Original Article

Synergistic effects of adjuvant A-134 on the herbicidal effects of glyphosate

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Undesirable side effects on ecosystems and strong selection for weed resistance demand an increase in the efficacy and a reduction in the dosage of glyphosate herbicide used. The synergistic effect of tank-mixed adjuvant KAO® A-134 (A-134) on the post-emergence activity of the commercial glyphosate formulation Roundup® (RDP) against crabgrass (*Digitaria sanguinalis*) was detected. Field study also showed that A-134 can increase the herbicidal effect of RDP. Meanwhile, A-134 concentration-dependently decreased the surface tension and increased the spreading area of RDP, causing faster penetration and improved uptake of glyphosate into crabgrass. Moreover, the tank mix with A-134 also increased the adhesion of spray droplets of glyphosate isopropylamine salt (GP) to the leaf surface after rainfall treatment, thus maintaining its herbicidal effect. Data suggested the necessity of using these synergistic properties of A-134 to reduce environmental exposure and glyphosate resistance selection.

Keywords: adjuvant A-134, synergistic effect, glyphosate, crabgrass, rain fastness.

Introduction

Herbicides currently provide not only a simple and cost-effective way of managing weeds but also a positive means of freeing up labor and improving crop quality.¹⁾ Since the use of pesticides in agriculture inevitably leads to the exposure of humans and other non-targeted organisms, undesirable side effects on communities or on ecosystems as a whole make it essential to reduce the dosage used and increase the efficacy and long-term control of herbicides.^{2,3)}

Herbicides must overcome a variety of barriers to their entry into plants in order to be effective. The addition of adjuvants to a herbicide formulation or tank mix can promote more even coverage of plant surfaces by the herbicide and increase the formulation's penetration through the cuticular wax, cell walls, and/or stomatal openings.^{4,5)} Organosilicone surfactants can reduce the surface tension of the spray solution, promote the infiltration of the herbicide into stomata, and increase the rate of droplet spread over the leaf surface.^{6,7)} The addition of the adjuvants At-

erbane at 0.5 and 0.25% v/v and Silwet L 77 at 0.1 and 0.05% v/v can increase the absorption of glyphosate and reduce the dose of glyphosate by more than 50% as compared with glyphosate used without adjuvants.⁸⁾ The previous experiments have already showed that surfactants that produce smaller spreading areas on plant foliage usually have a limited effect on enhancing the permeability of the cuticle or cell membrane.⁹⁾ In the wake of the potential toxicity of POEA (polyethoxylated tallow amine) in Roundup® (RDP), the most widely used herbicide worldwide, to humans, animals, and the environment,¹⁰⁾ a proper, health-oriented regulatory system has garnered increasing attention regarding the synergistic effects of herbicide mixtures.

Glyphosate is a systemic hydrophilic herbicide that has non-selective, broad-spectrum effects on many annual grasses and broadleaf weeds post emergently.¹¹⁾ Glyphosate-based herbicides in different formulations are the most widely used across the world, and this widespread use of glyphosate greatly increases the chances that it will be ingested by humans and farm animals.⁹⁾ Improved foliar uptake may reduce the usage dose and increase the efficacy of glyphosate. KAO A-134 (A-134) is an environmental friendly new botanical adjuvant innovated by Japan's Kao Corporation. Experiments in the present study were conducted to: a) identify the influence of A-134 on the surface tension and spreading area; b) compare the post-emergence activity with A-134; c) determine rain-leaching tolerance in the presence of A-134.

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Materials and Methods

1. Chemicals

KAO[®] adjuvant A-134 was commercially provided by Eco-Innovation Research, Kao Corporation, Japan. Forty-one per cent glyphosate isopropylamine salt AS (Roundup[®], Abs. RDP) purchased from Monsanto Company (St. Louis, MO, USA) and glyphosate isopropylamine salt (GP, $\geq 95\%$) obtained from Hanfu Biochemical Pharmaceutical Co., Ltd. (Weihai, China) were used in experiments.

2. Plants

Crabgrass (*Digitaria sanguinalis* L.) and barnyard grass (*Echinochloa crusgalli* L.) plants were grown from seed in a controlled environment ($25/16^{\circ}\text{C} \pm 2^{\circ}\text{C}$ day/night temperature and $70 \pm 5\%$ relative humidity). Experiments were performed when the crabgrass or barnyard grass reached the 3–3.5 leaf growth stage.

3. Assays of post-emergence activity in the greenhouse

The effects of RDP applied post emergently were tested on crabgrass at the 3–3.5 leaf growth stage in according with the reported assays. RDP was diluted with distilled water and applied to the foliage of the seedlings at concentrations of 100, 200, 300, 400, 500, or 600 g a.i./ha. In a parallel test, adjuvant A-134 was tank mixed with each dilution of RDP, and the concentration of A-134 in the mixture was set at 0.025%, 0.05%, 0.1%, or 0.2%. The tank mix was applied as described above. The diluted RDP and the mixtures were applied using a laboratory sprayer with a flat fan nozzle at a water volume of 450 L/ha. Treatments were replicated four times and arranged in completely random blocks within the greenhouse. Two weeks after treatment, the shoot portion of the crabgrass was collected. The efficacies of the diluted RDP and the mixtures were measured from the fresh weight of the shoot portions of the crabgrass. The % inhibition was then calculated using formula (1): $[1 - (\text{fresh weight in the treatment} / \text{fresh weight in the control})] \times 100$. The half efficacy concentration (EC_{50}) was calculated by probit analysis based on the regression percent inhibition and the log of test concentration of the diluted RDP and the mixtures. The relative toxic index was calculated using formula (2): $(\text{EC}_{50} \text{ of the diluted RDP} / \text{EC}_{50} \text{ of the mixture with A-134}) \times 100$.

4. Field study

Field studies were conducted in 2017 at the National South Pesticide Discovery Center (Shanghai) on an indigenous weed population. Every treatment involved plots (1.2 m long $\times$ 1.2 m wide). RDP was diluted with distilled water and applied to the foliage of the seedlings at concentrations of 500 and 700 g a.i./ha. In a parallel test, adjuvant A-134 was tank mixed with each dilution of RDP, and the concentration of A-134 in the mixture was set at 0.05%, 0.1%, or 0.2%. The tank mix was applied as described above. The same concentrations of RDP that contained no A-134 were used as the control. Diluted RDP and the mixtures were applied using a laboratory sprayer with a

flat fan nozzle at a water volume of 450 L/ha. The formulation was sprayed on June 14 and July 8, 2017 when the majority of weeds were 15–20 cm tall, and the weed density was 80–90%. Tractor-mounted boom sprayers applied the formulations at a speed of 4.8 km/hr.¹²⁾ Weed species present in 2017 were tendon grass (*Eleusine indica* L.), perennial ryegrass (*Lolium perenne* L.), and triangular grass (*Cyperus iria* L.). Visual mortality data were recorded periodically up to fourteen days after spraying on a 0–100 scale, where 0=no effect (healthy, green leaves), and 100=complete mortality (withered, yellow leaves).¹³⁾

5. Rainfastness test

The trials in the greenhouse were carried out on crabgrass and barnyard grass at 3–3.5 leaf growth stage in accordance with the previous method.¹⁴⁾ GP was diluted with distilled water and applied to the foliage at a concentration of 37.5, 50, or 75 g a.i./ha for crabgrass seedlings and 55.5, 75, and 111.0 g a.i./ha for barnyard grass seedlings. A tank mix of the diluted GP and adjuvant A-134 at a concentration of 0.1% or 0.2% was used as a standard treatment. The same amount of GP without A-134 was used as the control. After treatment for 1 hr, a rainfall simulation was made using a backpack CO₂ sprayer (30 psi) with a Lurmark nozzle. The applied water volume was 4.5 mm per hour, the application pressure was 0.2 MPa, and the total amount of water used during the experiment was 74 L/ha. Two weeks after treatment, the shoot portions of the grasses were weighed. The % inhibition of the fresh weight was then calculated using the above formula (1).

6. Determination of surface tension

The effect of adjuvant A-134 on the surface tension of RDP was examined using the previous method.¹⁵⁾ RDP at a concentration of 4000 mg/L was mixed with 0.05%, 0.1%, 0.2%, and 0.4% A-134 aqueous solution at a 1:1 ratio (v/v). The same volume of distilled water as for A-134 was used as the control. The surface tensions of the mixtures were determined by using a TX500C spinning drop interfacial tensiometer (USA KINO Industry Co., Boston, MA, USA). The temperature of the test mixture was set at $25 \pm 0.5^{\circ}\text{C}$. Each mixture was measured three times and the treatment replicated.

7. Determination of the spreading area

Droplets (5 μL) of RDP (4000 mg/L) mixed with 0.025, 0.05, 0.1, or 0.2% A-134 (1:1, v/v) were applied by a microsyringe to the wax surface. The same volume of distilled water as for A-134 was used as the control. After standing for 5 min, the diameter of the droplet spreading area was determined under a microscope. At least 10 replicates on separate droplets were used for each treatment.

8. Uptake assessment

Droplets (10 μL) of the mixture (1:1, v/v) of 4000 mg/L RDP and 0.1% A-134 were applied by a microsyringe to the central region of the adaxial surface of the fully expanded leaf on crab-

Table 1. Control efficacy (the % inhibition of the fresh weight) and median efficient concentration (EC_{50}) of RDP after mixed with water and 0.025, 0.05, 0.1, or 0.2% KAO® adjuvant A-134 against *Digitaria sanguinalis* in the greenhouse

	Conc. of A-134 (%)				
	Water	0.025	0.05	0.1	0.2
Dosage of Roundup® (g a.i./ha)					
100	−0.9±3.5 ^b	28.0±3.3a	26.3±2.9a	33.9±3.0a	42.9±3.4a
200	29.8±3.9c	36.8±3.6bc	42.8±3.1b	51.1±3.0b	62.6±5.8a
300	29.5±5.7c	50.6±4.9b	50.2±4.2b	67.8±2.5a	70.2±4.7a
400	71.7±1.2	69.9±1.9b	70.6±3.4b	70.4±1.7b	79.2±2.8a
500	79.4±2.2b	79.6±1.0b	83.2±1.6a	85.8±1.4a	83.7±1.7a
600	82.6±2.5a	83.5±2.9a	81.9±2.7a	83.3±2.7a	83.0±2.1a
ED_{50} (mg/L)	366.8a	233.5b	224.9bc	175.8c	175.8c
R.T.I. ^{a)}	100.0	157.1	163.1	208.7	260.5

^{a)} R.T.I. was the abbreviation of the relative toxic index. ^{b)} Means followed by the same letter in each column were not significantly different ($p < 0.05$, LSD test).

grass plants. The same volume of distilled water as for 0.1% A-134 was used as the control. Every treatment contained three replicate plants (one leaf per plant) and all plants were kept under constant growing conditions (20°C; 70% RH) for 10 min the uptake period. The treated leaves were excised and washed three times with 1 mL distilled water. The content of glyphosate in the washings was determined by high-performance liquid chromatography using methanol/water (PBS, PH2) (5:95) as the mobile phase, an Agilent ZORBAX SAX ion exchange column, 30°C column temperature, and 195 nm wavelength detection. Glyphosate uptake was calculated as the initial concentration minus the residual concentration in the water.

9. Data analysis

All data were presented as the mean ± S.E. and subjected to analysis of variance (ANOVA) using SPSS version 17.0. Mean separations among treatments were compared using the least significant difference (LSD) test. The level of significant difference (P) was set at 0.05.

Results

1. Bioefficacy study

1.1. Synergistic effect of A-134 on the post-emergence activity of RDP in the greenhouse

After spraying for 14 days, RDP at concentrations greater than 400 g a.i./ha killed crabgrass completely. With RDP treatments at concentrations lower than 300 g a.i./ha, A-134 strongly improved the control efficacy of RDP against crabgrass in a concentration-dependent manner (Table 1). The addition of A-134 also caused a corresponding decrease of the EC_{50} values of RDP, indicating that the increased toxicity of RDP was positively correlated to the concentration of A-134. The relative toxicity index showed that the toxicity of RDP was increased 2.6 times in the presence of 0.2% (v/v) A-134 as compared to the control.

1.2. Field study

The results of the first field experiment, on June 14, 2017, are shown in Figure 1a, and the results of the second field experi-

ment, on July 8, 2017, are shown in Figure 1b. The results of both experiments showed that glyphosate formulation activity varied significantly when mixed with A-134. In the first field experiment, under a condition with no rainfall, GP at concentrations of 500 and 700 g a.i./ha showed control efficacies of 47.3 and 72.3% against weeds, respectively. After tank mixing with

Fig. 1. Percent mortality on weeds. Weeds treated with RDP (tank-mixed with 0, 0.05, 0.1 and 0.2% (v/v) A-134, respectively) for 14 days in 2 concentration (500 and 700 g a.i./ha) on June 14, 2017 (a) and July 8, 2017 (b).

Fig. 2. Effect of rainfall on reduces the control efficacy of GP and GP+A-134 against *D. sanguinalis* (a) and *E. crusgalli* (b), respectively. Data were statistical values of 2 sets with 3 parallel replicates. Means followed by the same letter were not significantly different ($p < 0.05$, LSD test).

0.05%, 0.1%, and 0.2% (v/v) A-134, the control efficacies of 675 g a.i./ha GP increased to 62.3, 72.7, and 82.3%, respectively, and those of 700 g a.i./acre GP were enhanced to 90.3, 95.0 and 100.0%, respectively. In the second field experiment, under a condition with no rainfall, GP at concentrations of 500 and 700 g a.i./ha showed control efficacies of 56.3 and 66.3% against weeds, respectively. After tank mixing with 0.05%, 0.1%, and 0.2% (v/v) A-134, the control efficacies of 500 g a.i./ha GP were increased to 67.3, 73.3, and 87.0%, respectively, and those of 700 g a.i./acre GP were enhanced to 85.7, 91.7 and 100.0%, respectively.

1.3. Influence of A-134 on the rainfastness of GP

A tank mix with adjuvant A-134 enhanced the rain-scouring tolerance of GP for controlling crabgrass and barnyard grass. Rainfall could reduce the control efficacy of GP, and the waning reduction was positively dependent on the increasing concentration of A-134 (Figure 2). The presence of 0.2% (v/v) A-134 maintained the lowest reduction at about 14.1% on the control efficacy of 50 g a.i./ha GP against crabgrass (Figure 2a) and 11.5% on the control efficacy of 22.5 g a.i./ha GP against barnyard grass (Figure 2b) ($p < 0.05$).

2. Mechanism study

2.1. Influence of A-134 on the surface tension and spreading area of RDP

Adjuvant A-134 significantly decreased the surface tension and increased the spreading area of RDP in a concentration-dependent manner (Figure 3). After tank mixing with 0.2% (v/v) A-134, the surface tension of RDP at a concentration of 4000 mg/L was changed to approximately 75.42% of the control, and the spreading area of RDP was about 1.40 times greater than that of the control.

2.2. Influence of A-134 on glyphosate uptake

After mixing with A-134, the uptake of glyphosate through the surface of crabgrass leaves was significantly increased as compared to that of the control (Figure 4). After a drop of 10 μ L RDP mixed with 0.1% A-134 was placed on crabgrass leaves for 10 min, the remaining concentration of glyphosate on the crabgrass leaves was decreased from an initial 42.07 to 8.06%, while the control was decreased from an initial 40.28 to 20.74%. The rates of glyphosate uptake were increased 1.74 times by 0.1% (v/v) A-134 as compared with those of the control. Data suggested that tank mixing with A-134 caused faster penetration and improved uptake of glyphosate into the crabgrass as compared to those of RDP alone.

Discussion

Herbicides are almost always applied as formulated products.¹⁾ Optimized herbicide formulations should possess the preferred properties for enhancing dispersion in water, increasing coverage and absorption by plants, decreasing the time required to control weeds, and ensuring safety of herbicides to humans and animals.^{2,15)} The use of spray-tank adjuvants that increase droplet adhesion, retention, spreading, deposit formation, uptake, and translocation improves the efficacy of herbicides.^{4,9)} In order to improve the safe usage and efficiency of glyphosate, many innovative biogenic adjuvants that possess environmental compatibility, low aquatic toxicity, and easy biodegradation have been developed, such as alkyl polyglycosides, quaternary ammonium

Fig. 3. Surface tension and spread diameter of 4000 mg/L RDP after tank mixing with KAO® adjuvant A-134. Data were statistical values of 2 sets with 3 parallel replicates and expressed as means plus SE. Means followed by the same asterisk on the same panel were not significantly different ($p < 0.05$, LSD test).

Fig. 4. Adjuvant effect of 0.1% (v/v) A-134 on glyphosate uptake of 4000 mg/L RDP into the crabgrass leaves. The same volume of distilled water as for A-134 was used as the control. Data were statistical values of 2 sets with 3 parallel replicates and expressed as means plus SE. Means followed by the same asterisk on the same panel were not significantly different ($p < 0.05$, LSD test).

glycosides, esterified polyoxyethylene glycerols, citric acid alkylether esters, and methylated polyoxyethylene fatty acids.^{16–19)} These adjuvants have been expected to replace the currently widely used nonylphenol polyoxyethylene and fatty amine polyoxyethylene that harm the environment.¹⁰⁾ The present study explores KAO adjuvant A-134, a new alkyl ether obtained from coconut oil. As compared with other conventional organic adjuvants (HM 9110, HM 9120A, Agrimax 3, Agrimax 5, and X-77, all 5%) and alkylpolysaccharide adjuvants (LI 144, 2.5%; LI 700, 5%; ADE 702, 2.2%; ADE 804, 6.6%), the effective concentration of KAO A-134 is lower (the recommended amount is 0.1–0.2%).^{20,21)} A-134 significantly increased glyphosate activity through mechanisms such as a concentration-dependent decrease in the toxicity of RDP, a significant decrease in surface tension, and an increase in the spreading area.

The toxicity of glyphosate on mammals has been a concern worldwide.²²⁾ Glyphosate-based herbicides are toxic endocrine disruptors in human cell lines.²³⁾ Glyphosate induces the growth of human breast cancer cells *via* estrogen receptors, causes necrosis and apoptosis in mature rat testicular cells *in vitro*, and decreases testosterone at lower levels.^{24,25)} Moreover, long-term exposure to herbicides imposes a strong selection that enables weeds to produce resistance that must be minimized or it becomes a major limiting factor to food security in global agriculture.²⁶⁾ In the present study, A-134 significantly induced faster penetration and improved uptake of glyphosate into crabgrass. This property may reduce the dose of glyphosate and shorten the exposure time of glyphosate in the environment, humans, and other non-target organisms, even as it significantly increases the control efficacy of glyphosate.

Weather conditions, specifically precipitation before and after herbicide application, can significantly impact herbicide efficacy, making it necessary for the herbicide to penetrate weeds rapidly and providing suitable rainfastness for adequate weed control.²⁷⁾ Glyphosate herbicide lacks residual control, and, depending on the formulation, its efficacy can be reduced by precipitation after

application.²⁸⁾ Improved uptake and in-transport applications of glyphosate, however, are effective at controlling late-emerging weeds.²⁹⁾ In the present study, A-134 increased droplet adhesion and concentration-dependently prevented the decline of the control efficacy of GP against crabgrass and barnyard grass after rain-scouring treatment. Whether the application of A-134 is beneficial for glyphosate transformation in weeds is worthy of further investigation.

In conclusion, the control efficacy of glyphosate after tank mixing with KAO adjuvant A-134 was significantly improved against crabgrass and barnyard grass, and the same synergies are seen in the field study. This tank-mixing adjuvant also increases glyphosate rainfastness and reduces the dosage of glyphosate needed to achieve satisfactory efficacy, especially enhancing the application performance and decreasing the cost and environment selective pressure.

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