides without activity in monocots. The study resulted in 10 treatments, each replicated 4 times, and conducted at 5 sites: interaction.glufosinate.1 (Silverton), interaction.glufosinate.2 (Canby), interaction.glufosinate.3 (Salem), interaction.glufosinate.4 (Amity), and interaction.glufosinate.5 (Brownsville) (Table 1).

Interaction of Tiafenacil with ACCCase-inhibiting Herbicide Studies

ACCCase inhibitors disrupt fatty-acid biosynthesis in plants, leading to growth inhibition and eventual plant death (Meyer et al. 2021).

While there is limited literature on the specific interaction between ACCCase inhibitors and PPO inhibitors, exploring their potential interaction may improve weed control efficacy, especially with herbicide-resistant weeds. Studies have shown that ACCCase inhibitors often exhibit antagonistic effects when combined with other herbicide classes, especially ALS and PPO inhibitors (Zhang et al. 2005). However, tiafenacil was not included in these previous studies. Tiafenacil is unique in its activity against monocots, unlike most current PPO inhibitors.

The fourth field study evaluated the interaction between tiafenacil and ACCCase-inhibiting herbicides (Table 2). This was a two-factor RCBD factorial study (Ritz et al. 2021), with PPO-inhibiting herbicides and ACCCase-inhibiting herbicides as the variables. ACCCase inhibitors were assessed individually, including nontreated, clethodim at 135 g ai ha⁻¹, and fluazifop at 420 g ai ha⁻¹.

For PPO inhibitors, nontreated, tiafenacil at 75 g ai ha⁻¹, and carfentrazone at 35 g ai ha⁻¹ were evaluated. The study resulted in nine treatments, each replicated four times, and conducted at four sites: tia.ACCase.1 (Silverton), tia.ACCase.2 (Canby), tia.ACCase.3 (Salem), and tia.ACCase.4 (Brownsville) (Table 1).

Assessments for the Field Studies

Measurements of *L. perenne* ssp. *multiflorum* control and aboveground green area coverage were conducted at 35 DAT. Weed control was evaluated on a scale ranging from 0% to 100%, with 0% indicating no control and 100% representing complete control. The aboveground green coverage of *L. perenne* ssp. *multiflorum* was assessed by capturing two images within each plot using an iPhone 14 camera (Apple, Cupertino, CA), covering an area of approximately 6 m² per image. Canopeo software (Patrignani and Ochsner 2015) facilitated image analysis and determination of green coverage percentages, with these Canopeo parameters used to determine green area coverage: foliage type was “cover crops,” the red-to-green ratio was 1.00, the blue-to-green ratio was 0.80, and the minimum excess green was 20. The green area was validated visually in a subset of images. Lower aboveground green area coverage indicated reduced weed density and improved weed control. At 35 DAT, aboveground biomass samples were collected from *L. perenne* ssp. *multiflorum* plants within each plot using two 0.25-m² quadrats. Separate collections were made for shoots and inflorescences in all studies except for the efficacy of tiafenacil studies, where biomass was not separated. Biomass was dried and weighed. Inflorescence biomass was used as an indicator of seed production, highlighting the importance of implementing weed management strategies that focus on reduced weed seeds that replenish the weed seedbank (Norsworthy et al. 2012, 2018). While direct assessment of seed production and viability was not feasible in the field studies, inflorescence biomass provided valuable insights into the effectiveness of treatments in suppressing reproductive growth of *L. perenne* ssp. *multiflorum* populations. Reduction in shoot and inflorescence biomass relative to the nontreated control was calculated for each plot using Equation 1:

$$RB = \frac{OB}{AB_{NTC}} \times 100 \quad [1]$$

where RB represents the relative biomass expressed as a percentage compared with the nontreated control, OB is the observed biomass for the respective experimental unit, and AB_{NTC} is the average biomass of the nontreated control.

Statistical Analysis

All statistical analyses were performed in R v. 4.2.2 (R Core Team 2022).

Greenhouse Studies

Greenhouse survival data were analyzed using a two-parameter log-logistic model with a binomial distribution implemented through the DRC package (Ritz et al. 2015). Equation 2 describes this model:

$$y = \frac{1}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad [2]$$

where y represents plant survival, x is the herbicide rate (g ai ha⁻¹), b is the relative slope at the inflection point, and e is the herbicide rate

required for 50% plant survival (LD₅₀). Biomass dry weight was converted to relative biomass compared with the nontreated control for each growth stage using Equation 1. Data were combined across experiments, as no interactions of herbicide by growth stage and experimental run were observed. Relative biomass was modeled using a four-parameter log-logistic model in the DRC package (Ritz et al. 2015), depicted by Equation 3:

$$y = c + \frac{d - c}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad [3]$$

where y represents relative biomass; x is the herbicide rate (g ai ha⁻¹); b is the relative slope at the inflection point; e is the herbicide rate required for a 50% growth reduction (GR₅₀); and c and d are the lower and upper limits of the response, respectively. These models allowed us to calculate herbicide rates required to achieve a 50% (LD₅₀ or GR₅₀) and a 90% (LD₉₀ or GR₉₀) reduction in plant survival and growth for each treatment.

Field Studies

Field study data were subjected to ANOVA at $P < 0.05$. For field studies employing an RCBD, study site and treatments were considered fixed effects, while experimental blocks and interactions were treated as random. For field studies using a factorial design with RCBD, the study site, factors, and interactions were regarded as fixed effects, with experimental blocks considered random. Percentage-based data, which included weed control, aboveground green area coverage, shoot biomass reduction, and inflorescence biomass reduction, were beta-transformed, scaling data from 0 to 1. These beta-transformed data were analyzed using the GLMMTMB package (Brooks et al. 2017) with a generalized linear mixed model employing a beta error distribution. Weed control, shoot biomass reduction, and inflorescence biomass reduction for the nontreated control were excluded from the analysis, as their zero values lacked variance. Whenever significant treatment or factor interactions were detected, post hoc comparisons were conducted using Tukey's HSD method with the assistance of the EMMEANS package and the *cld* function (Lenth 2020). Additionally, Colby's method was used to evaluate herbicide interaction effects on *L. perenne* ssp. *multiflorum* control and inflorescence biomass reduction across all sites, as described by Equation 4 (Colby 1967; Ritz et al. 2021):

$$E = (X + Y) - \frac{XY}{100} \quad [4]$$

where E represents the expected value for the herbicide combination, with X and Y denoting the observed values for each herbicide separately. The expected and observed effects were compared using a two-sided t -test ($\alpha = 0.05$) through the *t.test* function from the base STATS package (R Core Team 2022). Herbicide combinations were classified as antagonistic if the observed value was significantly less than expected, additive if there was no significant difference between observed and expected values, and synergistic if the observed value significantly exceeded the expected value.

Results and Discussion

Greenhouse Studies

The greenhouse study provided valuable insights into the growth stage-dependent efficacy of tiafenacil in managing *L. perenne* ssp.

Table 3. Dose–response experiment estimates of tiafenacil efficacy at the BBCH 13, BBCH 23, and BBCH 33 growth stages on *Lolium perenne* ssp. *multiflorum*.^a

Growth stage	GR ₅₀	Ratio	GR ₉₀	Ratio	LD ₅₀	Ratio	LD ₉₀	Ratio
	g ai ha ⁻¹		g ai ha ⁻¹		g ai ha ⁻¹		g ai ha ⁻¹	
2–3 leaves (BBCH 13)	0.06	—	11.83	—	15.86	—	41.06	—
2–3 tillers (BBCH 23)	0.04	0.66	35.29	2.9	33.66	2.1	63.54	1.5
2–3 nodes (BBCH 33)	1,852	30866	13,956	1179	201.41	12.7	372.88	9.1

^aAbbreviations: GR₅₀, herbicide rate (g ai ha⁻¹) required for 50% growth reduction; GR₉₀, herbicide rate (g ai ha⁻¹) required for 90% growth reduction; LD₅₀, herbicide rate (g ai ha⁻¹) required for 50% plant mortality; LD₉₀, herbicide rate (g ai ha⁻¹) required for 90% plant mortality. Ratio, ratio of parameter relative to BBCH 13 parameter; —, not calculated.

multiflorum, revealing a clear trend: the susceptibility of *L. perenne* ssp. *multiflorum* to tiafenacil is inversely proportional to its maturity (Table 3). At BBCH 13, 0.06 g ha⁻¹ of tiafenacil was sufficient to reach the GR₅₀. The herbicide's efficacy was superior at this stage, as both GR₅₀ and GR₉₀ (11.83 g ha⁻¹) were well below the field rate (25 to 75 g ha⁻¹). Similarly, LD₅₀ and LD₉₀ were 15.86 and 41.06 g ha⁻¹, respectively. As the plants matured to BBCH 23, GR₅₀ did not change, but GR₉₀ increased by 3-fold relative to BBCH 13, indicating a growth stage–dependent response to tiafenacil. The LD₅₀ and LD₉₀ for BBCH 23 were recorded at 33.66 and 63.54 g ha⁻¹, respectively, indicating a 2.1 to 1.5 increased rate requirement. At BBCH 33, tiafenacil was not effective; the GR₅₀ escalated to 1,852 g ha⁻¹ and GR₉₀ to 13,956 g ha⁻¹. LD₅₀ and LD₉₀ values were 201.41 and 372.88 g ha⁻¹, respectively, which were 12.7- and 9.1-fold higher than those required at BBCH 13. This pattern of increased herbicide to control more mature *L. perenne* ssp. *multiflorum* could be attributed to physiological and morphological changes occurring during plant development (Ochoa-López et al. 2015). *Arabidopsis thaliana* (Jang et al. 2020), develops increasingly sophisticated defense responses, a phenomenon likely paralleled in *L. perenne* ssp. *multiflorum*, leading to reduced efficacy of tiafenacil in older plants. The results emphasize the importance of timely herbicide application for effective *L. perenne* ssp. *multiflorum* control. Early intervention, targeting the most vulnerable growth stage, can enhance the efficacy of tiafenacil, optimize its use, and contribute to sustainable weed management.

Field Studies

Efficacy of Tiafenacil

In the efficacy.1 study, tiafenacil provided greatest control at 150 g ha⁻¹ (95%) (Figure 2A). This was closely matched by tiafenacil at 50 g ha⁻¹ plus clethodim at 135 g ha⁻¹ or glufosinate at 1,150 g ha⁻¹. Tiafenacil at 75 g ha⁻¹ controlled 63% of *L. perenne* ssp. *multiflorum*. By contrast, tiafenacil at 25 and 50 g ha⁻¹ and clethodim and glufosinate exerted the least control, supporting the observation of a population with putative resistance to glufosinate and clethodim. The trends for ground cover and biomass reduction (Figure 2D and 2G) were consistent with the control assessments; the highest rate of tiafenacil (150 g ai ha⁻¹) and the combination of tiafenacil at 50 g ha⁻¹ with glufosinate or clethodim provided the greatest reductions.

In the efficacy.2 study, where a putative glyphosate-resistant biotype of *L. perenne* ssp. *multiflorum* was present, mixtures of 50 g ha⁻¹ tiafenacil with glufosinate or clethodim provided the greatest control of *L. perenne* ssp. *multiflorum* (>90%) (Figure 2B). The highest rate of tiafenacil tested, 150 g ha⁻¹, did not perform as well as tiafenacil mixtures but provided a similar level of control as glufosinate and clethodim. The tiafenacil mixture treatments also led to the greatest reductions in ground cover and biomass (Figure 2E and 2H).

In the efficacy.3 study, a site infested with a suspected glyphosate- and clethodim-resistant population, the mixture of tiafenacil at 50 g ha⁻¹ and glufosinate provided 75% *L. perenne* ssp. *multiflorum* control (Figure 2C), with the glufosinate treatment second in performance. Tiafenacil, clethodim, and tiafenacil mixed with clethodim were less effective (<60% control). Ground coverage and biomass reduction data supported these findings (Figure 2F and 2I), with the combination of tiafenacil at 50 g ha⁻¹ and glufosinate leading to the lowest ground cover by *L. perenne* ssp. *multiflorum* and the greatest (55%) reduction in biomass. These results hinted at better performance by mixtures over single active ingredients.

Impact of Carrier Volume on Tiafenacil Performance

Treatments with lower carrier volumes (187 L ha⁻¹), demonstrated greater control against *L. perenne* ssp. *multiflorum* in the carrier volume.1 study (Figure 3A). Inflorescence biomass reduction did not follow this trend, with no significant differences among herbicide treatments (Figure 3G). Shoot biomass reduction showed that low carrier volumes (i.e., 187 L ha⁻¹) paired with a midrange tiafenacil rate (50 g ha⁻¹) were less effective, whereas higher carrier volumes improved control (Figure 3I). At a low rate of tiafenacil (25 g ha⁻¹), the highest carrier volume (561 L ha⁻¹) diminished the herbicide's effectiveness in reducing shoot biomass compared with lower volumes.

In the carrier.volume.2 study, carrier volume from 187 to 561 L ha⁻¹ did not affect the performance of tiafenacil to control *L. perenne* ssp. *multiflorum* (Figure 3B). The data on ground coverage echoed these findings, where all herbicide treatments provided similar responses. One exception was the distinct reduction in inflorescence and shoot biomass with the highest rates of tiafenacil, 50 and 75 g ha⁻¹ (Figure 3H and 3J).

In the carrier.volume.3 study, the results were less consistent (Figure 3C). The highest tiafenacil rate, 75 g ha⁻¹, best controlled *L. perenne* ssp. *multiflorum* regardless of carrier volume. Ground cover data did not show a corresponding reduction, as all herbicide treatments resulted in similar levels compared with the nontreated control. The study was terminated before biomass collection, limiting further insights from this site.

Interaction of Tiafenacil with Glufosinate

In the interaction.glufosinate.1 study, the control of *L. perenne* ssp. *multiflorum* with putative resistance to glyphosate, paraquat, and ACCase inhibitors was significantly enhanced when PPO-inhibiting herbicides were added to glufosinate (Figure 4A). There was no significant difference between carfentrazone with glufosinate and tiafenacil (25 or 50 g ha⁻¹) with glufosinate. Only the highest rate of tiafenacil, 75 g ha⁻¹, added to glufosinate produced a significant enhancement in *L. perenne* ssp. *multiflorum* control compared with glufosinate plus carfentrazone or tiafenacil

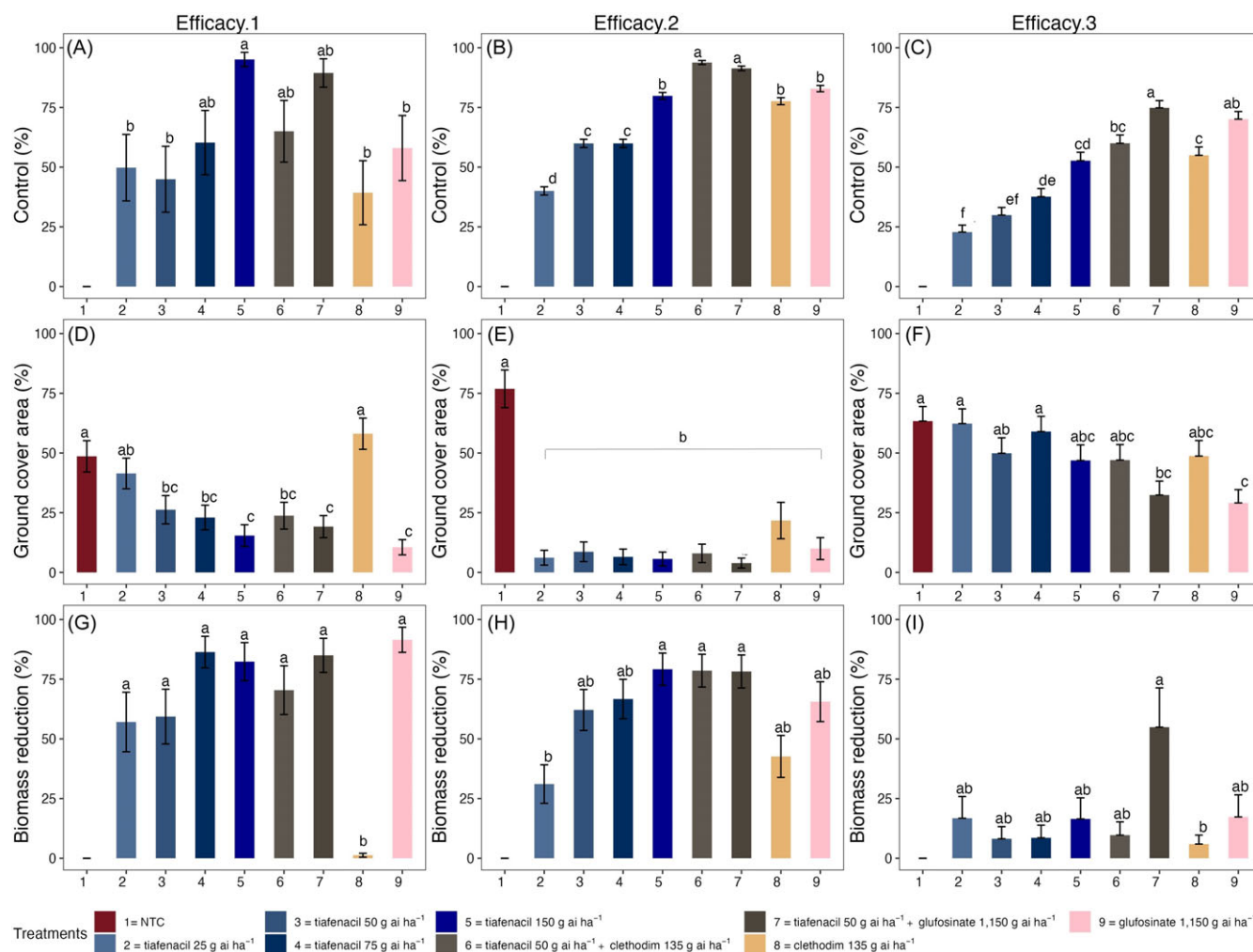


Figure 2. Tiafenacil performance for *Lolium perenne* ssp. *multiflorum* management: comparing the effectiveness of tiafenacil at varying rates alone and in mixture with glufosinate and clethodim. Assessments included control efficacy, ground cover rate, and biomass reduction at 35 d after treatment. NTC, nontreated control. Data presented are means ($\pm$ SEM); different letters within the same plot indicate significant differences at $P < 0.05$. *Lolium perenne* ssp. *multiflorum* control was visually estimated on a scale from 0% to 100%, where 0% represented no control and 100% represented complete control. Control data for the NTC were excluded from the analysis, as the values were 0%. Green area coverage was assessed using Canopeo software to analyze two random images per plot, with lower percentages indicating more effective weed suppression. Aboveground biomass was collected from two 0.25-m² quadrats.

at 25 or 50 g ha⁻¹. Ground cover results provided by any glufosinate-containing treatments were comparable and unaffected by the PPO-inhibiting herbicides. Paradoxically, the treatment providing the greatest control, tiafenacil at 75 g ha⁻¹ plus glufosinate, resulted in the lowest reduction in shoot biomass. Moreover, there was no significant difference in inflorescence biomass reduction between treatments containing glufosinate and those with tiafenacil.

In the interaction.glufosinate.2 study, the control of *L. perenne* ssp. *multiflorum* with putative resistance to glyphosate and ALS inhibitors presented an interesting scenario: combinations of tiafenacil (irrespective of the rate) with glufosinate enhanced control efficacy (Figure 4B). Yet this did not consistently reduce ground cover; only the highest rate of tiafenacil, 75 g ha⁻¹, combined with glufosinate, provided the greatest reduction (Figure 4G). There were no significant differences in shoot biomass reduction, and responses for inflorescence biomass were inconsistent. For inflorescence biomass reduction, glufosinate-containing treatments provided similar reductions, with no effects from the addition of PPO-inhibiting herbicides (Figure 4Q).

In study interaction.glufosinate.3, there was a clear distinction in *L. perenne* ssp. *multiflorum* control efficacy, particularly an increase in the control when tiafenacil was used in combination with glufosinate (Figure 4C). Ground cover rates did not exhibit the same level of distinction, but there was a trend where higher tiafenacil rates with glufosinate more effectively reduced *L. perenne* ssp. *multiflorum* cover (Figure 4H). Biomass reduction data further corroborated the effectiveness of treatments combining tiafenacil with glufosinate (Figure 4M and 4R). The population present on the site was suspected to be resistant to glufosinate and glyphosate; our results demonstrate the value of combining both herbicides to manage herbicide-resistant *L. perenne* ssp. *multiflorum*.

There were some significant differences among treatments concerning *L. perenne* ssp. *multiflorum* control in the interaction.glufosinate.4 study. Certain herbicide mixtures were more effective (Figure 4D). Tiafenacil at 25, 50, and 75 g ha⁻¹ and glufosinate exerted the lowest *L. perenne* ssp. *multiflorum* control. Control was improved by adding tiafenacil at 50 g ha⁻¹ or 75 g ha⁻¹ to glufosinate. The ground cover followed suit, with glufosinate-containing treatments providing the greatest reduction in ground

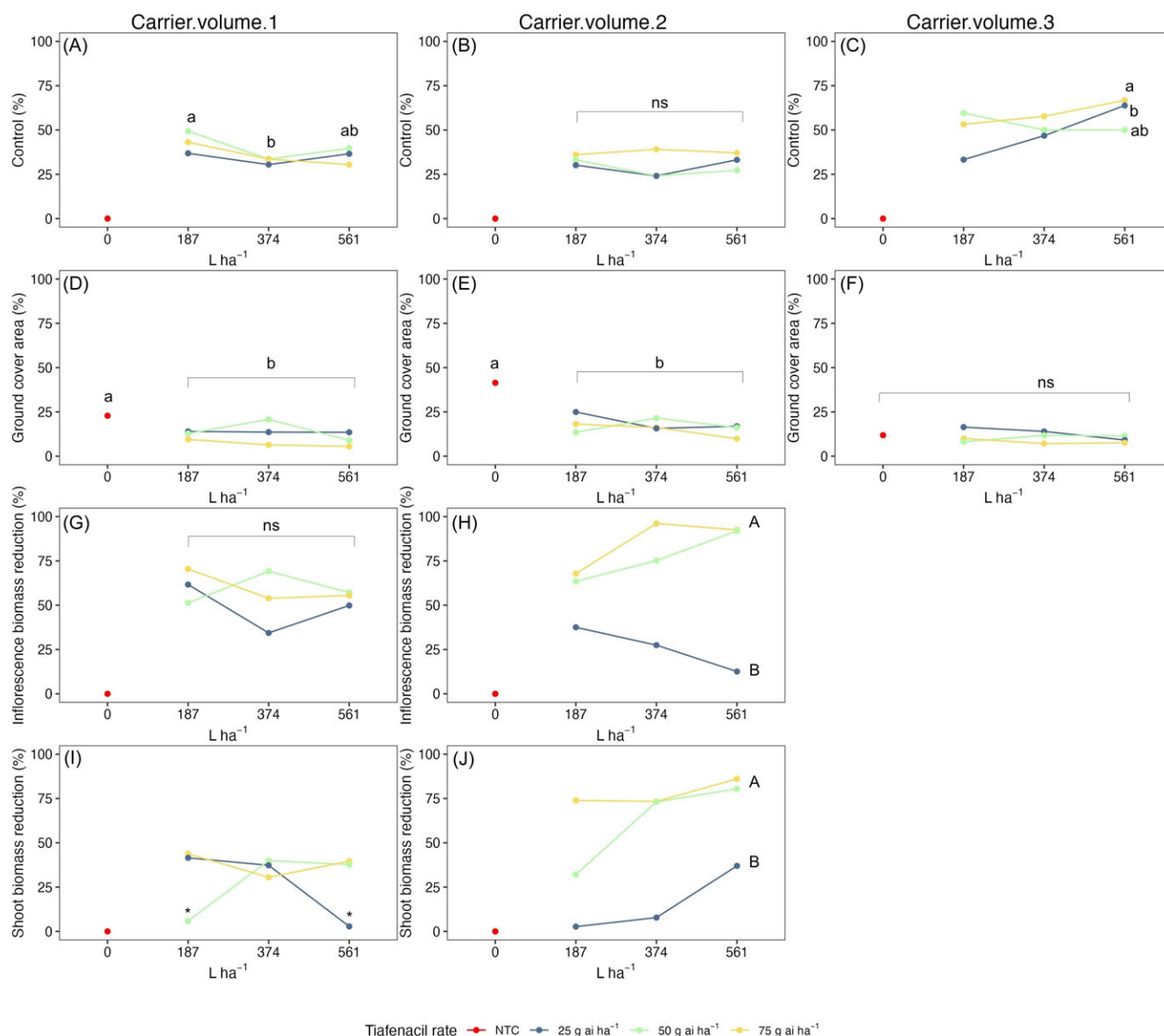


Figure 3. Evaluating the impact of carrier volume and tiafenacil rate on *Lolium perenne* ssp. *multiflorum*: effects on control efficacy, ground coverage, and biomass reduction of *L. perenne* ssp. *multiflorum* at 35 d after treatment. Abbreviations: ns, not significant; NTC, nontreated control. Data presented are means. Different lowercase letters within the same plot denote significant differences between carrier volumes ($P < 0.05$), while different uppercase letters indicate significant differences between tiafenacil rates ($P < 0.05$). An asterisk (*) in panel X represents significant difference ($P < 0.05$) between herbicide treatments. *Lolium perenne* ssp. *multiflorum* control was visually estimated on a scale from 0% to 100%, where 0% indicates no control and 100% complete control. Data for the NTC were omitted, as the control values were 0%. Ground coverage was determined using Canopeo software, which analyzed two random images per plot; lower percentages suggest more effective weed control. Biomass for shoots and inflorescences was separately collected from two 0.25-m² quadrats, with inflorescence biomass providing an indirect measure of potential seed production.

cover area among all herbicide treatments, indicating robust weed suppression (Figure 4I). However, there were no significant differences in shoot and inflorescence biomass reduction among treatments within the study's duration.

In the interaction.glyphosate.5 study, treatments containing glyphosate were most effective in controlling *L. perenne* ssp. *multiflorum*, regardless of the PPO-inhibiting herbicide (Figure 4E). This is pronounced in ground cover reduction, where glyphosate-containing treatments provided a stronger reduction (Figure 4J). Although there were no significant differences in shoot biomass reduction among treatments, those treatments containing glyphosate most greatly reduced inflorescence biomass (Figure 4T), with no additional effect observed by adding a PPO-inhibiting herbicide.

Across all study sites, glyphosate alone provided 54% control over *L. perenne* ssp. *multiflorum*, with an observed efficacy commensurate with the 57% reduction in inflorescence biomass (Table 4). As expected, carfentrazone provided no control. Tiafenacil showed increased control with higher rates, but this did not correspond to increased inflorescence biomass reduction. The narrative of combined treatments is more intricate. Glyphosate plus carfentrazone produced an additive interaction; the combination matched the expected control without exceeding it. This pattern was also reflected in biomass reduction. Glyphosate with tiafenacil at the lowest rate, 25 g ha⁻¹, maintained this additive trend, showing slightly greater control and inflorescence biomass reduction than expected outcomes, although the difference was not statistically significant. With increasing rates of tiafenacil, a synergistic shift was observed.

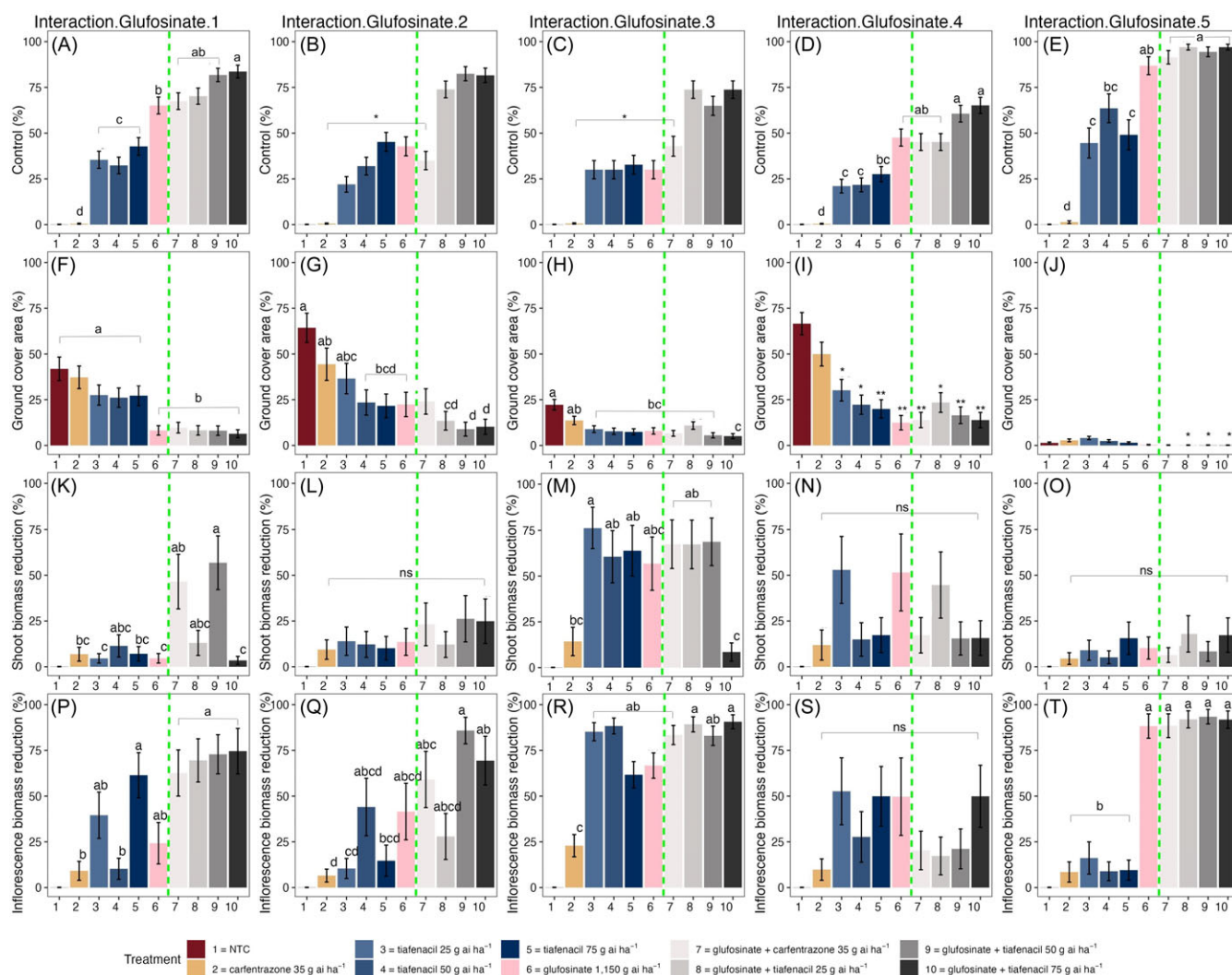


Figure 4. Interaction of glufosinate with protoporphyrin oxidase (PPO)-inhibiting herbicides on *Lolium perenne* ssp. *multiflorum* management: effects on control efficacy, ground coverage, and biomass reduction in shoots and inflorescences for *L. perenne* ssp. *multiflorum* at 35 d after treatment. Green dashed line separates the treatments that included herbicide mixtures, with herbicide mixtures being represented by gray to black colors. Abbreviations: ns, not significant; NTC, nontreated control. Data are shown as means ($\pm$ SEM); differences within the same plot are indicated by different letters, significant at $P < 0.05$. An asterisk (*) in I and J represents significant difference ($P < 0.05$) from the NTC, and double asterisks (**) denote significant differences from previous herbicide treatments. An asterisk (*) in B and C denotes significantly lower control than herbicide combinations. Control of *L. perenne* ssp. *multiflorum* was visually estimated from 0% to 100%, where 0% indicates no control and 100% complete control. Data for the NTC were omitted, as the control values were 0%. Ground coverage was analyzed using Canopeo software, assessing two random images per plot; lower percentages suggest more effective weed control. Biomass for shoots and inflorescences was collected separately from two 0.25-m² quadrats, with inflorescence biomass providing an indirect measure of potential seed production.

When combined with glufosinate at medium (50 g ha⁻¹) and higher (75 g ha⁻¹) rates, tiafenacil control of *L. perenne* ssp. *multiflorum* surpassed model predictions, indicating that the herbicides may be enhancing each other's efficacy. However, this synergistic effect did not translate to inflorescence biomass reduction, which continued additively.

Interaction of Tiafenacil with ACCase-inhibiting Herbicides

In the tia.ACCase.1 study, *L. perenne* ssp. *multiflorum* control was significantly greater, with combinations of ACCase inhibitors and tiafenacil (Figure 5A). Tiafenacil's control efficacy was comparable to that of clethodim and superior to that of fluzafop. Ground cover and shoot biomass reduction were not significantly different among treatments (Figure 5E and I). Similarly, there was no significant difference in inflorescence biomass reduction when

PPO inhibitors were combined with ACCase inhibitors (Figure 5L).

In the tia.ACCase.2 study, *L. perenne* ssp. *multiflorum* control improved significantly when ACCase inhibitors were combined with tiafenacil (Figure 5B). This combination significantly enhanced the reduction in ground cover, although adding tiafenacil to fluzafop did not yield improvement (Figure 5F). No significant differences were detected in shoot biomass reduction among herbicide treatments (Figure 5J). A similar trend was observed with inflorescence biomass reduction; no significant differences were found between ACCase inhibitors alone or in combination with PPO inhibitors (Figure 5M). The exception is the fluzafop-carfentrazone mixture, which exerted a lower reduction. The *L. perenne* ssp. *multiflorum* population at the site was suspected to be resistant to glyphosate and ALS inhibitors. While ACCase inhibitors remain a viable control option for this

Table 4. Observed and predicted means of *Lolium perenne* ssp. *multiflorum* control and inflorescence biomass reduction 35 d after treatment with glufosinate plus protoporphyrin oxidase (PPO)-inhibiting herbicides from study Interaction of Tiafenacil with Glufosinate Studies and averaged across all experimental sites.^a

Treatment ^b	Rate	Observed control ^c	Expected control ^d	P-value ^e	Interaction type ^f	Observed inflorescence biomass reduction ^g	Expected inflorescence biomass reduction ^d	P-value ^e	Interaction type ^f
	g ai ha ⁻¹	—	—	—	—	—	—	—	—
NTC	—	—	—	—	—	—	—	—	—
Gluf	1,150	54	—	—	—	57	—	—	—
Carf	35	1	—	—	—	15	—	—	—
Tiaf	25	30	—	—	—	49	—	—	—
Tiaf	50	35	—	—	—	39	—	—	—
Tiaf	75	40	—	—	—	49	—	—	—
Gluf + carf	1,150 + 35	57	54	0.574	Additive	68	59	0.349	Additive
Gluf + tiaf	1,150 + 25	72	67	0.669	Additive	65	71	0.817	Additive
Gluf + tiaf	1,150 + 50	78	69	0.692	Synergistic	72	68	0.601	Additive
Gluf + tiaf	1,150 + 75	80	71	0.706	Synergistic	83	87	0.395	Additive

^aAbbreviations: —, not applicable; NTC, nontreated control; gluf, glufosinate; carf, carfentrazone; tiaf, tiafenacil.

^bAll treatments included ammonium sulfate at 10 g L⁻¹ and methylated seed oil at 8.9 g L⁻¹, except the NTC.

^c*Lolium perenne* ssp. *multiflorum* control was visually estimated on a scale from 0% to 100%, where 0% represented no control and 100% represented complete control. Control data for the NTC were excluded from the analysis, as the values were 0%.

^dValues are the expected means calculated by Colby's equation.

^eP-value from paired t-test conducted on observed and expected values.

^fHerbicide combinations were classified as antagonistic if the observed value was significantly less than expected; additive if there was no significant difference between observed and expected values; and synergistic if the observed value significantly exceeded the expected value based on a paired t-test conducted on observed and expected values.

^gReduction in *L. perenne* ssp. *multiflorum* biomass was assessed relative to the NTC.

biotype, resistance to ACCase inhibitors is widespread. Resistance in *L. perenne* ssp. *multiflorum* to PPO inhibitors is unknown; the addition of tiafenacil to ACCase inhibitors may help delay the evolution of herbicide resistance.

In the tia.ACCase.3 study, the addition of PPO inhibitors to clethodim did not significantly influence *L. perenne* ssp. *multiflorum* control, but fluazifop did (Figure 5C). By adding 75 g ha⁻¹ tiafenacil to fluazifop, *L. perenne* ssp. *multiflorum* control was enhanced by 30%. Ground cover rates showed no significant differences among treatments (Figure 5G). *Lolium perenne* ssp. *multiflorum* biomass for this site was as previously stated.

In the tia.ACCase.4 study, *L. perenne* ssp. *multiflorum* control varied among treatments, with greater results provided by the addition of tiafenacil to ACCase-inhibiting herbicides compared with ACCase inhibitors alone (Figure 5D). Clethodim with tiafenacil and fluazifop with tiafenacil exerted the greatest *L. perenne* ssp. *multiflorum* control. These mixtures provided 15% to 33% greater control of *L. perenne* ssp. *multiflorum* than individual applications. Ground cover data reflected these findings (Figure 5H). There were no significant differences in shoot biomass reduction among the treatments; however, carfentrazone and tiafenacil alone resulted in the lowest reduction of inflorescence biomass (Figure 5K and 5N).

Across all study sites clethodim demonstrated strong stand-alone control (76%) against *L. perenne* ssp. *multiflorum* (Table 5). Tiafenacil exerted moderate control (49%), while fluazifop exerted slightly less control (46%). Clethodim and carfentrazone demonstrated an additive effect. Combined with carfentrazone, fluazifop created an antagonistic effect. The significant decrease in expected biomass reduction indicates an antagonistic response and highlights the importance of understanding herbicide mode of action interactions. By contrast, the combination of clethodim and tiafenacil produced a synergistic effect. This synergy, however, did not extend to biomass reduction, where an additive effect was found.

Finally, the combination of fluazifop and tiafenacil produced an additive outcome in both control and biomass reduction, suggesting a complementary interaction between the two herbicides.

The results showed inconsistencies between the variables assessed. Visual control, ground coverage, and biomass measurements play distinct but complementary roles in evaluating herbicide efficacy. Visual control focuses on the immediate appearance of the treated area as assessed by the naked eye, while ground cover reflects the area occupied by *L. perenne* ssp. *multiflorum*. However, high ground cover does not always indicate reproductive health. Shoot biomass indicates plant growth, with reductions suggesting effective stunting, but not reproductive potential. Inflorescence biomass, which quantifies the reproductive structures of weeds, is also critical for assessing long-term control strategies.

Synthesis of field study data revealed that tiafenacil's efficacy in managing *L. perenne* ssp. *multiflorum* is significantly enhanced when applied at higher rates and in combination with other herbicides. Despite its strengths, tiafenacil alone may not serve as a stand-alone management solution for *L. perenne* ssp. *multiflorum* (Soltani et al. 2021); even at its maximum label rate of 75 g ha⁻¹, it exerted <49% control across all experimental sites. Our findings, considered with those of Soltani et al. (2021) and Westerveld et al. (2021), emphasize the importance of integrating tiafenacil into herbicide combination programs instead of depending on it alone.

Results also revealed a complex relationship among tiafenacil rate, volumes of carriers used, and the varying effectiveness of these methods on *L. perenne* ssp. *multiflorum* control. Despite the varied response of *L. perenne* ssp. *multiflorum* across different sites, a consistent conclusion emerges: the rate of tiafenacil plays a pivotal role in its overall effectiveness, more so than the carrier volume. Carrier volume, dictating herbicide coverage and droplet density (Butts et al. 2018; Creech et al. 2015), has shown a complex and site-dependent impact on the effectiveness of tiafenacil, hinting at the influence of local environmental conditions and the *L. perenne*

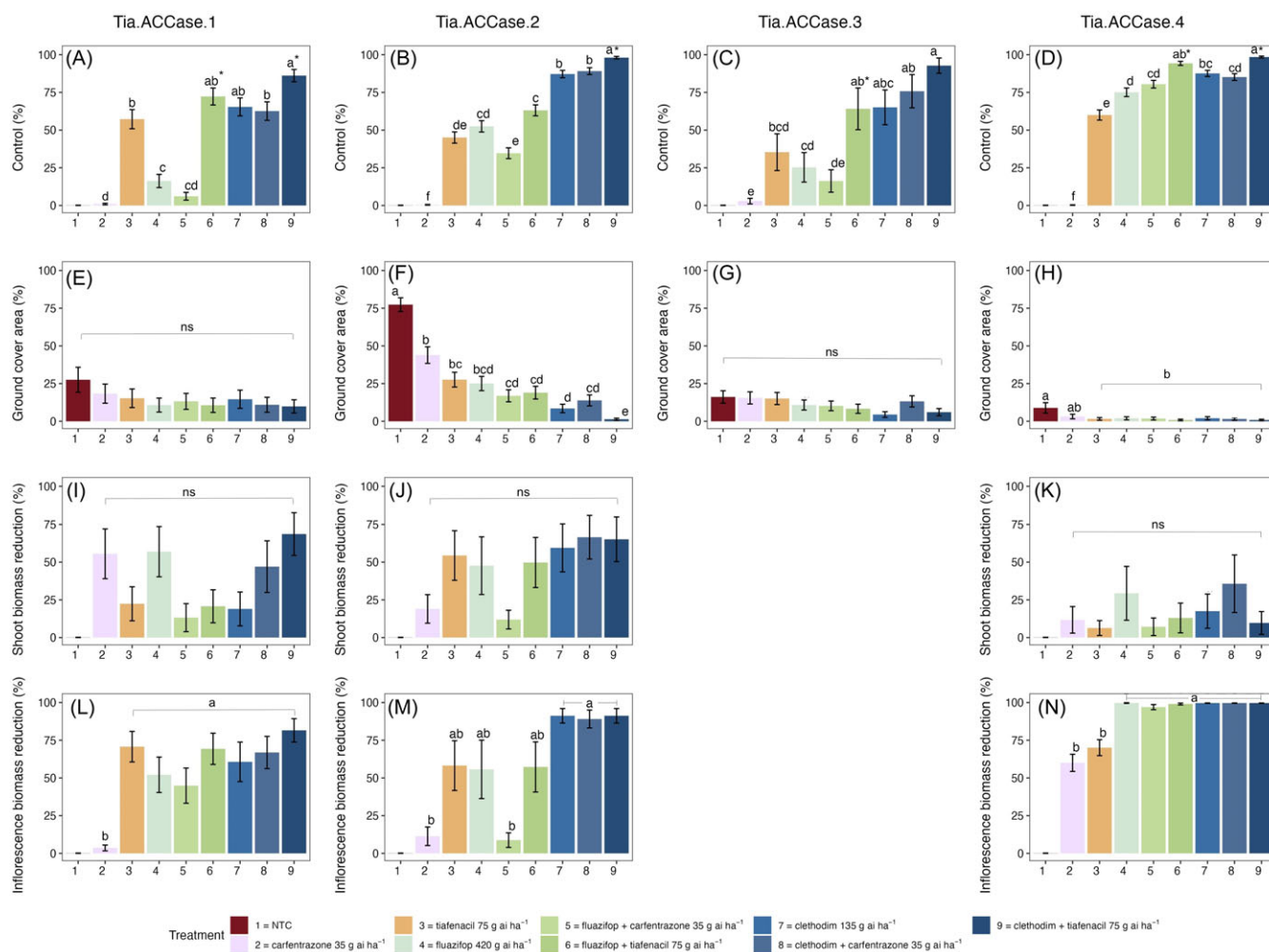


Figure 5. Interaction of acetyl-CoA carboxylase (ACCase)-inhibiting and protoporphyrin oxidase (PPO)-inhibiting herbicides on *Lolium perenne* ssp. *multiflorum* management: effects on control efficacy, ground coverage, and biomass reduction in shoots and inflorescences for *L. perenne* ssp. *multiflorum* at 35 d after treatment. Abbreviations: ns, not significant; NTC, nontreated control. Results are presented as means ($\pm$ SEM); significant differences within the same plot are denoted by different letters at $P < 0.05$. An asterisk (*) in A–D indicates results that are significantly greater ($P < 0.05$) than those achieved with the herbicides used individually. Control of *L. perenne* ssp. *multiflorum* was visually estimated from 0% to 100%, where 0% indicates no control and 100% complete control). Data for the NTC were omitted, as the control values were 0%. Ground coverage was analyzed using Canopeo software, assessing two random images per plot; lower percentages suggest more effective weed control. Biomass for shoots and inflorescences was collected separately from two 0.25-m² quadrats, with inflorescence biomass providing an indirect measure of potential seed production.

ssp. *multiflorum*'s population characteristics and physiological stage. Our results generally favor a carrier volume of 187 L ha⁻¹; higher volumes would require more frequent tank fills, increasing production costs.

The field studies exploring the interactions of tiafenacil with glufosinate reinforce the significance of integrating herbicide synergies into weed management strategies, particularly in managing herbicide-resistant weeds (Diggle et al. 2003; Ndikuryayo and Yang 2023). Previous findings by Dillitt et al. (2022) indicated limited to no benefit from adding sublethal rates of PPO-inhibiting herbicides to glufosinate to improve horseweed [*Conyza canadensis* (L.) Cronquist] control. In our research, combining tiafenacil, particularly at the highest labeled rate (75 g ha⁻¹), with glufosinate significantly enhanced the control of *L. perenne* ssp. *multiflorum* across multiple study sites where populations presented putative resistance to glyphosate, glufosinate, paraquat, ALS inhibitors, and ACCase inhibitors, highlighting the effectiveness of combining these mechanisms of action. These findings align with existing literature that emphasizes the benefits of herbicide synergy achieved by

combining these mechanisms of action for managing resistance and optimizing weed control (Takano et al. 2020). Incorporating such synergistic approaches into holistic integrated weed management (IWM) systems holds promise for enhancing the sustainability and effectiveness of weed management practices. However, the variability observed across sites underscores the need for tailored, site-specific weed management strategies to maximize efficacy and address localized challenges in herbicide-resistance management, representing a crucial step toward developing more targeted and sustainable weed control solutions in diverse agricultural contexts (Beckie and Harker 2017; Fernández-Quintanilla et al. 2018; Gerhards et al. 2022).

The field studies exploring the interactions between tiafenacil and ACCase-inhibiting herbicides demonstrated enhanced efficacy of herbicide combinations compared with individual applications and highlighted the synergistic effects of tiafenacil–clethodim and tiafenacil–fluzafop combinations for *L. perenne* ssp. *multiflorum* control. We found an antagonistic effect when combining carfentrazone with clethodim and fluzafop, consistent with previous research demonstrating that ACCase inhibitors

Table 5. Observed and predicted means for *Lolium perenne* ssp. *multiflorum* control and inflorescence biomass reduction 35 d after treatment for acetyl-CoA carboxylase (ACCase)-inhibiting herbicides plus protoporphyrin oxidase (PPO)-inhibiting herbicides from study interaction of Tiafenacil with ACCase-inhibiting Herbicides and averaged across all experimental sites.^a

Treatment ^b	Rate g ai ha ⁻¹	Observed control ^c	Expected control ^d	P-value ^e	Interaction type ^f	Observed inflorescence biomass reduction ^g	Expected inflorescence biomass reduction ^d	P-value ^e	Interaction type ^f
NTC	—	—	—	—	—	—	—	—	—
Cleth	135	76	—	—	—	89	—	—	—
Fluazi	420	46	—	—	—	70	—	—	—
Carf	35	1	—	—	—	36	—	—	—
Tiaf	75	49	—	—	—	76	—	—	—
Cleth + carf	135 + 35	78	76	0.652	Additive	90	90	0.639	Additive
Fluazi + carf	420 + 35	37	46	0.245	Additive	43	81	0.05	Antagonistic
Cleth + tiaf	135 + 75	95	87	<0.001	Synergistic	97	96	0.689	Additive
Fluazi + tiaf	420 + 75	76	71	0.268	Additive	83	90	0.078	Additive

^aAbbreviations: —, not applicable; NTC, nontreated control; cleth, clethodim; fluazi, fluazifop; carf, carfentrazone; tiaf, tiafenacil.
^bAll treatments included ammonium sulfate at 10 g L⁻¹ and methylated seed oil at 8.9 g L⁻¹, except for the NTC.
^c*Lolium perenne* ssp. *multiflorum* control was visually estimated on a scale from 0% to 100%, where 0% represented no control and 100% represented complete control. Control data for the NTC were excluded from the analysis, as the values were 0%.
^dReduction in *L. perenne* ssp. *multiflorum* biomass was assessed relative to the NTC.
^eValues are the expected means calculated by Colby's equation.
^fP-value from paired t-test conducted on observed and expected values.
^gHerbicide combinations were classified as antagonistic if the observed value was significantly less than the expected; additive if there was no significant difference between observed and expected values; and synergistic if the observed value significantly exceeded the expected value based on a paired t-test conducted on observed and expected values.

exhibit antagonistic effects when combined with broadleaf herbicides like PPO inhibitors (Lancaster et al. 2019; Zhang et al. 2005). The synergy found here improved control and underscores the potential for strategic herbicide mixing to overcome overreliance on single modes of action and effectively manage herbicide-resistant weeds (Busi and Beckie 2021; Diggle et al. 2003). To our knowledge, this is the first report of synergistic effects gained by combining PPO-inhibiting herbicides with ACCase-inhibiting herbicides for any weed anywhere.

Recent studies documented the superiority of herbicide combinations for managing herbicide resistance compared with herbicide rotations. Research investigating the evolution of herbicide resistance in barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] by Rigon et al. (2023) demonstrated that a fenoxaprop-*p*-ethyl and imazethapyr mixture at sublethal doses led to reduced control efficacy in subsequent generations, indicating an increased potential for resistance evolution. This underscores the importance of applying fully recommended herbicide rates in mixtures to mitigate the risk of resistance evolution. Studies by Busi and Beckie (2021) on rigid ryegrass (*Lolium rigidum* Gaudin) populations highlight the effectiveness of herbicide mixtures in delaying and mitigating herbicide resistance compared with stand-alone herbicides. They observed lower resistance frequencies and enhanced control efficacies of herbicide mixtures, demonstrating the potential of herbicide mixtures to mitigate resistance evolution.

In conclusion, this research across greenhouse and field environments revealed the nuanced efficacy of tiafenacil and its interactions with glufosinate and ACCase-inhibiting herbicides in controlling *L. perenne* ssp. *multiflorum*. The greenhouse results clearly illustrated a growth stage-dependent response to tiafenacil, with early application stages requiring significantly lower rates for effective control compared with later stages. Thus, the timing of tiafenacil application is critical, with early treatments potentially offering more efficient control and resource use. Field studies further emphasized the superiority of tiafenacil application in mixture with other herbicides, particularly glufosinate and ACCase inhibitors, over tiafenacil alone. These combinations enhanced *L. perenne* ssp. *multiflorum* control and highlighted the potential for synergistic effects to mitigate the risk of herbicide resistance. The specific characteristics of each *L. perenne* ssp. *multiflorum* population, along with the herbicide application strategies employed, significantly impact overall weed management. Nonetheless, tiafenacil mixtures controlled *L. perenne* ssp. *multiflorum* across multiple study sites where populations presented suspected resistance to glyphosate, glufosinate, paraquat, ALS inhibitors, and ACCase inhibitors. These combinations should be considered key components of any IWM strategy, tailored to specific environmental conditions and weed species. Further research should explore the underlying mechanisms of these herbicidal interactions to refine and optimize sustainable weed management strategies, ensuring effective control and the longevity of agricultural productivity.

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