A natural adjuvant shows the ability to improve the effectiveness of glyphosate application

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Glyphosate is a common herbicide used worldwide, but its adjuvant has not been studied much. A new adjuvant A-178® based on the coconut shell extracts, has been developed for glyphosate (glyphosate isopropylamine salt: GP). The potency of the new adjuvant was compared with traditional adjuvant polyethoxylated tallow amine (POEA). Field study has shown that A-178® can improve the herbicidal effect of GP formulation, and, as compared with 41% GP mixed with 7% POEA (GPP), 41% GP mixed with 7% A-178® (recommended dose, GPA) is more effective for weed control. GPA improved herbicidal activity against GP alone by 79.27% and against GPP by 27.38% at 500 g a.i./ha. A-178® decreased the surface tension, increased the spreading area of GP, and improved the uptake of GP in cockspur (Echinochloa crus-galli L.). Our results indicated that the new adjuvant shows better ability to improve glyphosate efficacy than does POEA. © Pesticide Science Society of Japan

Keywords: adjuvant, coconut shell extracts, glyphosate herbicide, pesticide reduction, efficiency mechanism.

Introduction

Glyphosate-based herbicides are broad-spectrum, post-emergence herbicides and are utilized both in agricultural and non-agricultural areas for perennial and annual weed control.1,2) Glyphosate inhibits a step in the biosynthetic pathway from shikimate to chorismate.3) Due to its susceptibility to microbial degradation,1) low persistence,4) high efficiency, and low toxicity,5) glyphosate remains one of the fastest growing herbicides, even after four decades. However, the extensive use of glyphosate is causing pressure on the environment. Glyphosate is water-soluble; maximum contamination were detected in storm sewers after rain.5) The concentration of glyphosate is higher than other commonly used herbicides, for example atrazine in the river system and in the urban drainage system.5) The most important thing is that the unexpected side effects of glyphosate formulations has been evidenced in non-target species, among other endocrine disruptions during pregnancy or spermatogenesis,7–14) which may be related to the adjuvant in the formulation.

An adjuvant is a reagent that can increase the efficacy of other reagents.15) In the particular case of agrochemicals, an adjuvant is usually broadly defined as any substance separately added to a pesticide product that will increase the performance of the pesticide product. People often use an adjuvant that is a non-herbicidal additive to enhance the effect of the herbicide.16) The main function of the adjuvant is to modify the physical properties of the herbicide or herbicide spray solution for the purpose of increasing the herbicide’s surface tension, wetting performance, adsorption performance, and weeding effect at the same concentration, thus increasing weed control. Adjuvants can be divided into two general types based on use states: formulation adjuvants and spray adjuvants. The first type of adjuvant is the part of the formulation; the second type is mixed with the pesticide in the spray tank15) before application on the field. The most common type of spray adjuvant is a surfactant that can reduce the surface tension of a spray droplet and the waxy surface of a leaf that causes more leaf surface area to be covered with droplets.16) Roundup® Pro Biactive and its recent replacement Roundup® Pro Bio already contain surfactants that are designed to improve adsorption performance and rain fastness, as compared with glyphosate formulations that do not contain surfactants.17) Mesnage et al.18) demonstrated that all glyphosate formulations are more toxic than glyphosate alone to three human cell lines, and the major adjuvant (the polyethoxylated tallow amine POEA) clearly appears to be the toxic principle in human cells. Generally, the problem of the toxicity of adjuvants in pesticides is increasingly recognized.19–21) In the quest to develop a new adjuvant, reducing the toxicology of glyphosate formulation is a necessary goal.

Our study entails researching the effectiveness of a new adjuvant for glyphosate in order to decrease the amount of glyphosate uses. In detail, we developed a new adjuvant A-178®, based...
on coconut shell extracts, that shows the ability to improve the effectiveness of glyphosate application. It was mixed with GP before application on the field. We did indoor trials and field trials, and the result showed that when A-178® was added to GP, the normal formulations could be more effective than GP alone; the magnitude of the difference was sufficient to provide some great practical benefits. Some additional research showed that it can increase penetration into the plant, which allowed us to do a series of studies, such as spread performance, static surface tension, and contact angle, to demonstrate this result.

Materials and Methods

1. Plants
 Crabgrass (Digitaria sanguinalis L.) plants were grown from seed in a controlled environment as reported previously, and experiments were performed when the crabgrass reached the four-leaf growth stage. The weed species present on June 10, 2018 were setaria viridis (Setaria viridis L.), crabgrass (D. sanguinalis L.), tendon grass (Eleusine indica L.), and triangular grass (Cyperus iria L.); weeds present on July 8, 2017 were tendon grass (E. indica L.), perennial ryegrass (Lolium perenne L.), and triangular grass (C. iria L.). The majority of weeds were 10 cm tall. All plants were grown at Shanghai Pesticide Research Institute (Shanghai, China).

2. Chemicals
 N-(phosphonomethyl)glycine (≥95%) was obtained from Hanfu Biochemical Pharmaceutical Co., Ltd. (Weihai, China). Polyethoxylated tallow amine (POEA) was obtained from Ak-hanfu Biochemical Pharmaceutical Co., Ltd. (Weihai, China). Adjuvant A-178®—Polyoxyethylene lauryl ether+Polyoxypropylene octyl ether (1+1 by weight, Patent Pub. No.: WO/2012/029893)—was obtained from Kao Corporation (Shanghai, China). Trifluoroacetic anhydride (TFAA) was obtained from Acros Organics (Geel, Belgium). 2,2,3,3,4,4,4-Heptfluorou-1-butanol (TFE) was obtained from TCI (Tokyo, Japan). 3,7-Dimethyl-2, 6-octadienal (Citral) was obtained from Adams Reagent Co., Ltd. (Shanghai, China).
 GP mixed with A-178®: GP+A-178®+water (41+7+52 by weight, GPA), GP mixed with POEA: GP+POEA+water (41+7+52 by weight, GPP).

3. Bioefficacy study
 Crabgrass was planted in 9 cm plastic pots using potting medium in the greenhouse under controlled conditions (25±2°C day/night temperature and 70±5% relative humidity). Plants were thinned 1 week after emergence, and one treatment included three replicate pots with about 35 plants per pot. All replicate pots were maintained for spraying. Weed species were sprayed at the four-leaf stage using three replicated pots treated with specified concentrations (100, 200, 300, 400, 500 g a.i./ha) of GP, GPA, and GPP. The chamber track sprayer which was fitted with a TeeJet 8002 flat fan spray nozzle sprayed at those pots, delivering 450 L/ha at 200 kPa. Plants were watered by the bottom watering method (keeping the moisture status at the saturated level) for optimum growth. Data were recorded periodically up to ten days 10 spraying.

4. Field studies
 Field studies were conducted in 2017 and 2018 at the National South Pesticide Discovery Center (Shanghai) on indigenous weed populations. Every treatment involved three plots (1.2 m long×1.2 m wide per plot). Weed species present in June, 2017 were setaria viridis (S. viridis L.), crabgrass (D. sanguinalis L.), tendon grass (E. indica L.), and triangular grass (C. iria L.); in July, 2017 species present were tendon grass (E. indica L.), perennial ryegrass (L. perenne L.), and triangular grass (C. iria L.). The majority of weeds were 10 cm tall. The 2017 experiment was conducted in conditions of 31/26±2°C day/night temperatures, and the 2018 experiment was conducted in conditions of 27/20±2°C day/night temperatures; in both experiments, there was no rain within 24 hr after spraying. GP, GPA, and GPP at 400, 500, and 600 g a.i./ha were sprayed on June 10, 2018 and July 8, 2017. Applications were made using double-distilled water as the carrier and a tractor-mounted boom sprayer at an application speed of 4.8 km/h. Visual mortality data were recorded periodically up to fourteen days after spraying on a 0–100 scale, where 0=no effect, and 100=complete mortality.

5. Static surface tension and contact angle
 The parameters studied in the laboratory included surface tensions (ST) for various combinations of GP, GPA, and GPP. Formulations were freshly prepared and diluted to 1000 mg a.i./L with double-distilled water. The same solutions were used for both static surface tension (ST) and contact angle (CA) measurements. Static surface tension was determined by the Wilhelmy plate method using a CNSHP Tensiometer (CNSHP Company, BYYZ-1, China). There were three replicate samples of 20 mL volume in plastic Petri dishes 6 cm in diameter. The platinum–iridium plate was flame heated every time before measuring the ST. Contact angles were measured with a Powereach Contact Angle Goniometer (Powereach, JC2000D3, Shanghai, China) and 5 µL drops of test solutions. Each sample was measured three times and then averaged. All observations were recorded at room temperature (25°C).

6. Spread performance
 Test formulations were freshly prepared in double-distilled water at 1000 mg a.i./L as used in the ST study. A 5 µL sample of each formulation was taken by a micropipette to drop on a paraffin surface. There were three replicate samples of each treatment. The maximum and minimum diameters of the droplets were measured using the V for Windows image analysis system after 5 min.

7. The determination of GP
 7.1. Gas chromatography-mass spectrometry system
 An Agilent 5975 mass spectrometer was equipped with a capil-
lary column (J&W Scientific DB-5, 30 m × 0.25 mm ID, 0.25 μm thick film). The chromatographic conditions used for the analysis of GP residues were as follows: detector temperature, 250°C; injector temperature, 200°C; oven temperature program: hold at 80°C for 1.5 min, increase to 260°C at 30°C/min, hold at 260°C for 1 min, increase to 300°C at 30°C/min; the total run time was 15 min. The injection volume was 2 μL, operated in the splitless mode, with the inlet purged 0.75 min after injection. The carrier gas, N₂, was maintained at a constant flow rate of 1.0 mL/min. The mass-selective detector, a quadrupole instrument, capable of providing positive electron impact (EI) mass spectra with selected-ion monitoring (SIM), was operated in the SIM, low-resolution mode and manually tuned for m/z 414, 502, and 614. The dwell time was 100 msec.

7.2. Working solution
For long-term storage, a stock solution (1.0 mg/mL) of GP was prepared in double-distilled water. Working calibration solutions of 25, 50, 100, 200 and 400 ng/mL were made by diluting them serially, as needed.

7.3. Analyte derivatization
TFAA+TFE (2:1 by volume) was added to a 10 mL glass container. The container was capped and gently inverted three times. A pipette was used to add 1.6 mL portions of the derivatization reagent mixture to 4.0 mL screw-top vials. Each vial was held at −80°C for half an hour. Fifty microliters standards were withdrawn into the pipette tip. The pipette tip was placed under the surface of the prechilled derivatization reagent, and the contents were slowly released. All vials were capped and allowed to equilibrate to room temperature (25°C), while the caps were tightened securely. The vials were placed for 1 hr in an oil bath at 90°C and shaken every 15 min. After heating, the vials were removed from the oil bath and allowed to cool to room temperature (25°C). Excess reagents were removed by a nitrogen evaporator at 40°C. After evaporation, the vials were capped and allowed to cool to room temperature (25°C). Using a 250 μL glass syringe, 225 μL ethyl acetate containing 0.2% citral was promptly added and then injected into GC-MS.

8. Test of penetration
Cockspur was planted in the same conditions as used in bioefficacy study. A 0.5 μL sample of each formulation that was used in bioefficacy study at 200 g a.i./ha (44.44 mg a.i./L) was taken by a micropipette to drop on the surface of a cockspur leaf (20 drops per leaf). The treated cockspur leaves were cut after ten minutes and then rinsed three times in 0.5 mL double-distilled water. The glyphosate residues in water were determined by gas chromatography-mass spectrometry after being derivatized.

The adsorption and permeation rate (%) = \(\frac{\text{Initial amount} - \text{Residue amount in water}}{\text{Initial amount}} \times 100\)

Table 1. Spread performance of glyphosate as influenced by the addition of A-178\(^\text{a,b}\)

| Treatments | Max | Min | Average | LSD (p=0.05)\(^\text{b}\) |
|------------|-----|-----|---------|-----------------------|
| GPA        | 4.06| 4.06| 4.14    | a                     |
| GP         | 4.38| 4.06|         |                       |
| GPP        | 4.22| 4.06|         |                       |
| GP         | 2.97| 2.72| 2.89    | c                     |
| GPA        | 2.97| 2.81|         |                       |
| GPP        | 2.97| 2.88|         |                       |
| GP         | 3.75| 3.28| 3.23    | b                     |
| GPA        | 3.28| 3.13|         |                       |
| GPP        | 3.12| 2.81|         |                       |

\(^{a}\) The mean values are calculated by three replicates. \(^{b}\) Different small letters in the same column indicate significant differences (p≤0.05) between any two groups.

9. Statistical Analysis
The whole statistical analysis process was run in the under SPSS version 17.0 statistical program. Date were subjected to ANOVA followed by Dunnett’s test (P≤0.05 indicates statistical significance).

Results
1. Bioefficacy study
A greenhouse study and a field study were used to test the adjuvant’s ability to increase the efficacy of glyphosate.

1.1. Greenhouse study
Similar statistical significances were observed with the arcsine transformation and the original data. Original data are presented in Table 1 with the least significant difference (LSD) values at a 5% level of significance. At 100, 200, 300, 400, and 500 g a.i. ha\(^{-1}\), the fresh weight inhibition rate of GP increased by 23.62%, 16.28%, 5.07%, 8.98%, and 6.31%, respectively, with the addition of 7% A-178\(^\text{®}\), as compared to glyphosate alone at 10 days after treatment, and GPA provided a similar weight inhibi

![Fig. 1. The inhibition rate of fresh weight on crabgrass. Crabgrass treated with GP, GPA and GPP at 100, 200, 300, 400, 500 g a.i./ha for 10 days. The fresh weight inhibition rate is expressed as the mean values (±S.D.) of three independent experiments, the result showed represent significantly differences relative to negative control with LSD values at 5% level.](image-url)
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1.2. Field study

In both experiments, the result showed that GP formulation activity varied significantly after mixing with A-178®, and its activity was higher than that of GPP at the same concentration (Fig. 2a and 2b). In the first field experiment, the addition of 7% A-178® to GP improved GP activity as compared to that of GP alone by 78.57% at 500 g a.i./ha. In the second field experiment, the addition of 7% A-178® to GP improved GP’s activity as compared to that of GP alone by 79.27% at 500 g a.i./ha.

2. Mechanism study

A series of date was measured to test the effect of adjuvant A-178® on the glyphosate formulations and to determine why it can improve glyphosate efficacy.

2.1. Static surface tension and contact angle

The ST and CA are the influencing factors for effectiveness; the reduction of the ST and CA of the spray on the plant surface can increase wetting ability and permit greater adsorption. The ST and CA of GP were significantly lowered with the addition of A-178®, as compared to those of GP and GPP (Fig. 3b). The addition of 7% A-178® to GP reduced the ST of the GP solution and GPP by 42.91% and 33.69%, respectively, and reduced the CA of the GP solution and GPP by 46.87% and 31.52%, respectively (data averaged over rates).

Decreased ST and CA with the addition of A-178® to GP improves the penetration of active ingredient. The lower surface tension of GP with the addition of 7% A-178® might have improved GP penetration in the weed species, which is reflected in increased mortality. A-178® enhances the uptake of GP. The CA of GP was lowered with the addition of A-178®, as compared to the GP formulation without the adjuvant, which substantiates increased weed control with the addition of surfactants.

2.2. Spread performance

It is necessary to experimentally determine the spread performance that results from reducing the ST and CA. The addition of A-178® significantly increased the spread performance of the GP solution (Table 1). The droplet diameter of GP with A-178® was 28.17% and 43.25% larger than those of GPP and GP alone, respectively. The increase in droplet diameter with the addition of A-178® to GP improves the spread performance.

2.3. Penetration test

A study must be conducted to determine the penetration of GP. In this study, we used GC-MS spectrometry. The log-linear standard curve prepared from calibration standards contained known concentrations of GP (5, 10, 20, 40, and 80 ng/mL). The linear equations are \( y = 45.40x + 1041 \) and \( R^2 = 0.998 \). The ability of the GP solution to penetrate into the cuticle membrane is an important indicator of the adjuvant’s effect. Our results showed that the adjuvant A-178® increased GP penetration into the tissues of cockspur leaves (Table 2). The penetration rate of GP with A-178® was 12.36% and 52.56% higher, respectively, as compared to those of GPP and GP alone.

Discussion

Glyphosate is the most popular herbicide in the world. However, the extensive use of glyphosate is causing mounting pressure on the environment. Glyphosate is water-soluble; maximum concentrations of glyphosate were detected in storm sewers after
rain. In river and urban drainage systems, the concentration of glyphosate is higher than that of atrazine. The most important thing is the unexpected side effects on non-target species of glyphosate formulations; the toxic effects of glyphosate formulations were evident in hepatic (HepG2), embryonic (HEK293), and placental (JEG3) cell lines, and cell apoptosis induced by glyphosate formulations in HepG2 cells has been demonstrated. It is very important to exploit a new glyphosate adjuvant. It is fortunate that we have developed a new adjuvant A-178 based on coconut shell extracts. We conducted some experiments to research this adjuvant.

From the results of greenhouse and field studies, we can understand that the addition of this adjuvant to glyphosate significantly increased glyphosate bioefficacy against target weeds. Regarding both mortality in the field experiment or the fresh weight inhibition rate in the greenhouse study, adjuvant A-178 can improve the effect of glyphosate. To discover why A-178 can increase the effect of glyphosate, we did some experiments, including static surface tension and contact angle, spread performance, and penetration testing in plants.

Surface tension is the elastic tendency of a fluid surface that makes it acquire the least surface area possible. The contact angle is the angle, conventionally measured through the liquid, where a liquid–vapor interface meets a solid surface. The ST and CA are the main factors affecting pesticide solutions. The reduction in ST and CA values of glyphosate with added A-178 was significantly less than those in the glyphosate formulation and Roundup. Next, we need more visualized result, for example, of spread performance.

We concluded that A-178 can increase the spread performance of a glyphosate solution by direct measurement. An increase in the spread area will result in a higher number of stomata or leaf surface area being covered, thus there will be a bigger area for absorption. Therefore, increasing penetration by A-178 is possible in a general way. The penetration test showed that the penetration rate of glyphosate with A-178 was 10.75% and 50.96% bigger than those of GP mixed with POEA and GP alone, respectively. The conjecture that A-178 can increase the penetration of glyphosate solution was verified. Previous studies have shown that adjuvants by themselves or in commercial formulations play a role in the toxicity attributed to herbicides. Developing new adjuvants to decrease the toxicity of glyphosate-based herbicides is critical.

In conclusion, our study introduced a new adjuvant, A-178, based on coconut shell extracts, which shows the ability to improve the effectiveness of glyphosate application. We did a series of physicochemical and biochemistry experiments in order to research the synergistic mechanism of this adjuvant. The results showed that the new glyphosate adjuvant (A-178) can decrease the ST and CA of glyphosate solutions, improve their spread performance, and make the target plant uptake more active ingredients. More toxicology research will be carried out in the future.

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Table 2. Effect of adjuvant A-178 on glyphosate penetration

| Treatments | Residues concentration (ng/mL) | Penetration rate (%) | LSD (p=0.05) |
|------------|-------------------------------|---------------------|--------------|
|            | Repeat 1 | Repeat 2 | Repeat 3 | Average |                         |                   |
| GPA        | 22.58    | 19.74    | 21.99    | 21.44    | 73.20 | a               |
| GP         | 41.96    | 42.78    | 40.11    | 41.62    | 47.98 | b               |
| GPP        | 28.15    | 26.50    | 29.00    | 27.88    | 65.15 | c               |

a) The mean values are calculated by three replicates. b) Different small letters in the same column indicate significant differences (p≤0.05) between any two groups.
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