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2,4-D antagonizes glyphosate in glyphosate-resistant barnyard grass *Echinochloa colona*

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Glyphosate is often tank-mixed with auxinic herbicide 2,4-D for grass and broadleaf weed control. Here we examined the possible interaction of 2,4-D and glyphosate in barnyard grass, *Echinochloa colona* (L.) Link. The results showed that 2,4-D antagonizes glyphosate remarkably in glyphosate-resistant populations but only marginally in susceptible populations. This antagonism is related to reduced glyphosate uptake and (to a lesser extent) translocation. As 2,4-D has multiple, unpredictable effects on other herbicides, care must be taken when tank-mixing herbicides with 2,4-D.

Keywords: antagonism, glyphosate resistance, tank mixing, uptake and translocation.

Electronic supplementary material: The online version of this article contains supplementary material (Supplemental Fig. S1), which is available at <http://www.jstage.jst.go.jp/browse/jpestics/>.

Introduction

Herbicide tank-mixing is a common practice for weed control in many situations.^{1,2)} Herbicide tank mixtures can broaden the range of weed species targeted, thus reducing weed-control costs, and have been recommended as components for best management practices to delay herbicide-resistance evolution.^{2–4)} However, synergism or antagonism can occur with certain herbicide mixtures.^{2,5,6)} Herbicide mixture synergism or antagonism can be due to altered herbicide uptake and/or translocation^{5,7–17)} and metabolism.^{5,18–20)} Both synergistic and

antagonistic effects of 2,4-D (2,4-dichlorophenoxyacetic acid) and glyphosate [N-(phosphonomethyl) glycine] have been reported and are weed species specific. For example, 2,4-D showed synergy with glyphosate for the control of bindweed (*Convolvulus arvensis*) due to greater glyphosate translocation to the roots.⁷⁾ Such 2,4-D/glyphosate synergism was also reported in poison ivy [*Toxicodendron radicans* (L.) Kuntze], although its mechanism is unknown.²¹⁾ Recently, 2,4-D choline/glyphosate synergism was patented to control many weed species.²²⁾ In contrast, 2,4-D antagonism of glyphosate was found in Johnson grass (*Sorghum halepense*) due to reduced glyphosate uptake and translocation^{8,16)} and in the Micro Tom tomato due to reduced glyphosate translocation.¹⁶⁾

As glyphosate is often tank-mixed with other herbicides (such as 2,4-D), this 2,4-D/glyphosate interaction was investigated in glyphosate-resistant and -susceptible barnyard grass [*Echinochloa colona* (L.) Link], and the underlying mechanism was examined.

Materials and Methods

1. Plant materials

A glyphosate-susceptible (S) population²³⁾ and two glyphosate-resistant (R) barnyard grass populations were used in this study. One R population originated from Queensland, with 2-fold low-level resistance endowed by the EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) target-site mutation of the Pro-106-Leu (P106L),²³⁾ and the other R population (WAE4)²⁴⁾ from Western Australia with 8-fold moderate resistance mainly due to nontarget-site resistance mechanisms.^{25,26)}

2. Herbicide treatment

Seeds of the R and S populations were germinated on wet filter paper at 30°C for 4 days, and germinating seeds were transferred to plastic trays containing a potting mix of 50% composted fine pine bark, 30% coco peat, and 20% river sand. Trays were placed in a greenhouse with average day/night temperatures of 33/25°C. When seedlings reached the 3-leaf stage, they were treated with the potassium salt of glyphosate (Roundup Powermax, Monsanto) alone, 2,4-D alone in the form of 2-ethyl hexyl ester or dimethylamine and monomethylamine salts (Estericide Xtra 680 or Amicide Advance 700, Nufarm), or a tank mixture of glyphosate and 2,4-D. The tank mixture of glyphosate and 2,4-D was prepared by dissolving 2,4-D first in a small amount of water and then adding glyphosate to the required volume, and the surfactant, BS1000 (0.2% v/v), was added last. No precipitation or formation of foam or gel was observed in the mixture during preparation or afterward. Each treatment had three replicate trays, and each tray contained 30–50 plants. The herbicides were applied using a cabinet sprayer with a spray volume of 118 L ha⁻¹ at a pressure of 200 kPa and a speed of 1 m s⁻¹.

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Plant mortality was determined 3 weeks after treatment. Plants that produced no new growth after treatment were recorded as dead.

3. Glyphosate uptake and translocation

The 2-leaf stage R seedlings (P106L) were transplanted into plastic cups (60×60×100 mm, one seedling per cup) filled with potting mixture and grown in a controlled-environment room with 35/25°C day/night temperatures, a 12/12 hr day/night photoperiod, 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, and 75% relative humidity. When the seedlings reached the 3-leaf stage, a single droplet (1 μL) of ^{14}C -glyphosate treatment solution (0.37 kBq μL^{-1}) with or without the addition of 700 g ai ha $^{-1}$ 2,4-D amine was placed onto the midpoint of the second fully expanded leaf. Glyphosate was ^{14}C -labeled in the phosphonoethylene with a specific radioactivity of 55.18 mCi mmol $^{-1}$ (PerkinElmer, Inc.). The ^{14}C -glyphosate was diluted in a commercial glyphosate formulation (Roundup Powermax) to yield a final glyphosate concentration of 22 mM, equivalent to the field rate of 540 g ai ha $^{-1}$.

Plants were harvested 24, 48, and 72 hr after treatment, and each harvest contained 6 plants. The treated leaf surface of each plant was rinsed with 10 mL of washing buffer containing 20% (v/v) methanol and 0.2% (v/v) Triton X-100 to remove unabsorbed ^{14}C -glyphosate. The radioactivity in the leaf wash was quantified using a liquid scintillation counter (LSC) (Packard 1500, Tri-carb $^{\text{®}}$, PerkinElmer). Similarly, the roots of 6 treated plants were rinsed with 50 mL of washing buffer, and the radioactivity in the root wash was quantified. The plants were then blotted dry with paper towels, pressed, and oven dried for 2 days at 60°C. Translocation of ^{14}C -glyphosate within each plant was visualized using phosphor imaging (Personal Molecular Imager $^{\text{TM}}$, Bio-Rad). After imaging, each plant was sectioned into three parts: the treated leaf, the untreated leaf and shoot, and the root. These plant sections were combusted in a biological oxidizer (RJ Harvey Instrument Corporation), and the radioactivity in each section was quantified using an LSC. The average recovery of applied ^{14}C -glyphosate (from the leaf and root wash and combustion) across the three time points in the R and S samples was 86±1.0%. The glyphosate leaf uptake was expressed

as a percentage of the total amount recovered and translocation as a percentage of the total amount absorbed.

4. Statistics

The significant difference in glyphosate dose response, uptake, and translocation between glyphosate treatment alone and treatment with glyphosate plus 2,4-D was determined by *t*-test ($\alpha=0.05$) using Prism $^{\text{®}}$ software (version 5.0, GraphPad Software, Inc.). Herbicide dose experiments were repeated at slightly different glyphosate rates with similar results, and therefore, only one representative experiment was presented.

Results

1. 2,4-D antagonizes glyphosate control of glyphosate-resistant barnyard grass populations

The field-recommended 2,4-D rate of 700 g ha $^{-1}$ was used alone or in a tank mix with glyphosate. As expected, glyphosate-susceptible and -resistant plants were unaffected by 2,4-D. For glyphosate, the S plants were controlled at half (270 g ha $^{-1}$) of the recommended (540 g ha $^{-1}$) glyphosate rate, although there were signs of antagonism in the presence of 2,4-D amine at this low glyphosate rate (Table 1). However, for the glyphosate-resistant plants, 2,4-D remarkably antagonized glyphosate efficacy in both the target and nontarget site-based glyphosate-resistant populations (Table 1; Fig. 1). For example, target site-based R plants (P106L) were controlled by glyphosate alone at the recommended (540 g ha $^{-1}$) rate, whereas up to 90% of plants survived at this rate when glyphosate was tank-mixed with 2,4-D amine or ester. Similarly, this antagonistic effect of 2,4-D on glyphosate was evident for the nontarget site-based glyphosate-resistant plants (Table 1; Fig. 1). These nontarget site-resistant plants were controlled at the higher glyphosate rate of 1620 g ha $^{-1}$. However, in the presence of 2,4-D amine, 77% of plants survived at this rate, albeit with some degree of injury, and even 4 times the recommended glyphosate rate (2160 g ha $^{-1}$) failed to control them. Therefore, at least for these biotypes, it is clear that 2,4-D is antagonistic to a low degree toward glyphosate in glyphosate-susceptible barnyard grass at the lethal glyphosate doses tested, but 2,4-D is highly antagonistic to

Table 1. Survival (%) of glyphosate-resistant (R: P106L and WAEC4) and susceptible (S) barnyard grass populations following glyphosate treatment with (+) or without (–) tank mix with 2,4-D amine or ester. The plants were treated at the 3-leaf stage and survival determined 3 weeks after treatment

Treatment Glyphosate (g ha $^{-1}$)	S population		P106L population				WAEC4 population	
	2,4-D amine		2,4-D amine		2,4-D ester		2,4-D amine	
	–	+	–	+	–	+	–	+
0	100 (0) a	100 (0) a	100 (0) a	100 (0) a	100 (0) a	100 (0) a	100 (0) a	100 (0) a
270	0 (0) b	11 (1.2) a	81 (2.7) b	100 (0) a	75 (1.7) b	100 (0) a	–	–
540	–	–	0 (0) b	90 (1.5) a	0 (0) b	85 (1.4) a	–	–
1080	–	–	–	–	–	–	17 (2.0) b	90 (0.9) a
1620	–	–	–	–	–	–	0 (b)	77 (1.7) a
2160	–	–	–	–	–	–	0 (b)	34 (2.0) a

Each value is the mean (S.E.) ($n=3$). Means with different letters in a row for each population are significantly different (*t*-test, $p<0.05$).

Fig. 1. Reduced glyphosate control of glyphosate-resistant barnyard grass populations P106L (a–d) and WAEC4 (e and f) by tank-mixing glyphosate with 2,4-D ester (a and b) or amine (c–f) 3 weeks after treatment.

glyphosate in glyphosate-resistant barnyard grass populations.

2. 2,4-D reduces glyphosate uptake and translocation

To examine the basis of 2,4-D antagonism toward glyphosate, plants with the target site-based glyphosate R (P106L) population were used for ¹⁴C-glyphosate uptake and translocation experiments. Glyphosate leaf uptake reached up to 80% within 24 hr without further increase over 48 to 72 hr, and 45% glyphosate was translocated to untreated leaves and the stem (Table 2;

Supplemental Fig. S1). However, when applied with 2,4-D, glyphosate uptake was significantly reduced, by up to 20%, in these P106L plants (Table 2; Supplemental Fig. S1). By 48 and 72 hr after treatment, glyphosate translocation to untreated leaves and the stem was reduced by up to 12% and was significantly lower in the presence of 2,4-D than in the absence of 2,4-D (Table 2; Supplemental Fig. S1). Therefore, it is likely that 2,4-D antagonizes glyphosate in glyphosate-resistant barnyard grass at least in part due to reduced glyphosate uptake and translocation.

Table 2. Absorption and translocation of [¹⁴C]-glyphosate applied with or without 2,4-D to a single leaf of the glyphosate-resistant barnyard grass population (P106L), 24, 48 and 72 hr after treatment

Treatment	Treatment duration (hr)	Absorption (% recovered)	Translocation (% absorbed)		
			Root	Untreated leaf plus stem	Treated leaf
Glyphosate alone	24	80.4 (4.7) a	22.2 (1.7) a	45.1 (3.3) a	32.7 (3.8) a
2,4-D+glyphosate		60.9 (5.5) b	25.8 (2.1) a	39.9 (1.4) a	34.3 (1.7) a
Glyphosate alone	48	72.5 (3.1) a	24.4 (1.4) a	49.8 (1.4) a	25.8 (0.7) b
2,4-D+glyphosate		60.1 (2.5) b	28.2 (1.9) a	37.9 (1.1) b	33.9 (2.5) a
Glyphosate alone	72	78.5 (2.7) a	22.8 (1.1) a	45.6 (1.3) a	31.6 (1.4) a
2,4-D+glyphosate		58.1 (3.3) b	30.3 (3.6) a	38.2 (2.0) b	31.5 (2.1) a

Each value is the mean (S.E.) ($n=3$). Means with different letters in a column for each time point are significantly different (t -test, $p<0.05$).

Discussion

Glyphosate controls a wide range of plant species, including grassweeds, whereas 2,4-D is effective on dicot species. Tank-mixing glyphosate with 2,4-D is a common practice to achieve excellent control of both grasses and dicots. However, 2,4-D antagonism of glyphosate was reported in some weed species, such as Johnson grass,⁸⁾ wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and wild oats (*Avena fatua* L.).²⁷⁾ In this study, 2,4-D/glyphosate antagonism was confirmed in barnyard grass. A small antagonistic effect was found in susceptible populations, and a remarkable effect in glyphosate-resistant populations, at the lethal rates used. For instance, 270 g ha⁻¹ glyphosate achieved ≥88% control of susceptible plants, with or without 2,4-D (Table 1). However, the two glyphosate-resistant populations, P106L and WAEC4, were much less affected by their respective lethal glyphosate doses if co-treated with 2,4-D (Table 1). This indicates that the 2,4-D antagonism is dependent on the plant's intrinsic sensitivity to glyphosate. As there are now many glyphosate-resistant barnyard grass populations,²³⁾ as well as glyphosate-resistant grass weed species,²⁸⁾ care should be taken with 2,4-D/glyphosate mixtures.

Decreased glyphosate uptake and translocation associated with 2,4-D/glyphosate antagonism was reported in Johnson grass and tomatoes.^{8,16)} In addition, reduced glyphosate translocation by the auxin herbicide dicamba was recently reported in *Kochia scoparia*.¹⁷⁾ Here, a similar mechanism was observed in glyphosate-resistant barnyard grass (Table 2; Supplemental Fig. S1). In our study, the up to 20% reduction of glyphosate uptake is modest; however, this plus a further reduction (up to 12%) in translocation may account for the antagonism. Nevertheless, other unidentified mechanisms may also be possible. The mechanisms of reduced glyphosate uptake and translocation by 2,4-D are not clear but may be due to the chemical complex through the cation exchange²⁹⁾ and the physical or physiological interactions.^{8,16,29,30)}

The chemical forms of 2,4-D and glyphosate were reported to have an impact on the interactions. For instance, previous research indicated that 2,4-D in both butoxyethyl ester and diethanolamine formulations conferred antagonism against glyphosate isopropylamine salt in wheat but no such antagonism with the isopropylamine formulation of 2,4-D.²⁹⁾ Moreover, isooctyl ester and dimethylamine formulations of 2,4-D antagonized the efficiency of glyphosate isopropylamine salt in barley.³⁰⁾ In our current study, interactions between the 2-ethyl hexyl ester or dimethylamine plus monomethylamine formulation of 2,4-D and glyphosate potassium salt were examined in barnyard grass, and it was found that both the ester and amine forms of 2,4-D were antagonistic to glyphosate potassium salt (Table 1; Fig. 1). Additionally, previous research showed that nonionic surfactants can enhance glyphosate phytotoxicity,^{29,31)} but without any effect on 2,4-D/glyphosate antagonism.²⁹⁾ Whether the nonionic surfactant BS1000, which was used in our study, has an impact on 2,4-D/glyphosate antagonism needs evaluation.

In conclusion, 2,4-D (amine/ester) may compromise glyphosate (potassium salt) efficacy in controlling barnyard grass, depending on the plant's intrinsic glyphosate sensitivity. 2,4-D has also been reported to antagonize other herbicides, such as ACCase-inhibiting herbicides in wild oats,^{5,18,32,33)} *Lolium rigidum*,^{19,20)} and other weed species.³⁴⁾ Therefore, tank-mixing 2,4-D with other herbicides should be exercised with caution.

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