





Rainfastness of a 2,4-D Plus Glyphosate Mixture on Various Weed Species


Resistência à Chuva da Mistura de 2,4-D e Glifosato em Várias Espécies de infestantes


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Abstract

Environmental factors such as rainfall occurring soon after herbicide spraying can decrease weed control efficiency. The objective of this study was to evaluate the effect of simulated rain on the rainfastness of an herbicide mixture composed of 2,4-D plus glyphosate, used to control *Amaranthus* spp., *Commelina benghalensis*, and *Glycine max* L. Merrill soybean at two developmental stages. The herbicides used were dimethylamine salt (DMA806 BR[®] 670 g L⁻¹) and glyphosate potassium salt (Roundup Transorb R[®], 480 g L⁻¹). The mixture was sprayed using a TTI11002 nozzles (300 kPa) at an application rate of 150 L ha⁻¹. The plants were subjected to simulated rain at 5, 15, 30, 45, 120, and 240 minutes after herbicide application. Control efficiency assessments were performed visually at 35 days after application. The initial growth stage of the plants showed higher control efficiency and lower susceptibility to the effects of rainfall. Rainfall up to 240 minutes after 2,4-D plus glyphosate mixture application reduced the control efficiency for *G. max* and *C. benghalensis* weed species. The control of *Amaranthus* spp. was satisfactory after 30 minutes without rainfall. Therefore, the decreased control efficiency of the 2,4-D plus glyphosate herbicide mixture is influenced by the interval between rainfall, plant species and developmental stage.

Keywords: Rain Losses. Application Technology. Weed. Simulated Rain.

Resumo

A ocorrência de chuvas logo após a pulverização de herbicidas pode diminuir a eficiência de controle de plantas daninhas. O objetivo deste trabalho foi avaliar o efeito da chuva simulada sobre a resistência da mistura de herbicidas composta por 2,4-D e glyphosate, utilizada para o controle de *Amaranthus* spp., *Commelina benghalensis* e de soja voluntária (*Glycine max* L. Merrill), em dois estádios de desenvolvimento. Os herbicidas utilizados foram o sal de dimetilamina (DMA806 BR[®] 670 g L⁻¹) e o sal de potássio de glyphosate (Roundup Transorb R[®], 480 g L⁻¹). A mistura foi pulverizada com uma ponta TTI11002 (300 kPa) a uma taxa de aplicação de 150 L ha⁻¹. As plantas foram submetidas a chuva simulada aos 5, 15, 30, 45, 120 e 240 minutos após a aplicação do herbicida. As avaliações da eficiência de controle foram realizadas visualmente aos 35 dias após a aplicação. O estágio inicial de desenvolvimento das plantas daninhas apresentou maior eficiência de controle e menor suscetibilidade aos efeitos da chuva. A chuva até 240 minutos após a aplicação da mistura 2,4-D + glyphosate reduziu a eficiência no controle de soja voluntária e *C. benghalensis*. O controle da *Amaranthus* spp., foi satisfatório após 30 minutos sem precipitação. Conclui-se que, a diminuição da eficiência de controle da mistura dos herbicidas 2,4-D e glyphosate é influenciada pelo intervalo entre a precipitação, pela espécie e estágio de desenvolvimento da planta.

Palavras-chave: Perdas por Chuva. Tecnologia de Aplicação. Plantas Daninhas. Chuva Simulada.

1 Introduction

Weeds are any plants that interfere with the man's interests and affect agricultural crops, potentially causing decreases in productivity (Presoto; Andrade; Carvalho, 2020). Weed infestation can lead to crop productivity losses of up to 80%, depending on the weed species and competition type (Galon *et al.*, 2020, Zain; Dafaallah; Zaroug, 2020).

The species of the genus *Amaranthus* spp. have become a growing concern in agricultural areas where farmers have reported challenges in their management (Silva *et al.*, 2016). This challenge is largely due to their high environmental adaptability, discontinuous emergence pattern, rapid growth, and resistance to multiple herbicide modes of action, which can reduce crop productivity (Heap, 2024; Lauren *et al.*, 2016).

Similarly, *Commelina benghalensis* L. is considered a problematic weed in agricultural systems, with infestations reported in 25 crops in 29 countries (Mandeep *et al.*, 2016). They can cause significant reduction in production and quality of soybean, corn wheat and bananas fields due to the reproductive flexibility, glyphosate resistance and seeds produced both above and below ground (Fibrich; Lall, 2020; Isaac *et al.*, 2013).

Studies have demonstrated the significant impact of *Amaranthus* spp. and *Commelina benghalensis* L. interference on crop productivity (Amini *et al.*, 2014; Berger *et al.*, 2015; Teixeira *et al.*, 2017; Webster *et al.*, 2007; Zandoná *et al.*, 2022). The authors concluded that depending on time of emergence and population density, *Amaranthus palmeri* can reduce 65% the productivity in cotton fields while *Amaranthus retroflexus* can achieve up to 85% in soybean fields. Already *C. benghalensis* L. caused up to 51% yield loss in peanuts and 85% the grain yield (g plant⁻¹) in *Cicer arietinum* L.

Therefore, to avoid these losses, control measures must be utilized.

Chemical weed control has been one of the main methods adopted in conventional agriculture. However, factors related to coverage of herbicide, penetration, absorption, weed species present in the area and environmental factors such as rain immediately after spraying, can interfere with the efficiency of the herbicide and reduce the effectiveness and control of weeds (Ferreira *et al.*, 2017; Mirgorodskaya *et al.*, 2020; Souza *et al.*, 2014). Thus, rainfastness, which is the ability of products to resist removal after the occurrence of rainfall and/or other related environmental phenomena, is considered essential for pesticide formulation (Andrade *et al.*, 2021).

Among the herbicides most used in weed management is 2,4-dichlorophenoxyacetic acid, considered the first selective and systemic herbicide applied in post-emergence, acting mainly on the plasticity of the plant cell membrane (Oliveira *et al.*, 2021; Peterson *et al.*, 2016). Another widely used herbicide in weed management is glyphosate, a non-selective with high control rates that acts on annual and perennial weeds with narrow or broad leaves (Silva *et al.*, 2022). Some studies to evaluate the effect of rainfall on the application of isolated herbicides 2,4-D and glyphosate were carried out. It was observed that the herbicide 2,4-D requires a minimum of 30 minutes without rainfall after spray application to ensure the efficacy in controlling *Senna obtusifolia* (fedegoso) (Souza *et al.*, 2014).

For the control of *Ipomoea grandifolia*, 15 minutes without precipitation is enough for 2,4-D to work properly, when subjected to simulated precipitation with a 15 mm depth, but the glyphosate herbicide had its control efficiency reduced in the occurrence of the same precipitation, up to eight hours after the application of the herbicide (Souza; Martins; Pereira, 2013).

It was also found that the application of Roundup Transorb R[®] (potassium salt) alone did not provide efficient control (22.8%) of *B. decumbens* plants with up to 20 cm and 8 leaves, if 20 mm of rain fell 15 minutes after spraying (Costa *et al.*, 2017).

The occurrence of simulated rainfall after 240 min reduces the glyphosate efficiency on the control by 30%, 15% and 60% for the isopropylamine, potassium and ammonium salt formulations, respectively, in hairy fleabane plants (Dalazen *et al.*, 2020). Already volumes of simulated rainfall of 90 mm reduced the phytotoxicity of dicamba in soybean (Silva *et al.*, 2020).

Considering the importance of tank mixtures of 2,4-D plus glyphosate herbicides in weed management, scientific information updated is needed to verify the rainfastness of these herbicides on weed control, considering the developmental stages of these plants, as well as the effect of rain on the process, the absorption of herbicides, and the spray efficacy.

Thus, the objective of this study was to characterize the rainfastness effect on plants of *Amaranthus* spp., *Commelina benghalensis* and *Glycine max* L. Merrill, at two developmental stages,

with the application of the herbicides 2,4-D plus glyphosate followed by the occurrence of rainfall at different intervals after spraying.

2 Material and Methods

The experiment was conducted in a $3 \times 2 \times 6$ factorial arrangement, and the evaluated factors were: (1) weed species, (2) plant developmental stage, and (3) rainfall interval after herbicide application. The experiment also included a control applied without precipitation and a control without herbicide application, with six replications in a randomized block design.

The plant species used in this study were *C. benghalensis*, *Amaranthus* spp., and *G. max* variety BRS 543 RR or voluntary soybean, which is considered a weed. All the plants were grown in a greenhouse. They were seeded on a commercial substrate, thinned after emerging, and then transplanted to 1 L plastic containers filled with topsoil. To ensure that plant competition did not interfere in the growth and development, the experimental setup maintained specific planting densities. Two seedlings of *Amaranthus* spp. and *Commelina benghalensis* were kept per pot, while only one *Glycine max* plant was allocated per pot.

Herbicides were sprayed during two distinct development stages of plants (Table 1).

Table 1 - Description of plant development stages during the experiments

Plant Species	Plant Development Stages Description	
	Initial Stage (S1)	Final Stage (S2)
<i>Glycine max</i> L.	Vegetative (V3/V4)	Reproductive (R1)
<i>Amaranthus</i> spp.	≤ 7 cm	> 10 cm
<i>Commelina benghalensis</i> L.	≤ 6 leaves	> 20 leaves

Source: research data.

The mixture of herbicides used were 2,4-D (dimethylamine salt, DMA806 BR[®] 670 g L⁻¹, 1 L ha⁻¹) plus glyphosate (glyphosate potassium salt, Roundup Transorb R[®], 480 g L⁻¹, 3 L ha⁻¹). An automatic test sprayer for laboratory applications with a 15-meter displacement track, a hydraulic system consisting of a manual pressure controller, and a three-piston hydraulic pump was used for herbicide application. The system was powered by a 1.5 kW electric motor, equipped with a two-meter bar and four spray tips spaced 0.5 m apart and placed 0.5 m above plant tops. The setting for the spray boom was configured to guarantee a 100% overlap between the nozzles and ensure an efficient deposition and coverage of the herbicides. A flat jet spray nozzle with air induction (TTI11002 Model Turbo Teejet Induction[®]), ultra-thick droplet class, 300-kPa pressure (0.79 L min⁻¹), 1.75 m s⁻¹ travel speed, and a constant application rate of 150 L ha⁻¹ was used.

Rain simulation was conducted using the same laboratory automatic sprayer, equipped with three deflecting nozzles (TK-SS FloodJet Stainless Steel), spaced 0.35 m apart and set a 2.0 m above

the weeds canopy. Water was pumped from a reservoir, with outlet pressure kept constant at 1,000 kPa.

After herbicide application, S1 and S2 plants were subjected to 10 mm simulated rain at six different intervals: 5, 15, 30, 60, 120, and 240 minutes.

The efficiency of weed control was determined up to 35 days after application (DAA), using a visual scale ranging from 0 to 100%, where 0 means no control and 100% means total control (Alam, 1974). Visual assessments were conducted until the phytointoxication symptoms were consolidated or disappeared.

The data were subjected to F-test analysis of variance, and the interactions among the species, developmental stages, and rainfall intervals after herbicide application were analyzed. In the comparison structure, the mean values for the species were compared using the Tukey test ($p < 0.05$) and those for the developmental stages were compared using the student's *t*-test for independent data ($p < 0.05$). For the rainfall, interval a quadratic model was adjusted and validated using the parameter significance and coefficient of determination (R^2).

3 Results and Discussion

Significant effects were observed for weed species control on isolated factors (species, stage of development and time of occurrence of rain). Additionally, were observed, the interactions between species and stage of development, species and timing of rainfall occurrence, and stage and timing of rainfall occurrence after the application of the 2,4-D plus glyphosate mixture (Table 2).

Table 2 - Analysis of variance of interaction among plant species, developmental stage, and rainfall interval after 2,4-D plus glyphosate application

Variation Source	DF	SS	MS	Pr > FC
Species	2	84383.46	42191.73	0.0001
Stage	1	30166.71	30166.71	0.0001
Precipitation	5	22015.89	4403.17	0.0001
Species*Stage	2	1230.53	615.26	0.0001
Species*Precipitation	10	20113.80	2011.38	0.0001
Stage*Precipitation	5	4069.76	813.95	0.0001
Species*Stage*Precipitation	10	16074.89	1607.48	0.0001
Error	180	8052.40	44.73	
CV (%) = 11.97 Overall mean (%) = 55.88				

*DF: degree of freedom; SS: sum of square; MS: mean square; Pr > FC corresponds to the significance probability value associated with the F-value.

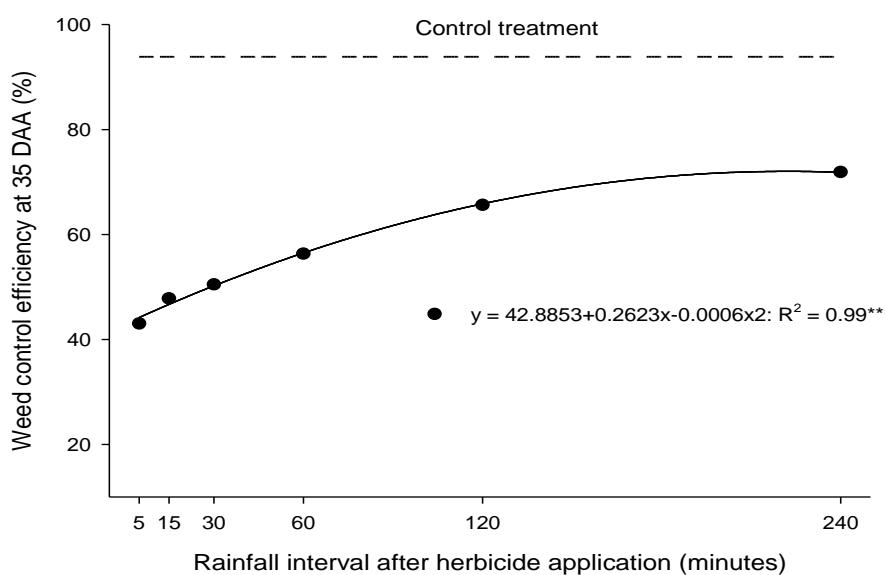
Source: research data.

The effects of triple interaction were also significant. Therefore, to better understand the results, we analyzed the double interactions of the factors tested in the experiment. The experiment had an

average experimental variability ($10\% < CV < 20\%$), according to Pimentel Gomes (2009).

Visual assessments of weed control demonstrated that 35 DAA species have consolidated phytointoxication symptoms. The average general control of the species, depending on the time of rainfall occurrence after the application of 2,4-D plus glyphosate, when compared to the control (without precipitation), was decreased for all treatments (Figure 1).

Figure 1 - General means of control efficiency at 35 DAA according to the rainfall interval after 2,4-D plus glyphosate application.



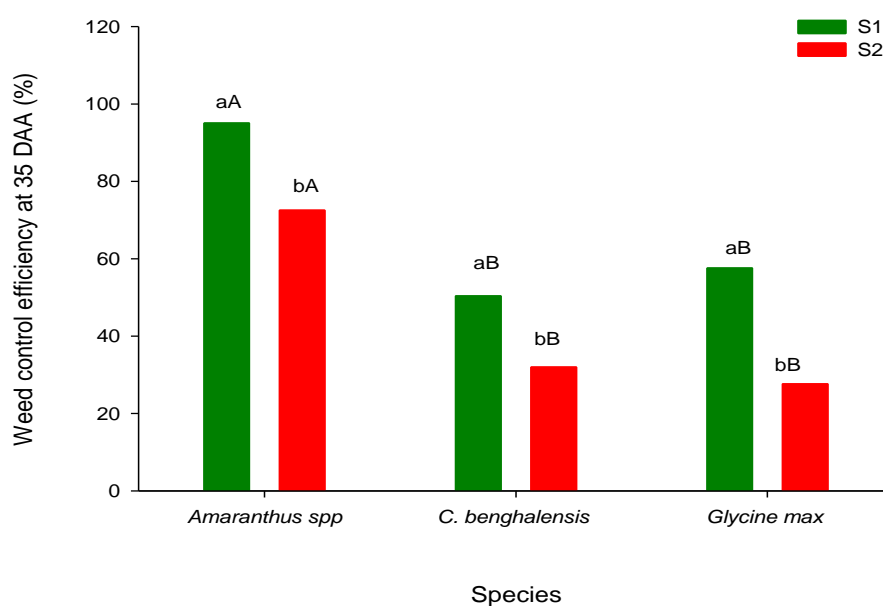
**Significant ($p < 0.01$)

Source: research data.

Weed control was reduced more significantly when rainfall was applied minutes after herbicide spraying. The lower rainfastness observed soon after herbicide application occurred because there was not an ideal contact period between the herbicide drop and the leaf surface to allow sufficient absorption of the active ingredient. Between the rainfall simulation intervals, there was an increase in the control percentage of 66.2% for the time of 240 minutes without rainfall compared to 5 minutes without precipitation.

The analysis of the interaction between species and developmental stage based on the general average of the different rainfall intervals between species and plant developmental stages showed that rainfastness was most affected at the later developmental stage (S2) (Figure 2).

Figure 2 - Control at 35 DAA according to the interaction between species and developmental stage based on the general average of the different rainfall intervals after 2,4-D plus glyphosate application. Different capital letters indicate significant difference between species by the SNK test ($p < 0.05$), and different lower-case letters indicate significant difference between the developmental stages by the student's t-test ($p < 0.05$)



Source: research data.

In stage S1, rainfall after herbicide application decreased the control of *Amaranthus spp.* to 95%, *C. benghalensis* to 50% and in the species *G. max*, control to 57% (Figure 2 and 3). Others research about the control of *Commelinas spp.* Plants were performed, with the mixture of herbicides 2,4-D plus glyphosate, subjected to 20mm precipitation, and concluded that a minimum period of 12 hours without precipitation was necessary for the absorption of the mixture and plant control >90% (Costa *et al.*, 2011), similar to control *C. benghalensis*, in the absence of precipitation with averages that arrived 95%, for the mixture of 2,4-D plus glyphosate (Jerônimo *et al.*, 2021; Perissato *et al.*, 2023). Already, control of this *G. max* reached 95% when using the 2,4-D herbicide alone, without precipitation interference (Dan *et al.*, 2009).

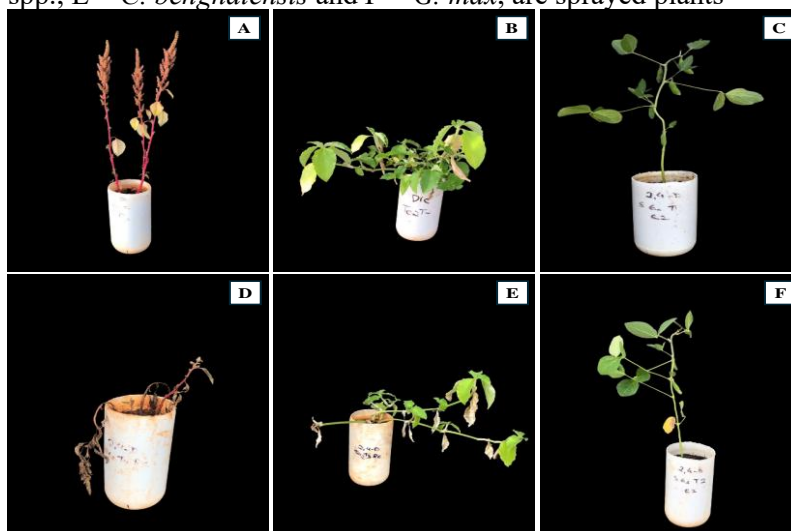
Figure 3 - Weed control at 35 DAA according to the interaction between species and developmental stage, after 2,4-D plus glyphosate application and 10 mm simulated rain. Plants on the initial stage - S1: A= *Amaranthus* spp., B = *C. benghalensis* and C = *G. max*, are control (without spray application) and D = *Amaranthus* spp., E = *C. benghalensis* and F = *G. max*, are sprayed plants



Source: research data.

At S2, it was found that the rainfall occurrence provides greater interference in control, reducing control to 73, 32 and 27% for species *Amaranthus* spp., *C. benghalensis* and *G. max*, respectively (Figure 2 and 4). Therefore, the rainfall occurrence can be a limiting factor in rainfastness and weed control in agricultural areas, as it is common to find weeds of the same species at different stages of development.

Figure 4 - Weed control at 35 DAA according to the interaction between species and developmental stage, after 2,4-D plus glyphosate application and 10 mm simulated rain. Plants on the second stage - S2: A= *Amaranthus* spp., B = *C. benghalensis* and C = *G. max*, are control (without application) and D = *Amaranthus* spp., E = *C. benghalensis* and F = *G. max*, are sprayed plants



Source: research data.

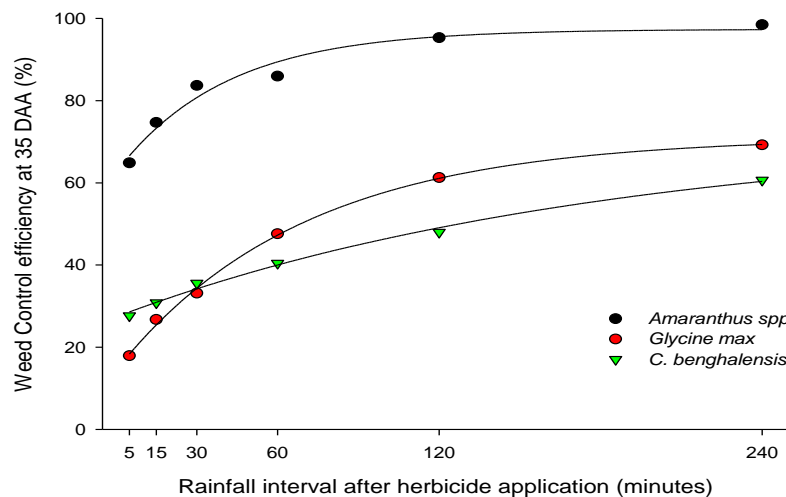
The effect of rainfall on the action of herbicides in the *Amaranthus* spp was altered at all intervals, with an increase in control as precipitation was more spaced out, reaching 97% at 240 minutes (Table 3 and Figure 4). In *C. benghalensis*, control was observed at 60% with an interval of 240 minutes after herbicide application. It was observed for *G. max* that control increases 2.3 times when plants spend 120 minutes after herbicide application, compared to plants that spend only 5 minutes. These differences in control may be related to the different leaf surfaces that plants have, influencing product absorption and washing by rainfall (Ferreira *et al.*, 2023; Monquero; Cury; Chistoffoleti, 2005; Monquero *et al.*, 2004).

Table 3 - Equations and coefficient determination (R^2) of the control according to the interaction between species and rainfall interval after 2,4-D plus glyphosate application

Species	Equation	R ² (%)	p-value
<i>Amaranthus</i> spp.	$y = 62.5628 + 34.8079(1-0.9756^x)$	96.56	0.05
<i>Glycine max</i>	$y = 14.3819 + 56.7318(1-0.9856^x)$	99.83	0.05
<i>Commelina benghalensis</i>	$y = 27.3610 + 45.1369(1-0.9945^x)$	99.43	0.05

Source: research data.

Figure 4 - Control at 35 DAA according to the species and rainfall interval after 2,4-D plus glyphosate spray application



Source: research data.

It is important to note that true weeds such as *C. benghalensis* and *Amaranthus* have distinct characteristics in terms of anatomy. Such as *C. benghalensis* that is characterized by a low number of stomata, an epicuticular wax layer of, in addition to elongated trichomes (Ferreira *et al.*, 2017), while *Amaranthus* plants have the characteristics of a smooth leaf surface, without trichomes (Ozimeide; Obute; Nyananyo, 2019). These structural differences had a significant impact on the effectiveness

of herbicides, particularly in the case of *C. benghalensis*.

In addition, *G. max*, classified as a eudicotyledon, displays a high degree of susceptibility to auxinic herbicides, particularly at low concentrations of 2,4-D (Silva *et al.*, 2018). However, despite this sensitivity, the effectiveness of herbicide control was diminished by the rain after spray application. Notably, precipitation of 10 mm interfered with the herbicide absorption and translocation within the plant, requiring an interval greater than 240 minutes without rain to ensure effectiveness.

In this way, it is the responsibility of the rural producer to determine the most appropriate weed control strategy for their cultivation areas. When opting for chemical management, it is crucial to consider that herbicide resistance to rain is directly influenced by the interval between spray application and the occurrence of precipitation, as well as by the weed species and their developmental stage.

4 Conclusion

The level of control inflicted on plants by the 2,4-D plus glyphosate herbicide mixture depends on the rainfall interval after application, weed species, and developmental stage. After 240 minutes of application, it is still critical for herbicide absorption, indicating that longer intervals are required to avoid wash-off and the consequent decrease in weed control efficiency.

Considering these findings, herbicide spray applications should be planned for periods with no rainfall to ensure optimal absorption and maximize weed control efficacy.

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