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# Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules

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A field study was conducted during 2000 and 2001 at Stoneville, MS, to determine the effects of isopropylamine, trimethylsulfonium (Tms), diammonium, and aminomethanamide dihydrogen tetraoxosulfate (Adt) salt formulations of glyphosate on weed control, growth, chlorophyll content, nodulation, nitrogen content, and grain yield in glyphosate-resistant soybean and to assess potential glyphosate accumulation in soybean nodules. Glyphosate-Tms and glyphosate-Adt injured soybean, and visible injury ranged from 29 to 38% 2 d after late postemergence (LPOST) application; however, soybean recovered by 14 d. Glyphosate formulations had no effect on chlorophyll content, root and shoot dry weight, or nodule number but reduced nodule biomass by 21 to 28% 14 d LPOST. Glyphosate levels in nodules from treated plants ranged from 39 to 147 ng $g^{-1}$ (dry weight), and leghemoglobin content was reduced by as much as 10%. Control of five predominant weed species 14 d after LPOST was > 83% with one application and > 96% with two applications regardless of the glyphosate salts used. Soybean yields were generally higher with two applications than with one application regardless of glyphosate formulation. These results indicate that soybean injury and inhibition of nodule development with certain glyphosate formulations can occur, but soybean has the potential to recover from glyphosate stress.

**Nomenclature:** Glyphosate; soybean, *Glycine max* (L.) Merr. 'DP 5806 RR'.

**Key words:** Chlorophyll, formulation, injury, leghemoglobin, nodules, weed control, transgenic soybean.

Glyphosate is a nonselective, broad-spectrum herbicide that is widely used, but it causes crop injury when applied directly to foliage. Transgenic soybean resistant to glyphosate was developed by stable integration of a foreign gene that codes insensitive 5-enolpyruvylshikimate-3-phosphate synthase (Delannay et al. 1995; Padgette et al. 1995). Transgenic soybean, resistant to glyphosate, represents a revolutionary breakthrough in weed control technology. Glyphosate-resistant soybean as a weed management tool has provided farmers with the opportunity and flexibility to manage a broad spectrum of weeds. There are several reasons for the rapid increase in glyphosate-resistant soybean acreage in the United States: simplicity of weed control (one herbicide controls a broad spectrum of broadleaf, grass, and sedge weeds), flexibility in glyphosate application rate (depending on weed species and growth stage) and timing (soybean emergence to flowering stage), lower herbicide cost, and lack of crop rotation restrictions (Baldwin 2000; Barnes 2000; Delannay et al. 1995; Reddy 2001).

Although transgenic soybean is resistant to glyphosate, application of glyphosate has resulted in significant soybean injury under certain conditions and with certain salt formulations of glyphosate. Glyphosate has decreased chlorophyll content (Pline et al. 1999; Reddy et al. 2000), nodule biomass and leghemoglobin content (Reddy et al. 2000), and nitrogen fixation and accumulation (King et al. 2001) in some glyphosate-resistant soybean cultivars. No significant yield reductions due to the glyphosate-resistant gene occurred in extensive field trials of transgenic soybeans (Delannay et al. 1995; Elmore et al. 2001a; Krausz and Young 2001; Nelson and Renner 1999, 2001; Reddy and Whiting 2000). However, a few recent studies have reported reduced soybean yield under stress conditions such as low water availability and in certain glyphosate-resistant soybean cultivars (Elmore et al. 2001b; King et al. 2001).

Physiological effects associated with injury caused by glyphosate application to glyphosate-resistant soybean are not fully understood. Glyphosate at 0.5 mM decreased chlorophyll content in hypocotyls of nontransgenic soybean grown in liquid culture (Hoagland 1980). Glyphosate can reduce plant growth, and it can concomitantly reduce nodulation in glyphosate-resistant soybean (King et al. 2001; Reddy et al. 2000). Reduction in nodulation can be an indirect result of glyphosate injury to the plant, from direct action of glyphosate on rhizobial symbionts or from action against both soybean and rhizobial populations (Moorman 1986). Glyphosate has been shown to affect the bacterial symbiont (*Bradyrhizobium japonicum*) of soybean through accumulation of hydroxybenzoic acids within the *B. japonicum* cells (Moorman et al. 1992). In other studies, glyphosate reduced the nitrogenase activity of *B. japonicum* bacteroids, with inhibition proportional to in vitro sensitivity of these strains under culture conditions (Hernandez et al. 1999). These researchers also demonstrated the accumulation of protocatechuic acid in soybean nodules of glyphosate-treated soybean plants, suggesting that glyphosate was translocated to nodules. However, under field conditions, glyphosate accumulation in glyphosate-resistant soybean nodules from routine glyphosate application used for weed control has not been investigated. Recently, Duke et al. (2003) found that glyphosate applied at label use rates accumulated in seed of glyphosate-resistant soybean.

Isopropylamine (Ipa) salt of glyphosate was used in development of glyphosate-resistant soybean (Delannay et al. 1995; Padgette et al. 1995). With the expiration of the glyphosate patent, several other salt formulations of glyphosate are now commercially available for use in glyphosate-resistant soybean. Trimethylsulfonium (Tms) salt of glyphosate was approved for commercial use in glyphosate-resistant soybean in 1999. Several researchers have observed glyphosate-resistant soybean injury from glyphosate-Tms (Etheridge et al. 2000; Mulkey et al. 1999). Recently, Krausz and Young (2001) reported that both glyphosate-Ipa and glyphosate-Tms caused 0 to 20% chlorosis, and in some instances, glyphosate-Ipa caused more chlorosis than did glyphosate-Tms. In 2001, glyphosate-Tms was discontinued, and a diammonium (Dia) salt of glyphosate was commercialized for use in glyphosate-resistant soybean. Aminomethanamide dihydrogen tetraoxosulfate (Adt) salt of glyphosate also debuted in 2001 for noncrop use.

Effects of these newer salt formulations of glyphosate on glyphosate-resistant soybean growth and nodulation parameters under field conditions merit investigation. The objectives of this study were to compare the effects of Ipa, Tms, Dia, and Adt salts of glyphosate on (1) weed control, (2) glyphosate-resistant soybean injury, chlorophyll content, nodulation, nodule leghemoglobin content, nitrogen content, and yield, and (3) glyphosate accumulation in soybean nodules.

## Materials and Methods

### Field Study Description

Research was conducted in 2000 and 2001 at the USDA Southern Weed Science Research Unit farm, Stoneville, MS (33°N latitude). The soil was a Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) with pH 6.3, 1.1% organic matter, a cation exchange capacity of 15 cmol $kg^{-1}$, and soil textural fractions of 26% sand, 56% silt, and 18% clay, and it contained an abundant native population of *B. japonicum*. The experimental area was naturally infested with barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], browntop millet [*Brachiaria ramosa* (L.) Stapf], pitted morningglory (*Ipomoea lacunosa* L.), smooth pigweed (*Amaranthus hybridus* L.), and yellow nutsedge (*Cyperus esculentus* L.). Field preparation consisted of tillage in the fall and spring with a disk harrow and in spring with a field cultivator before planting. Soybean cultivar DP 5806 RR was planted on May 17, 2000 and May 1, 2001. A different experimental site with similar soil conditions was used in 2001.

Herbicide treatments consisted of early postemergence (EPOST) or EPOST followed by late postemergence (LPOST) applications of four glyphosate salt formulations. The commercial formulations of Ipa,[1] Tms,[2] Dia,[3] and Adt[4] salts of glyphosate were used. Glyphosate EPOST was applied at 0.84 kg ae $ha^{-1}$ at the one-trifoliolate leaf stage (14 d after planting). Glyphosate LPOST was applied at 0.84 kg ae $ha^{-1}$ at the three- to four-trifoliolate leaf stage (28 d after planting). These early growth stages were selected for glyphosate treatment to accentuate any effects on soybean growth and nodulation. An untreated control was included. No preemergence herbicides were used in the study. Herbicide treatments were applied with a tractor-mounted sprayer with TeeJet 8004 standard flat spray tips[5] delivering 187 L $ha^{-1}$ water at 179 kPa. A nonionic surfactant[6] at 0.5% (v/v) was added to Adt salt formulation of glyphosate as suggested by the manufacturer. No surfactant was added to Ipa, Tms, and Dia salt formulations of glyphosate because the labels do not explicitly recommend surfactant additions.

### Chlorophyll

Chlorophyll measurements were made on the fifth trifoliolate leaf 9 d after LPOST and on the ninth trifoliolate leaf 23 d after LPOST. Distal leaflets of the fifth and ninth trifoliolates were sampled from three randomly selected soybean plants in each plot. Chlorophyll was extracted using the method of Hiscox and Israelstam (1979). Excised leaf tissue was soaked in 10 ml of dimethyl-sulfoxide for 24 h in the dark to extract chlorophyll, and chlorophyll concentration was determined spectrophotometrically. Chlorophyll content was calculated using the equation of Arnon (1949) and expressed as milligrams of chlorophyll per gram of leaf tissue (fresh weight).

### Soybean Shoot and Root Weight

Ten soybean plants were randomly sampled from each plot 14 d after LPOST. After the plants were dug, with roots intact, shoots were excised for dry weight determination. The roots were stored in a plastic bag and transported to the laboratory on ice. Roots were collected by washing off soil with water, and nodules were harvested on the same day of field sampling. Both shoot and roots were oven dried at 70 C for 72 h, and the weights were recorded.

### Nodule Parameters

Ten plants sampled 14 d after LPOST, as described above, also were used for characterizing nodule parameters. Nodules were harvested after roots were washed with water to remove soil and counted, and then fresh weights were recorded. Nodule samples were homogenized in aliquots of Drabkin's reagent[7] (1:1 to 1:2 ratio; w/v) and leghemoglobin quantified spectrophotometrically at $A_{540}$ as described by Wilson and Reisenauer (1963). Human hemoglobin[7] was used as a standard, and values are expressed as milligrams per gram of nodule mass (fresh weight).

### Glyphosate Assay

Nodules collected from the 10 plants sampled 14 d after LPOST, as above, were used to determine the presence of glyphosate. Briefly, nodules (1 g fresh weight) and 5 ml of 20% aqueous methanol were added to glass centrifuge tubes, macerated with a glass rod, and sealed with a teflon-lined cap. Samples were extracted on a rotary shaker (125 rpm, room temperature) for 1 h, centrifuged at 5,900 × *g* for 10 min, and the supernatant filtered using a Gilman Acrodisc (0.2 μm) syringe filter.[8] Glyphosate was quantified on a duplicate 50-μl aliquot of extract by enzyme-linked immunosorbent assay,[9] using protocols developed by the man-

TABLE 1. Effect of glyphosate-Ipa, glyphosate-Tms, glyphosate-Dia, and glyphosate-Adt on glyphosate-resistant soybean [*Glycine max* (L.) Merr.] injury and yield.[a,b]

| Glyphosate formulation | Application timing | Soybean injury: Days after EPOST 2 | Soybean injury: Days after EPOST 12 | Soybean injury: Days after LPOST 2 | Soybean injury: Days after LPOST 14 | Soybean yield |
|---|---|---|---|---|---|---|
| | | % | | | | kg ha$^{-1}$ |
| No herbicide | | 0 | 0 | 0 | 0 | 720 |
| Glyphosate-Ipa | EPOST | 0 | 0 | 0 | 0 | 1,540 |
| | EPOST + LPOST | 0 | 0 | 1 | 0 | 1,630 |
| Glyphosate-Tms | EPOST | 14 | 5 | 0 | 0 | 1,490 |
| | EPOST + LPOST | 14 | 5 | 29 | 0 | 1,750 |
| Glyphosate-Dia | EPOST | 0 | 0 | 0 | 0 | 1,480 |
| | EPOST + LPOST | 0 | 0 | 0 | 0 | 1,620 |
| Glyphosate-Adt | EPOST | 8 | 4 | 0 | 0 | 1,510 |
| | EPOST + LPOST | 9 | 5 | 38 | 0 | 1,700 |
| LSD (0.05) | | 3 | 1 | 3 | — | 180 |

[a] Abbreviations: Ipa, isopropylamine; Tms, trimethylsulfonium; Dia, diammonium; Adt, aminomethanamide dihydrogen tetraoxosulfate; EPOST, early postemergence; LPOST, late postemergence.

[b] Data represent the average of the years 2000 and 2001.

ufacturer. The aliquot was derivatized and incubated with enzyme conjugate and a suspension of paramagnetic particles containing antibodies specific to glyphosate in disposable plastic tubes. A competitive reaction occurred between the glyphosate in the sample and the enzyme-labeled glyphosate analog for the antibody-binding sites on the magnetic particles. After 30 min of incubation, the reaction tubes were placed in a magnetic rack to sediment the paramagnetic particles (with glyphosate bound to the antibodies on the particles). Unbound conjugate and reagents were decanted, and the particles were washed three times. Glyphosate concentrations were determined after incubation with substrate solution, termination of the reaction, and measurement of absorbance at 450 nm. The range of detection of this assay was 4 to 100 ppb, with a sensitivity of 0.8 ppb. Glyphosate concentration in nodules was expressed as a dry weight equivalent. Because the glyphosate kit debuted in 2001, glyphosate was only determined in nodules for the 2001 experiment.

### Nitrogen Content

The fifth trifoliolate leaf 9 d after LPOST and the ninth trifoliolate leaf 23 d after LPOST were sampled from 10 randomly selected soybean plants in each plot in both years. Soybean grain from each plot was sampled at harvest only in 2001. Oven-dried leaves and grain were ground, and total nitrogen was determined in the samples by the Kjeldahl method (Baker and Thompson 1992). Nitrogen analysis was made at the Plant Analysis Laboratory, University of Arkansas, Fayetteville, AR. Total nitrogen was expressed as milligrams of nitrogen per gram of leaf tissue (dry weight).

### Weed Control and Soybean Injury and Yield

Soybean injury (yellowing, speckling, and necrosis) and control of individual weed species were visually estimated on a scale of 0 (no soybean injury or no weed control) to 100% (soybean death or complete weed control). Soybean was harvested from the entire plot using a combine, and grain yield was adjusted to 13% moisture.

### Statistical Analysis

Each treatment consisted of eight 12.2-m soybean rows spaced 51 cm apart. Treatments were arranged in a randomized complete block design with four replications. The data were subjected to analysis of variance using Proc Mixed, and the least squares means were calculated (SAS 1998). Treatment means were separated at the 5% level of significance using Fisher's LSD test. Data were averaged across years because treatment by year interactions were not significant.

## Results and Discussion

### Soybean Injury

Glyphosate-Tms and glyphosate-Adt injured soybean, and visible injury (yellowing, speckling, and necrosis) ranged from 8 to 14% 2 d after EPOST and from 29 to 38% 2 d after LPOST (Table 1). Soybean injury symptoms differed between glyphosate-Tms and glyphosate-Adt. Glyphosate-Adt caused necrosis within hours of application, and glyphosate-Tms caused yellowing and bleaching usually within a day or two. Soybean injury decreased over time, and soybean completely recovered from injury 14 d after LPOST. Apparently, yellowing, speckling, and necrosis were restricted to leaves that intercepted glyphosate spray because new growth after application did not exhibit injury. Because glyphosate is readily translocated to metabolic sinks (Duke 1988) such as young leaves, rapid development of injury symptoms after application and absence of injury symptoms in new growth after glyphosate application suggest that injury in soybean may be due to the Tms and Adt salt portion of the formulation of glyphosate rather than due to glyphosate itself. Krausz and Young (2001) reported no chlorosis from glyphosate-Tms and glyphosate-Adt at label use rates, but they reported chlorosis at higher rates or at applications beyond the four-trifoliolate growth stage. Glyphosate-Ipa and glyphosate-Dia resulted in no crop injury in glyphosate-resistant soybean.

TABLE 2. Effect of glyphosate-Ipa, glyphosate-Tms, glyphosate-Dia, and glyphosate-Adt on number, biomass, leghemoglobin content, and glyphosate content of nodules 14 d after LPOST in glyphosate-resistant soybean [*Glycine max* (L.) Merr.].[a,b]

| Glyphosate formulation | Application timing | Nodule | | | |
|---|---|---|---|---|---|
| | | Number | Fresh weight | Leghemoglobin | Glyphosate |
| | | no. plant$^{-1}$ | mg plant$^{-1}$ | mg g$^{-1}$ fresh tissue | ng g$^{-1}$ dry tissue |
| No herbicide | | 41 | 706 | 9.65 | 9 |
| Glyphosate-Ipa | EPOST | 33 | 544 | 9.54 | 58 |
| | EPOST + LPOST | 35 | 524 | 8.68 | 147 |
| Glyphosate-Tms | EPOST | 30 | 521 | 9.40 | 79 |
| | EPOST + LPOST | 35 | 508 | 8.84 | 39 |
| Glyphosate-Dia | EPOST | 39 | 524 | 9.47 | 67 |
| | EPOST + LPOST | 35 | 536 | 8.79 | 123 |
| Glyphosate-Adt | EPOST | 36 | 556 | 8.81 | 75 |
| | EPOST + LPOST | 34 | 529 | 8.82 | 47 |
| LSD (0.05) | | NS | 146 | 0.80 | 78 |

[a] Abbreviations: Ipa, isopropylamine; Tms, trimethylsulfonium; Dia, diammonium; Adt, aminomethanamide dihydrogen tetraoxosulfate; EPOST, early postemergence; LPOST, late postemergence; NS, not significant.
[b] Data represent the average of the years 2000 and 2001, except that glyphosate data is for 2001 only.

## Chlorophyll

Chlorophyll content of the fifth trifoliolate (9 d after LPOST) and the ninth trifoliolate (23 d after LPOST) leaves of untreated plants was 2.03 and 2.42 mg g$^{-1}$ leaf, respectively. Glyphosate had no effect on chlorophyll content of the fifth and ninth trifoliolate leaves regardless of number of applications or formulation (data not shown). Under greenhouse conditions, a reduction in chlorophyll content in glyphosate-resistant soybean has been reported at rates above 2 kg ai ha$^{-1}$ (Pline et al. 1999; Reddy et al. 2000).

## Soybean Shoot and Root Dry Weight

Dry weight of shoots and roots of untreated plants was 5.5 and 0.8 g per plant, respectively, 14 d after LPOST. Shoot and root dry weights of glyphosate-resistant soybean 14 d after LPOST were unaffected by glyphosate regardless of number of applications or formulation (data not shown). In greenhouse studies, glyphosate at 2.24 kg ai ha$^{-1}$ (Reddy et al. 2000) and 8.4 kg ai ha$^{-1}$ (King et al. 2001) reduced shoot and root dry weights of glyphosate-resistant soybean.

## Nodule Parameters

Glyphosate-Ipa, glyphosate-Tms, glyphosate-Dia, and glyphosate-Adt had no effect on nodule number, but they reduced nodule biomass compared with the untreated control 14 d after LPOST (Table 2). One application of glyphosate-Ipa, glyphosate-Tms, and glyphosate-Dia had no effect on leghemoglobin content, but two applications reduced leghemoglobin content by 8 to 10% compared with the untreated control. However, both applications of glyphosate-Adt reduced leghemoglobin content by 9% compared with the untreated control. Reductions in both nodule mass and leghemoglobin content indicate that glyphosate did not affect nodule initiation and formation, but it inhibited nodule development. In this study, the glyphosate effects on nodulation were compared between soybeans treated with various glyphosate salts providing excellent weed control and soybeans in a weedy plot that received no herbicide. Nodulation and nitrogen fixation in legumes can be inhibited if subjected to water and light stress (Sprent 1976). In this study, the low density of small weeds present in glyphosate-treated plots at the time of nodulation assessment would have had minimal effect on shading of soybean and reduction of photosynthesis. Thus, these weeds had negligible impact on nodulation. Conversely, the high density of large weeds present in the untreated control plots may have caused competition for water and light and reduced nodulation in soybeans. Therefore, differences in nodulation directly attributable to glyphosate would likely have been greater if comparisons were made between glyphosate treatments and a hand-weeded no herbicide control. Other studies under greenhouse conditions have shown that glyphosate applied above 2.24 kg ha$^{-1}$ reduced nodulation parameters in glyphosate-resistant soybean that was grown weed-free (King et al. 2001; Reddy et al. 2000).

## Glyphosate Accumulation in Soybean Nodules

Glyphosate was observed in nodules regardless of the number of applications and formulations, and the concentration ranged from 39 to 147 ng g$^{-1}$ nodule dry weight (Table 2). Movement of glyphosate to metabolic sinks such as developing and mature nodules is not surprising. Two applications of glyphosate-Ipa had higher glyphosate concentration in nodules than two applications of glyphosate-Tms and glyphosate-Adt. Similarly, two applications of glyphosate-Dia had higher glyphosate concentration in nodules than two applications of glyphosate-Tms. These results may have been due to decreased movement of photosynthates in soybean plants that were under stress from glyphosate-Tms and glyphosate-Adt applications (Table 1). Glyphosate accumulation in soybean grain (0.1 to 3.1 $\mu$g g$^{-1}$) due to label use rate of glyphosate in glyphosate-resistant soybean has been reported recently (Duke et al. 2003). Glyphosate does cause accumulation of certain benzoic acids in nodules of glyphosate-treated soybean (Hernandez et al. 1999) and *B. japonicum* cells under culture conditions (Moorman et al. 1992). Glyphosate concentration of less than 1 mM was inhibitory and that greater than 5 mM was lethal to *B. japonicum* (Moorman et al. 1992). The combination of *B. japonicum* sensitivity and potential accumulation of gly-

Table 3. Effect of glyphosate-Ipa, glyphosate-Tms, glyphosate-Dia, and glyphosate-Adt on leaf and grain nitrogen content in glyphosate-resistant soybean [*Glycine max* (L.) Merr.].[a,b]

| Glyphosate formulation | Application timing | Nitrogen content: Fifth trifoliolate leaf | Ninth trifoliolate leaf | Soybean grain |
|---|---|---|---|---|
| | | mg $g^{-1}$ dry tissue | | |
| No herbicide | | 42.4 | 51.5 | 67.3 |
| Glyphosate-Ipa | EPOST | 46.5 | 51.1 | 65.7 |
| | EPOST + LPOST | 45.8 | 51.0 | 63.7 |
| Glyphosate-Tms | EPOST | 45.1 | 51.7 | 66.5 |
| | EPOST + LPOST | 46.4 | 51.3 | 64.8 |
| Glyphosate-Dia | EPOST | 48.1 | 51.0 | 65.6 |
| | EPOST + LPOST | 45.9 | 51.2 | 65.2 |
| Glyphosate-Adt | EPOST | 45.8 | 52.4 | 65.8 |
| | EPOST + LPOST | 47.7 | 51.2 | 66.8 |
| LSD (0.05) | | 2.7 | NS | 1.9 |

[a] Abbreviations: Ipa, isopropylamine; Tms, trimethylsulfonium; Dia, diammonium; Adt, aminomethanamide dihydrogen tetraoxosulfate; EPOST, early postemergence; LPOST, late postemergence; NS, not significant.

[b] Data represent the average of the years 2000 and 2001, except that nitrogen content in soybean grain is for 2001 only.

phosate or benzoic acids could interfere with nodule development and nitrogen fixation in soybean. King et al. (2001) have observed decreased nodule biomass and delayed nitrogen fixation in glyphosate-resistant soybean treated with glyphosate. Glyphosate also was detected in nodules from untreated plants (one of four replicates) but at a concentration less than 10 ng $g^{-1}$. This may have been due to drift from glyphosate application made around the experimental site.

## Nitrogen Content

Nitrogen content of the fifth trifoliolate leaf was higher with glyphosate application than in the untreated control (Table 3). One exception was treatment with one application of glyphosate-Tms, which was similar to the untreated control. Glyphosate had no effect on nitrogen content of the ninth trifoliolate leaf regardless of number of applications or formulation type. This is in agreement with soybean injury (Table 1) and chlorophyl content (see the Chlorophyll section) data that indicate recovery of stressed soybeans after treatment with glyphosate-Tms and glyphosate-Adt formulations.

## Weed Control

Control of pitted morningglory, smooth pigweed, and yellow nutsedge was $> 94\%$ 14 d after LPOST regardless of number of applications or formulation (Table 4). Barnyardgrass control was higher with two than with one application of glyphosate-Ipa, glyphosate-Tms, and glyphosate-Dia. Control of browntop millet was lower with one application (83 to 89%) than with two applications ($> 99\%$) regardless of glyphosate salt formulations. However, in a greenhouse study, Norris et al. (2001) found slightly higher levels of barnyardgrass and pitted morningglory control with glyphosate-Tms than with glyphosate-Ipa.

## Soybean Yield and Nitrogen Content

Soybean yields were higher with all glyphosate formulations than with the untreated control (Table 1). Soybean

Table 4. Barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], browntop millet [*Brachiaria ramose* (L.) Stapf], pitted morningglory (*Ipomoea lacunose* L.), smooth pigweed (*Amaranthus hybridus* L.), and yellow nutsedge (*Cyperus esculentus* L.) control 14 d after LPOST with glyphosate-Ipa, glyphosate-Tms, glyphosate-Dia, and glyphosate-Adt in glyphosate-resistant soybean [*Glycine max* (L.) Merr.].[a,b]

| Glyphosate formulation | Application timing | Control: Barnyardgrass | Browntop millet | Pitted morningglory | Smooth pigweed | Yellow nutsedge |
|---|---|---|---|---|---|---|
| | | % | | | | |
| Glyphosate-Ipa | EPOST | 91 | 83 | 98 | 96 | 94 |
| | EPOST + LPOST | 100 | 100 | 99 | 100 | 98 |
| Glyphosate-Tms | EPOST | 90 | 84 | 96 | 99 | 95 |
| | EPOST + LPOST | 100 | 100 | 100 | 100 | 100 |
| Glyphosate-Dia | EPOST | 93 | 85 | 95 | 100 | 95 |
| | EPOST + LPOST | 100 | 100 | 100 | 100 | 96 |
| Glyphosate-Adt | EPOST | 95 | 89 | 94 | 98 | 96 |
| | EPOST + LPOST | 100 | 99 | 100 | 100 | 99 |
| LSD (0.05) | | 6 | 6 | NS | NS | NS |

[a] Abbreviations: Ipa, isopropylamine; Tms, trimethylsulfonium; Dia, diammonium; Adt, aminomethanamide dihydrogen tetraoxosulfate; EPOST, early postemergence; LPOST, late postemergence; NS, not significant.

[b] Data represent the average of the years 2000 and 2001.

yields were similar between one and two applications of glyphosate-Ipa and glyphosate-Dia. Soybean yields were higher with two applications of glyphosate-Tms and glyphosate-Adt than with one application of glyphosate regardless of formulations, with the exception of glyphosate-Ipa. Interestingly, these same formulations had caused greater soybean injury 14 d after LPOST. Nitrogen content of soybean grain was assessed only in 2001, and it ranged from 63.7 to 67.3 mg $g^{-1}$ grain (dry weight) among treatments (Table 3). Despite the narrow range, nitrogen content was slightly lower with two applications of glyphosate-Ipa, glyphosate-Tms, and glyphosate-Dia when compared with the untreated control.

The presence of weeds in this study may have confounded the effects of glyphosate on soybean yield and nitrogen content. Competition for light, nutrients, and moisture from weeds may have limited photosynthesis and subsequent yield potential. Glyphosate effects on both *Bradyrhizobium* and endomycorrhizal symbiosis in glyphosate-resistant soybean in the absence of weeds under field conditions needs further investigation. However, glyphosate effects on the bacterial symbiont (*B. japonicum*) of soybean, nodule parameters, and $N_2$ fixation activity have been studied under culture and greenhouse conditions. Furthermore, it is important to note that several researchers, including the authors, have observed injury when glyphosate was used for weed control at label rates in glyphosate-resistant soybean. Also, it is unlikely that soybean producers will apply glyphosate for anything but weed control. Therefore, the focus of this study was to assess changes in physiological parameters in glyphosate-resistant soybean with changes in weed populations as a consequence of glyphosate usage for weed control.

This study demonstrated that label use rates of glyphosate-Ipa, glyphosate-Tms, glyphosate-Dia, and glyphosate-Adt in a glyphosate-resistant soybean cultivar caused subtle reductions in nodulation parameters. Visible injury to soybean from glyphosate-Tms and glyphosate-Adt was restricted to leaves that intercepted spray, and new growth after application was completely devoid of injury. Glyphosate salt formulation had no effect on nodule number but reduced nodule biomass compared with the untreated control. Soybean yields were similar for one and two applications of glyphosate-Ipa and glyphosate-Dia. Soybean yields were higher with two applications of glyphosate-Tms and glyphosate-Adt than with one application of glyphosate regardless of formulations, with the exception of glyphosate-Ipa. Currently, hundreds of glyphosate-resistant cultivars from different maturity groups are commercially available. The physiological and yield responses of these cultivars to different salts of glyphosate may vary and also may depend on geographical location, environmental conditions, soil types, sensitivity of native populations of *B. japonicum*, etc. However, most soybean farmers in the midsouthern United States do not use supplemental rhizobium culture or nitrogen fertilizer in soybean production. Results of Hernandez et al. (1999) suggest that use of a tolerant rhizobial strain may overcome glyphosate interference with soybean symbiosis; however, U.S. soils typically contain abundant *B. japonicum* populations, and introduced strains offer little competition for formation of nodules on soybean (Berg et al. 1988; Klubeck et al. 1988). Extensive research under a wide range of environments indicated no yield reductions due to glyphosate application on glyphosate-resistant soybean (Delannay et al. 1995; Elmore et al. 2001a; Krausz and Young 2001; Nelson and Renner 1999, 2001; Reddy and Whiting 2000). These results indicate that soybean injury and reduction in nodulation with certain glyphosate formulations can occur, but soybean has the potential to recover from glyphosate stress.

## Sources of Materials

[1] Roundup Ultra®, isopropylamine salt of glyphosate, Monsanto Agricultural Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167.

[2] Touchdown® 5, trimethylsulfonium salt of glyphosate, Syngenta Crop Protection, 410 Swing Road, Greensboro, NC 27419.

[3] Touchdown® IQ, diammonium salt of glyphosate, Syngenta Crop Protection, 410 Swing Road, Greensboro, NC 27419.

[4] Engame™, 1-aminomethanamide dihydrogen tetraoxosulfate salt of glyphosate, Entek, 6835 Deerpath Road, Elkridge, MD 21075.

[5] TeeJet 8004 standard flat spray tips, Spraying Systems Co., North Avenue and Schmale Road, Wheaton, IL 60189.

[6] Induce® nonionic low foam wetter/spreader adjuvant contains 90% nonionic surfactant (alkylaryl and alcohol ethoxylate surfactants) and fatty acids and 10% water, Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38119.

[7] Drabkin's reagent and human hemoglobin, Sigma Chemical Company, 3050 Spruce Street, St. Louis, MO 63178.

[8] Gelman Acrodisc PVDF syringe filters, Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219.

[9] Glyphosate high-sensitivity kit, Abraxis LLC, 2935 Byberry Road, Hatboro, PA 19040.

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