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Interaction of Glyphosate and Diquat in Ready-To-Use Weed Control Products

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Glyphosate-based, ready-to-use weed control products frequently contain diquat (typically, 0.04 by weight relative to glyphosate) under the supposition that the diquat, ''makes glyphosate work faster.'' However, in light of the known modes of actions of glyphosate and diquat, we hypothesize that diquat may be antagonistic to glyphosate activity. Greenhouse experiments using longstalked phyllanthus were conducted to test this hypothesis. Glyphosate was applied at a series of rates, ranging from 0.11 to 1.12 kg ae/ha, either alone or tank-mixed with either 0, 0.02, 0.04, and 0.06 diquat. Onset of visual injury was more pronounced with the glyphosate + diquat tank mixtures compared with glyphosate alone. However, long-term control, as expressed by regrowth suppression, was greater with glyphosate alone. Regression analysis indicated that, at marginally effective glyphosate rates, the amount of glyphosate must be increased by approximately 60% to compensate for the diquat-based antagonism. Absorption and translocation studies using ^{14}C-glyphosate revealed that the antagonism of diquat toward glyphosate can be attributed to reduced translocation of absorbed glyphosate.
Nomenclature: Diquat; glyphosate; longstalked phyllanthus, *Phyllanthus tenellus* Roxb PYLTE.
Key words: Linear regression, nonlinear regression, synergism and antagonism.

A wide variety of ready-to-use weed control products that are marketed to home owners have glyphosate as their main component. Glyphosate is ideal for such products for several reasons. Glyphosate is relatively nonselective and, therefore, is effective against a wide diversity of weed species. Because of the systemic nature of glyphosate, it can kill the roots and other tissues not directly contacted by the spray. Glyphosate has low mammalian toxicity and is correctly perceived by the public as relatively safe. An informal survey of these ready-to-use weed control products revealed that glyphosate is rarely marketed as a single component product and that diquat is a common additive (G. Wehtje et al., personal observation). Sales personnel claim that the small amount of diquat (typically 0.04 by weight relative to the glyphosate) ''makes the glyphosate work faster.'' Glyphosate inhibits aromatic amino acid production (Jaworski 1972; Steinrücker and Amrhein 1980). One aspect of this mode of action is that treated plants generally do not exhibit injury until several days after application (Senseman 2007b). This delay is actually beneficial because it allows for extensive translocation and, ultimately, more complete control. However, this delay in symptom occurrence does not give the consumer the immediate visual satisfaction that the product is working. Diquat diverts energy from photosynthesis, producing peroxide radicals, which result in rapid cell collapse and onset of phytotoxic symptoms (Black and Meyers 1966; Funderburk and Lawrence 1964). Symptoms such as rapid wilting and desiccation can occur within several hours in full sunlight (Senseman 2007a). We surmise that the addition of diquat in these glyphosate-based products serves to rapidly cause injury in treated plants, thereby increasing consumer satisfaction.

A compilation of the reported interactions of herbicide tank mixtures has been prepared by Hatzios and Penner (1985), where glyphosate was tank-mixed with 16 different and diverse herbicides (diquat not included). Fifteen of these combinations were deemed antagonistic. The one exception was glyphosate tank-mixed with naptalam, which was deemed synergistic with respect to yellow nutsedge (*Cyperus esculentus* L.) control. In more recent studies, Chachalis and Reddy (2004) reported that, with respect to trumpetcreeper [*Campsis radican* (L.) Seem. *ex* Bureau] control, the addition of pelargonic acid to glyphosate did not improve control nor did it improve glyphosate absorption and translocation relative to glyphosate alone. Norris et al. (2001), in a greenhouse study, evaluated the interaction of several formulations of glyphosate when combined with selected POST-active herbicides. Target species were barnyardgrass [*Echinochloa crus-galli* (L.) Beauv], prickly side (*Sida spinosa* L.), pitted morningglory (*Ipomoea lacunosa* L.), and hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex. A. W. Hill]. Control was evaluated at 2 and 4 wk after treatment (WAT), and interactions were evaluated by the Colby procedure (1967). Many synergistic and antagonistic interactions were detected. The occurrence of synergistic or antagonistic interactions varied with the glyphosate formulation, targeted species, and length of time between application and evaluation.

In light of the literature precedent for glyphosate to be antagonized by the addition of other herbicides, and the known ability of diquat to rapidly destroy tissue, we hypothesized that diquat would be antagonistic toward glyphosate when the time was sufficient for the activity of glyphosate to be fully manifested. Verifying this hypothesis and quantifying suspected synergistic and antagonistic interactions was our first research objective. If our suspected interactions were valid, glyphosate foliar absorption or subsequent translocation should be inhibited by the addition of diquat. Determining to what extent (if any) glyphosate foliar absorption and translocation is altered by diquat addition was our second objective.

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Materials and Methods

General Information. Studies were conducted between April and June of 2007. Longstalked phyllanthus (*Phyllanthus tenellus* Roxb.), which is problematic in nurseries and landscapes across the southeastern United States and, thus, a common target of ready-to-use weed control products, was selected as the test species. Test plants were grown and maintained after treatment in a double-layer polyethylene greenhouse located at Auburn University, Auburn, AL. The greenhouse was equipped with evaporative cooling, which operated whenever the temperature within the greenhouse exceeded 23 C. Only natural lighting was received, and day length (latitude = 32°N) ranged from 12.3 to 14.0 h.

Longstalked phyllanthus plants were grown in 10-cm, square, plastic pots, filled with a pine-bark and sand (7 : 1 v/v) substrate, which had been amended with a controlled-release granular fertilizer,[1] dolomitic limestone, and a micronutrient fertilizer[2] at 10.9, 5.1, and 1.2 kg/m^3, respectively. Media-filled containers were placed under mist irrigation, and were seeded with approximately 20 longstalked phyllanthus seed per container. Seed had been collected by the authors the previous season and stored at 3 C. All experiments were repeated at least once. Data were subjected to ANOVA using the general-linear model procedure in SAS.[3] For all experiments, data were pooled over the two repetitions because no treatment by experimental repetition interactions were detected in the initial ANOVA. This was followed by linear or nonlinear regression. SigmaPlot[4] was used to summarize and present data.

Interaction of Glyphosate–Diquat Tank Mixtures. Treatments consisted of a factorial arrangement of six glyphosate rates and four diquat levels. Glyphosate[5] was applied at 1.12, 0.63, 0.40, 0.25, 0.16, and 0.11 kg ae/ha. Glyphosate rates were supplemented with either 0, 0.02, 0.04, or 0.06 by weight of diquat[6] relative to the glyphosate rate. For example, glyphosate at 1.12 kg ae/ha contained diquat at 0.022, 0.045, and 0.067 kg ai/ha, respectively. Four percent diquat is the amount contained in the commercially available, ready-to-use products. A nontreated control was included. No additional surfactant was included. Treatments were applied with an enclosed-cabinet sprayer calibrated to deliver 280 L/ha at 193 kPa.

Data collected included injury rating at 4 d after treatment (DAT), followed by a control rating at 2 WAT. After the 2-WAT rating, plants were clipped at approximately 15 cm above the soil surface and allowed to regrow for an additional 2 wks. At this time, regrowth (if any) was removed, and its fresh weight determined. Regrowth was expressed as a percentage relative to that of the nontreated control.

Glyphosate Absorption and Translocation as Influenced by Diquat. This study used ^{14}C-labeled glyphosate, which was supplied by the manufacturer; specific activity was 2.04 GBq/mmol. A portion of the spray solutions that were used to apply glyphosate at 0.25 kg/ha, either alone or tank-mixed with diquat at 0.01 kg/ha (i.e., the 0.04 diquat rate) were retained. These two solutions were then supplemented with ^{14}C-glyphosate, so that the concentration of radioactivity was 75 Bq/µl. At treatment, a single 2-µl drop of the radiolabeled spray solution was applied to a single leaflet using a micropipette. Treated plants were harvested 36 h after treatment. A completely random design with six single-plant replicates was used. At harvest, treated plants were removed from pots, and the roots were washed free of the media. Treated plants were partitioned into treated leaflet, remainder of the treated leaf, all remaining foliage, and the roots. One milliliter of a water–methanol solution [50 : 50 (v/v)] was placed into a 20-ml scintillation vial, and the treated leaflet was placed into that vial and agitated with a swirling motion for 30 s to remove any unabsorbed glyphosate. After removing the disk, 10 ml of scintillation fluid was added into the vial in preparation for counting through liquid-scintillation spectrometry.[7] All tissue samples were dried at 45 C (24 h) and combusted in a biological tissue oxidizer,[8] and the recovered radioactivity was quantified through liquid-scintillation spectrometry. Radioactivity was summed over the wash and that recovered from the tissues of the partitioned plant, and expressed as a percentage of the total amount applied. In preliminary trials by the authors (data not shown), recovery of radioactivity ranged from 93 to 105% of that applied. In light of the nearly complete recovery, data were normalized to 100%, based upon the amount applied. Data were subjected to multivariate analysis of variance using the general linear-model procedure in SAS so that the ^{14}C-glyphosate sorption and distribution of ^{14}C-glyphosate could be compared between that applied alone and that applied in combination with diquat. Multivariate analysis of variance was used to compare treatment effects on ^{14}C distribution throughout the plant, in consideration of the interrelatedness of those response variables (Littell et al. 1991). Wilks' λ was used as the test statistic for the multivariate comparison of the ^{14}C distribution between the two treatments (Littell et al. 1991). Assuming the multivariate distribution was declared significantly different, univariate ANOVA was conducted on individual parameters.

Results and Discussion

Interaction of Glyphosate–Diquat Tank Mixtures. Preliminary data examination indicated a likely linear relationship between early injury (i.e., visual rating at 4 DAT) and the log of glyphosate rate, whether applied alone or in combination with diquat. Furthermore, this relationship appeared equivalent across the diquat levels (data not shown). Linear regression and the statistical procedure, as described by Littell et al. (1991), were used to test for homogenate of both slope and intercept within the three diquat levels. Within the three diquat levels, glyphosate rate was significant ($P < 0.001$); however, diquat level and the glyphosate–diquat interaction were not significant; $P = 0.052$ and 0.142, respectively. Thus, the regression responses for all three diquat levels were equivalent with respect to both slope and intercept. Because glyphosate plus either 0.02, 0.04, or 0.06 diquat produced essentially equivalent early injury, we chose to focus only on the 0.04 level in subsequent statistical procedures and data presentation because that is the concentration used commercially. A linear relationship was detected between injury at 4 DAT and glyphosate applied alone ($r^2 = 0.82$), and

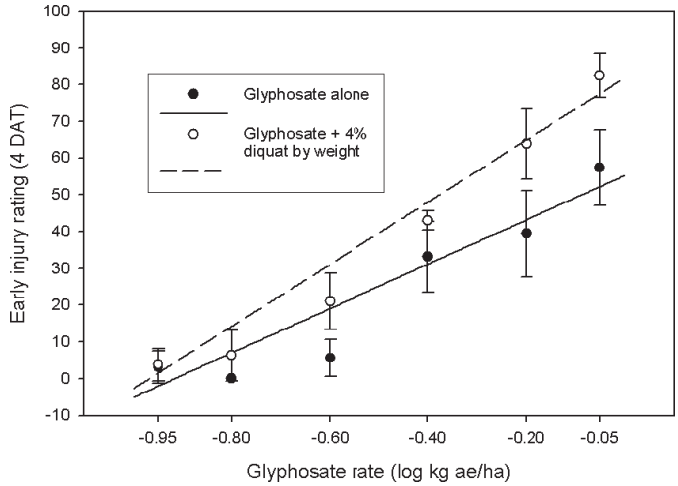

Figure 1. Linear regression of injury at 4 d after treatment on longstalked phyllanthus between glyphosate rate applied alone or in combination with 0.04 diquat (by weight); glyphosate alone, injury = 55.4 + 60.3 (log of glyphosate rate), r^2 = 0.82; glyphosate + diquat, injury = 81.9 + 84.4 (log of rate); r^2 = 0.93; glyphosate log rates of −0.95 and −0.05 = 0.11 and 1.12 kg/ha, respectively.

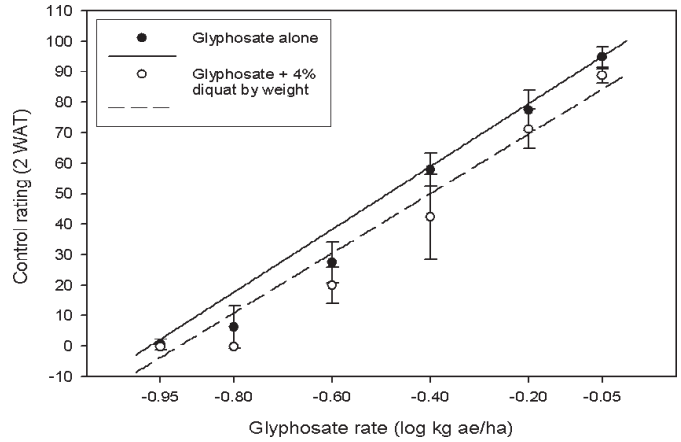

Figure 2. Linear regression of control of longstalked phyllanthus at 2 wk after treatment between glyphosate rate applied alone or in combination with 0.04 diquat (by weight); glyphosate alone, control = 100 + 103 (log of rate), r^2 = 0.93; glyphosate + diquat, control = 89 + 97 (log of rate); r^2 = 0.94; glyphosate log rates of −0.95 and −0.05 = 0.11 and 1.12 kg/ha, respectively.

glyphosate + 0.04 diquat (r^2 = 0.93; Figure 1). The procedures of Littell et al. (1991) were then used to compare these two treatment series. Within these two treatment series, glyphosate rate, diquat level, and the interaction of glyphosate rate and diquat level were all significant ($P < 0.001$), indicating that the response differed in both slope and intercept (Figure 1). Addition of diquat to glyphosate resulted in significantly more early injury compared with glyphosate alone and, thus, can be considered as a synergist to glyphosate.

Preliminary data examination again indicated a likely linear relationship between control at 2 WAT and the log of glyphosate rate for all treatment series. Furthermore, the relationship between control and glyphosate rate also appeared equivalent across all diquat levels. The procedure described by Littell et al. (1991) was used to test for homogenate of both slope and intercept within the three diquat levels. Within these three treatment series, glyphosate rate was significant ($P < 0.001$); however, neither diquat level nor the interaction of glyphosate and diquat were significant (P = 0.134 and 0.464, respectively). Thus, the regression responses for all three diquat levels were equivalent. Again, we chose to focus only upon the 0.00 and 0.04 diquat levels in further statistical analyses and presentation. A linear relationship was detected between control and glyphosate applied alone (r^2 = 0.93), and glyphosate + 0.04 diquat (r^2 = 0.94; Figure 2). The procedures of Littell et al. (1991) were then used to compare these two treatment series. Within these two treatment series, both glyphosate rate and diquat level were significant ($P < 0.001$); however, the interaction of glyphosate and diquat was not (P = 0.348; Figure 2). Thus, with respect to linear regression, the slopes were equivalent, but the intercepts differed, i.e., the lines of best fit were parallel (Figure 2). This indicates that after sufficient time for the phytotoxicity of glyphosate to be fully realized, glyphosate was more effective when applied alone.

Regrowth control could best be described by the log-logistic dose–response curve as described by Seefeldt et al. (1995). Visual comparison indicated that the response was likely equivalent for all three diquat levels. Comparisons of these three responses by the statistical technique described by Seefeldt et al.(1995) confirmed their equivalency (P = 0.60). Glyphosate applied alone was compared only to glyphosate + 0.04 diquat in subsequent analyses and presentation. Regrowth control, as a function of the log of glyphosate rate, whether applied alone or tank-mixed with diquat, could be described as a log-logistic dose response (Figure 3) (no diquat, r^2 = 0.67; with diquat, r^2 = 0.79). Comparing the two responses using the statistical technique described by Seefeldt et al.(1995) established that the responses were different ($P < 0.01$). However, the only parameter that was signifi-

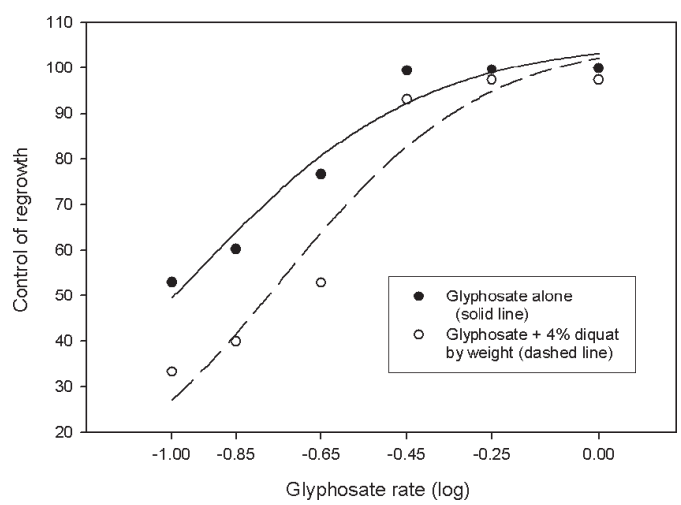

Figure 3. Log-logistic response of regrowth control of longstalked phyllanthus and glyphosate rate when applied alone or in combination with 0.04 diquat (by weight) evaluated 1 m after treatment and 2 wks after clipping; glyphosate log rates of −0.95 and −0.05 = 0.11 and 1.12 kg/ha, respectively. See Table 1 for summary.

Table 1. Parameter estimates for the log-logistic dose–response analysis obtained in comparing regrowth control between treatments using the nonlinear regression procedures as described by Seefeldt et al. (1995).

Treatment	r^2	Parameter estimates (units)[a]				
		Upper limit	Lower limit	Slope	LD_{50}[b]	LD_{50}
		% control			log of rate	kg ae /ha
Glyphosate alone	0.67	100.8	54.5	5.4	−0.93	0.12
Glyphosate + 0.04 diquat[c]	0.79	96.0	36.6	7.3	−0.72	0.19
Significance	—	NS	NS	NS	< 0.01	

[a] Abbreviation: NS, not significant.

[b] Refer to Figure 3.

[c] By weight relative to glyphosate weight.

cantly different ($P < 0.01$) was LD_{50} (i.e., −0.93 for glyphosate alone vs. −0.72 for glyphosate + 0.04 diquat; Table 1). Taking the antilog of −0.93 and −0.72 yields the rates 0.12 and 0.19 kg/ha, respectively. Thus, the glyphosate rate had to be increased by approximately 60% to compensate for the addition of diquat. However, inspection of the data (Figure 3) reveals that the diquat-based antagonism was most pronounced at lower, marginally effective rates. Conversely, the responses were equivalent, indicating no antagonism, at higher rates (≥ 0.6 kg/ha; log = −0.2).

Glyphosate Absorption and Translocation as Influenced by Diquat. Applied alone, 63.7% of the applied ^{14}C-glyphosate was recovered within tissues of the treated plant, and 39.9% of applied was recovered in areas that indicated translocation away from the treated leaflet (Table 2). When diquat was added, 78.5% of that which was applied was recovered within the treated plant, but only 6.1% was recovered away from the site of entry. With diquat, the greatest proportion of the applied ^{14}C-glyphosate remained in the treated leaflet. We speculate that the diquat resulted in rapid tissue necrosis, which served to sorb and retain the ^{14}C-glyphosate at the point of entry and, in turn, prevented entry into vascular tissues. The net result being a substantial reduction in the translocation of the absorbed ^{14}C-glyphosate away from the site of entry.

Table 2. Absorption and distribution of ^{14}C-glyphosate alone and when tank mixed with diquat in longstalked phyllanthus.[a]

Sample	^{14}C-glyphosate alone	^{14}C-glyphosate + diquat	Univariate probability
	% of applied		
Leaf wash	36.3	21.5	0.030
Treated leaflet	23.8	72.4	< 0.001
Remainder of treated leaf	6.2	1.4	< 0.001
Remainder of foliage	23.2	2.7	0.011
Roots	10.5	2.0	< 0.001
Multivariate probability	< 0.001		

[a] ^{14}C-glyphosate (with and without diquat) was applied to an individual leaflet. Glyphosate concentration simulated 0.25 kg ae/ha rate; diquat simulated 0.01 kg ai/ha, or 0.04 by weight relative to glyphosate. Plants were harvested 3 d after treatment.

Diquat can be deemed a synergist to glyphosate with respect to the production of rapid visual injury. However, diquat is antagonist with respect to long-term control and the prevention of regrowth. This antagonism can be largely attributed to reduced translocation. The theory that diquat makes glyphosate work faster is incorrect. Rapid onset of diquat-based injury is independent and inhibits the activity of glyphosate. If diquat is included, higher glyphosate rates must be used to avoid any loss of long-term control. Although higher rates are likely easily achieved in these ready-to-use products, it does represent excessive herbicide usage for a transitory benefit.

Sources of Materials

[1] Polyon 17N–6P–12K, available from Pursell Technologies Inc., 203 West 4th Street, Sylacauga, AL 35105.

[2] Micromax, O. M. Scott Corp., 14111 Scotts Lawn Road, Marysville, OH 43401.

[3] Statistical Analysis System (SAS) software, release 8.3, SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

[4] SigmaPlot 2000 for Windows, version 6.00, Systat Software Inc., 501 Canal Blvd., Suite E, Point Richmond, CA 94804-2058.

[5] Roundup PRO, 479 g ae/L of glyphosate as an isopropylamine salt, Monsanto Co., 800 North Lindberg Blvd., St Louis, MO 63167.

[6] Reglone, 240 g/L of diquat as a dibromide salt, Syngenta, P.O. Box 8353, Wilmington, DE 19803-8353.

[7] Model LS6500, Beckman Coulter, Inc., Analytical Instrument Systems, 4300 North Harbor Blvd., Fullerton, CA 92834.

[8] Model OX700, R. J. Harvey Instrument Corp. 123 Patterson St., Hillsdale, NJ 07642.

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