Glyphosate Efficacy, Absorption, and Translocation in Selected Four Weed Species Common to Florida Citrus

Megh Singh, Shiv D. Sharma, Analiza H.M. Ramirez, and Amit J. Jhala[1]

ADDITIONAL INDEX WORDS. ^{14}C-glyphosate absorption, biomass reduction, glyphosate tolerance, leaf wax content, percent control, surfactant

SUMMARY. Glyphosate is the most widely used herbicide for postemergence weed control in Florida citrus (*Citrus* spp.). Variation in susceptibility of certain weed species to glyphosate has been observed in last few years. Therefore, understanding the mechanism underlying such phenomenon is required. Experiments were conducted to evaluate differences in tolerance of four weed species to glyphosate by quantifying glyphosate efficacy, the amount of epicuticular wax, absorption, and translocation of carbon-14-labeled glyphosate (^{14}C glyphosate). The results of glyphosate efficacy study suggested that application of glyphosate at 3 oz/acre resulted in 99%, 90%, and 84% control of florida beggarweed (*Desmodium tortuosum*), spanishneedles (*Bidens bipinnata*), and johnsongrass (*Sorghum halepense*), respectively. Increasing application rate and addition of nonionic surfactant (NIS) usually did not improve glyphosate efficacy. Ivyleaf morningglory (*Ipomoea hederacea*) was the most tolerant and resulted in 0% and 25% control when glyphosate applied at 3 and 24 oz/acre, respectively. Biomass reduction in all weed species reflected a similar trend to percent control in response to all glyphosate treatments. Glyphosate absorption and translocation in the weed species were differed with the quantity of wax extracted. Ivyleaf morningglory had the lowest leaf wax content (10.8 μg·cm^{-2}) and showed less absorption (62% to 79%) and translocation (15% to 39%) of ^{14}C-glyphosate compared with other weed species. The absorption of ^{14}C-glyphosate was in the range of 87%, 71% to 83%, and 72% to 83%; and translocation was 34% to 50%, 32% to 52%, and 53% to 58% in florida beggarweed, spanishneedles, and johnsongrass, respectively. Increasing glyphosate application rate from 6 to 12 oz/acre and addition of NIS usually increased ^{14}C-glyphosate translocation.

Glyphosate, a foliar applied, broad-spectrum herbicide was discovered in 1970 and commercialized in 1974 (Franz et al., 1996). Since then, glyphosate use has increased rapidly, and it has become the most popular herbicide worldwide (Baylis, 2000; Duke and Powles, 2008). The combination of effective control of many annual and perennial weed species, a favorable environmental profile, and economical cost to the growers has resulted in the widespread use of glyphosate for weed control. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase [EPSPS (EC 2.5.1.19)], resulting in shikimate accumulation and reduced production of aromatic amino acids (Schönburnn et al., 2001). Before commercialization of glyphosate-resistant crops in 1996, glyphosate was primarily used for pre-plant burndown of weeds as a nonselective herbicide (Dill, 2005). However, a rapid adoption of glyphosate-resistant crops including but not limited to glyphosate-resistant soybean (*Glycine max*), cotton (*Gossypium hirsutum*), and maize (*Zea mays*) in the United States has resulted in widespread use of glyphosate as a selective in-crop herbicide (Fernandez-Cornejo and McBride, 2000; Green, 2009).

Over-reliance on a single herbicide or herbicide with same mode of action could result in loss of efficacy on certain weeds because of selection pressure which results in evolution of herbicide resistant weeds (Powles, 2008). Currently, 21 weed species have evolved resistant to glyphosate worldwide (Heap, 2011). The evolution of glyphosate-resistant weeds in the United States and many other countries led to an increased need for a program for integrated management of glyphosate-resistant weeds (Beckie, 2006). Growers usually adopt integrated weed management strategies only after development of herbicide resistance. Weed control can cost significantly more once herbicide resistant weeds fully developed especially when few alternative herbicides are available (Orson, 1999).

Florida is the largest producer of citrus in the United States. In 2010, citrus was grown on more than 550,000 acres in Florida [U.S. Department of Agriculture (USDA), 2010a] with the production of ≈159 million boxes, which accounted for ≈65% of the total U.S. production (USDA, 2010b). Because of warm weather and relatively higher rainfall, weed control is an important aspect for citrus growers in Florida. Because of its broad weed spectrum and relatively low cost, glyphosate is the most commonly used herbicide in citrus groves (Sharma and Singh, 2007). There is no confirmed report of glyphosate-resistant weed in Florida citrus; however, some citrus growers are facing problem of reduced efficacy of glyphosate for controlling certain weed species. For example, brazil pusley (*Richardia brasiliensis*), day flower (*Commelina benghalensis*), and milkweed vine (*Morrenia odorata*) are problem weeds in citrus groves, and glyphosate does not provide adequate control of these species.

Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850-2299

[1]Corresponding author. E-mail: amit@ufl.edu.

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
10	%	g·L^{-1}	0.1
0.4047	acre(s)	ha	2.4711
29,574	fl oz	μL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
9.3540	gal/acre	L·ha^{-1}	0.1069
2.54	inch(es)	cm	0.3937
70.0532	oz/acre	g·ha^{-1}	0.0143
4.3942×10^6	oz/inch^2	μg·cm^{-2}	2.2757×10^{-7}
6.8948	psi	kPa	0.1450
(°F − 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

There are many factors affecting differential tolerance of weed species to glyphosate. Differences in susceptibility of weed species to glyphosate could be due to mutations of the EPSPS gene, lower absorption rate, and reduced glyphosate translocation (Powles and Preston, 2006). Decreased absorption rate may be due to the physical differences, chemical differences, or both in the plant cuticle (Norsworthy et al., 2001). The primary barrier to glyphosate absorption is the leaf cuticle, composed of a lipophilic waxy layer (Kirkwood, 1991). Glyphosate is negatively charged and cannot effectively penetrate the waxy layer in certain species (Franz, 1985). For example, tolerance of hemp dogbane (*Apocynum cannabinum*) and leafy spurge (*Euphorbia esula*) was due to low glyphosate absorption (Wyrill and Burnside, 1976).

The absorption and translocation of glyphosate are greatly enhanced by the addition of appropriate adjuvants (Sharma et al., 1996; Sharma and Singh, 1999). Relatively higher glyphosate absorption in certain weed species might be due to less epicuticular wax and presence of stomata and trachoma on the adaxial leaf surface (Wyrill and Burnside, 1976). Addition of NIS to glyphosate may increase herbicidal activity (Kirkwood, 1993; Riechers et al., 1994). It is speculated that the plasma membrane is a barrier to the foliar uptake of glyphosate and biologically active surfactants contribute to glyphosate flux across the plasma membrane (Riechers et al., 1994). On the other hand, it was reported that removing the epicuticular wax from milkweed (*Asclepias syriaca*) and hemp dogbane leaves did not increase glyphosate absorption (Wyrill and Burnside, 1976). Therefore, there is a need to fully understand the mechanism of tolerance of different weed species to glyphosate based on absorption and translocation studies.

The change in relative frequency of weeds within a species (abundance) or among species (diversity) in response to weed management tactics is referred to as weed species shifts (Reddy and Norsworthy, 2010). About 90% of citrus groves are treated with glyphosate and weed species shift would affect all glyphosate users. Weed shifts have been seen in citrus groves in Florida (personal observation) and is a step before resistance. In recent years, lower activity

of glyphosate has been observed on some citrus weeds such as goatweed (*Scoparia dulcis*), hairy beggarticks (*Bidens pilosa*), ivyleaf morningglory, and florida beggarweed (personal observation). Although glyphosate-resistant weed is not documented in Florida citrus yet, it is likely that because of extensive use of glyphosate and reduced susceptibility of certain weed species to glyphosate, it may appear in near future. Both, the weed species shifts and evolution of resistance require careful management practices and typically increases weed control costs for growers.

Research was required to study the differences in weed tolerance to glyphosate which might involve different mechanisms when glyphosate applied at various rates with and without adjuvants. The objectives of this study were 1) to examine whether rates of glyphosate and addition of NIS increased efficacy to control selected four weed species: florida beggarweed, ivyleaf morningglory, johnsongrass, and spanishneedles, 2) to investigate whether the differences in glyphosate tolerance in four weed species were due to reduction in absorption and translocation, and 3) to verify the correlation between leaf wax content and glyphosate absorption.

Materials and methods

PLANT MATERIALS. Seeds of selected four weed species including florida beggarweed, ivyleaf morningglory, johnsongrass, and spanishneedles were collected from a citrus grove near Lake Alfred, FL. These weed species were chosen because they are problem weeds in Florida citrus, their variability in susceptibility to glyphosate, and their apparent differences in leaf surface characteristics. Experiments were conducted under greenhouse conditions at the Citrus Research and Education Center, University of Florida, Lake Alfred. Six to eight seeds of each weed species were planted in a 15 × 10-cm plastic pots containing commercial potting mix (Conrad Fafard, Agawam, MA). The pots were kept in a greenhouse, maintained at 25/16 °C (± 0.5 °C) day/night temperature, 70% (± 5%) day/night relative humidity (RH), and a light intensity of 250 μmol·m^{-2}·s^{-1} photosynthetically active radiation (*PAR*). Plants of each weed species used in all studies were watered regularly and fertilized once (2 weeks

after planting) with 20N–8.7P–16.6K fertilizer (Tracite; Helena Chemical Co., Collierville, TN) to promote optimum plant growth.

HERBICIDE AND SURFACTANT. Glyphosate (Roundup Original; Monsanto, St. Louis) was used in all experiments. The adjuvant Ortho X-77 (Chevron Chemical Co., Richmond, CA), a mixture of alkylaryl polyoxyethylene glycols, was used as NIS and added to glyphosate at 0.25% v/v. Glyphosate at 6 and 12 oz/acre applied alone and with NIS (0.25% v/v) was evaluated for a ^{14}C-glyphosate absorption and translocation study. In a radiolabel study, ^{14}C-glyphosate isopropylamine salt with 23.87 curies per mole specific activity was used. The solubility of glyphosate isopropylamine salt was 900 g·L^{-1} at pH 7, water temperature of 25 °C, and octanol–water partition coefficient (K_{ow}) of 0.0006–0.0017 (Vencill, 2002).

EFFICACY STUDY. To examine the effects of different rates of glyphosate with and without NIS on control of four weed species, glyphosate was applied at 3, 6, 12, and 24 oz/acre alone or with NIS (Ortho X-77 at 0.25 v/v) using a chamber track sprayer fitted with a flat fan spray nozzle (Teejet 8002; Spraying System Co., Wheaton, IL) delivering 20 gal/acre at 30 psi. Deionized water was used as a carrier for all spray solution. All species were thinned to four plants per pot. Glyphosate treatments were applied to 15-cm-tall (three to four leaves) ivyleaf morningglory, 12-cm-tall (four leaves) florida beggarweed, 10-cm-tall (four leaves) spanishneedles, and 12- to 15-cm-tall (three to four leaves) johnsongrass. Percent control was estimated visually at 14 d after treatment (DAT) based on a scale of 0% to 100%, where 0% indicates no damage and 100% indicates complete plant death (Frans et al., 1986). Aboveground biomass from each pot was harvested by cutting the plants from the base at 14 DAT, and fresh weight was recorded. Harvested samples were oven dried at 40 °C for 10 d, and dry weight of each weed species (weed biomass) was recorded. Percent biomass reduction over untreated control was calculated using the following equation: percent biomass reduction = $[(a - b)/a] \times 100$, where a is biomass of untreated plants and b is biomass of the treated plants.

ABSORPTION AND TRANSLOCATION. One week before applying ^{14}C

treatments, plants were transferred from greenhouse to a growth chamber and maintained at 25/16 °C (± 0.5 °C) day/night temperature, 55%/70% (± 5%) day/night RH, and 200 μmol·m^{-2}·s^{-1} *PAR*. For each plant, the leaf to be treated with ^{14}C was carefully covered with aluminum foil, before over the top application of glyphosate at 6 or 12 oz/acre with or without NIS (Ortho X-77 at 0.25% v/v) using an air pressured chamber track sprayer as mentioned in the efficacy study. After removing the aluminum foil, five 2-μL droplets (10 μL contained 300 to 335 Bq) of ^{14}C-glyphosate were applied to a discrete area of the adaxial leaf surface in the median part of the third fully expanded leaf at four leaves stage using a 10-μL microsyringe. The quantity of ^{14}C applied to leaves was calculated by dispensing a similar number of droplets directly into 5 mL scintillation cocktail in a 7-mL polyvinyl vial. At 72 h after the ^{14}C application, plants were dissected into two sections (treated leaf and remaining tissues). The excised treated leaf was washed twice with a mixture of 4 mL water and ethanol (1:1 v/v) to recover unabsorbed ^{14}C-glyphosate and then rinsed twice with 3 mL ethanol solution. A 200 μL subsample from each washing was dispensed to 7-mL polyvinyl vial containing 6 mL scintillation liquid and afterward radio-assayed using a liquid scintillation counter. Plant samples were oven dried at 50 °C for 48 h and were combusted using a Biological Oxidizer OX 500 (R.J. Harvey Instrument Corp., Hillsdale, NJ). Evolved ^{14}CO$_2$ was trapped in Carbon-14 Cocktail (R.J. Harvey Instrument Corp.) flushed in 20-mL glass vials. The trapped ^{14}CO$_2$ was quantified by liquid scintillation spectrometry to determine distribution pattern of ^{14}C-glyphosate. Foliar absorption (percent applied) was defined as ^{14}C not recovered by washing the treated leaves and was calculated using the following equation: percent foliar absorption = (^{14}C in all plant tissues/^{14}C applied) × 100.

Translocation of ^{14}C-glyphosate was expressed as percent of applied as well as a percent of the total quantity absorbed. The recovery efficiency of ^{14}C-glyphosate was calculated by including all the oxidized radio assayed fractions and ^{14}C-glyphosate present in washing. Total recovery of ^{14}C-

glyphosate was ≥94%, and there was no difference in recovery among the treatments.

CHLOROFORM EXTRACT. Plants of the four weed species were grown in the greenhouse under similar conditions to those in the glyphosate efficacy study. At the three to four leaves stage of each weed species, 10 to 15 leaves were harvested and leaf area was determined using a portable leaf area meter. Harvested leaves were immersed in 30 mL chloroform for 20 s at room temperature in preweighed vials. The surface wax was extracted according to the procedure by Bukovac et al. (1979). Because of the difficulty in determining if only epicuticular waxes were extracted, all extracted wax was referred as "chloroform extract" (Westwood et al., 1997). Each treatment was replicated four times, and the experiment was repeated three times.

STATISTICAL ANALYSIS. All the studies were conducted in a randomized complete block design with four replications and were repeated. There was a nonsignificant experiment-by-treatment interaction; therefore, data were pooled and represented the mean of the repeated experiments. The data from efficacy study (percent control and biomass reduction) and ^{14}C absorption study were subjected to two-way factorial analyses with glyphosate treatments and weed species as two factors. All data were subjected to analysis of variance and means were separated using Fisher's protected least significant difference test (P = 0.05) in SAS (version 9.2; SAS Institute, Cary, NC). The data of percent weed control and biomass reduction were arcsine transformed before analysis; however, nontransformed percentages are presented with mean separation based on transformed data.

Results and discussion

GLYPHOSATE EFFICACY. Glyphosate treatments had variable effects on the tested weed species, and there was a significant interaction between weed species and glyphosate application rates; but the addition of NIS did not improve control of any species (Table 1). Compared with untreated control, glyphosate applied alone or with NIS at any rate provided significant control of florida beggarweed, spanishneedles, and johnsongrass, indicating that these species were not

glyphosate tolerant. Similar results were obtained in a study that evaluated the response of four weed species including barnyardgrass (*Echinochloa crus-galli*), hemp sesbania (*Sesbania exaltata*), pitted morningglory (*Ipomoea lacunose*), and prickly sida (*Sida spinosa*) to glyphosate with or without NIS (Latron AG-98; Rohm and Hass, Philadelphia) (Norsworthy et al., 2001). Florida beggarweed was the most sensitive and recorded 99% control even at the lowest rate (3 oz/acre), and there was no difference among treatments (Table 1). The application of glyphosate at 3 oz/acre resulted in 90% and 84% control of spanishneedles and johnsongrass, respectively. Increasing glyphosate rates from 6 to 24 oz/acre provided 98% to 100% control of these two weed species (Table 1).

Ivyleaf morningglory was the most tolerant to glyphosate among the four weed species. For example, ivyleaf morningglory was not controlled when glyphosate applied alone or with NIS at 3 oz/acre (Table 1). Increasing glyphosate rate to 24 oz/acre resulted in ≤25% control. Similar to this result, Shaw and Arnold (2002) reported 32% control of pitted morningglory with 12 oz/acre glyphosate. In contrast to this, a previous study reported >90% control of pitted morningglory (Koger and Reddy, 2005); however, glyphosate was applied at almost double rate (39 oz/acre) than the maximum rate used in this study.

Percent biomass reduction in all the tested weed species followed almost identical trend to percent control in response to glyphosate treatments (Table 2). The effect of glyphosate application rates on weed biomass was significant; however, there was no difference because of addition of NIS. For example, ivyleaf morningglory showed the lowest biomass reduction compared with other weed species and recorded <10% and <65% biomass reduction at 3 and 24 oz/acre glyphosate, respectively (Table 2). Biomass reduction was 78% to 84% in florida beggarweed, 64% to 75% in spanishneedles, and 76% to 88% in johnsongrass with the application of 3 oz/acre glyphosate (± NIS), and the biomass reduction was higher with increasing application rates (Table 2). Similar to this result, a study reported 24% and 69% biomass reduction in pitted morningglory when glyphosate was applied at 240 and 1680 g·ha^{-1} (3.43

and 23.98 oz/acre), respectively (Norsworthy et al., 2001). Field experiments conducted by Blackshaw and Harker (2002) reported that glyphosate applied at 445 g·ha^{-1} (6.35 oz/acre) provided significant biomass reduction in green foxtail (*Setaria viridis*), wild oat (*Avena fatua*), redroot pigweed (*Amaranthus retroflexus*), and wild mustard (*Sinapis arvensis*) without injury to glyphosate-resistant wheat (*Triticum aestivum*).

Overall results of glyphosate efficacy study suggested that species sensitivity to glyphosate was ranked as florida beggarweed > spanishneedles > johnsongrass > ivyleaf morningglory, averaged over use of NIS and glyphosate rate.

ABSORPTION AND TRANSLOCATION. Differences in absorption and translocation of ^{14}C-glyphosate among four weed species were evaluated. The results suggested that ivyleaf

morningglory absorbed the lowest ^{14}C-glyphosate (63% of applied) at the 6 oz/acre compared with other weed species (Table 3); however, at the highest rate (12 oz/acre), it was comparable with spanishneedles and johnsongrass. The glyphosate efficacy data suggested that ivyleaf morningglory was the most tolerant (Table 1) and had the lowest biomass reduction compared with other species (Table 2). However, it has to be noticed that

Table 1. Effects of application rates of glyphosate with and without nonionic surfactant (NIS) on control of four weed species.

Treatment	Rate (oz/acre)^z	Ivyleaf morningglory	Florida beggarweed	Spanishneedles	Johnsongrass
			Control (%)^y		
Untreated control	—	0 i	0 i	0 i	0 i
Glyphosate	3	0 i	99 a	90 b	84 c
Glyphosate	6	10 gh	100 a	98 a	99 a
Glyphosate	12	14 g	99 a	100 a	100 a
Glyphosate	24	25 e	100 a	100 a	100 a
Glyphosate + NIS^x	3	0 i	95 a	88 bc	75 d
Glyphosate + NIS	6	6 h	99 a	100 a	95 a
Glyphosate + NIS	12	20 f	100 a	100 a	100 a
Glyphosate + NIS	24	23 ef	100 a	100 a	100 a

^{z}1 oz/acre = 70.0532 g·ha^{-1}.
^{y}Treatment comparison within weed species or weed species comparison within treatment. Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference test at $P < 0.05$.
^{x}Ortho X-77 (Chevron Chemical Co., Richmond, CA) at 0.25% v/v.

Table 2. Effects of application rates of glyphosate with and without nonionic surfactant (NIS) on biomass reduction of four weed species.

Treatment	Rate (oz/acre)^z	Ivyleaf morningglory	Florida beggarweed	Spanishneedles	Johnsongrass
			Biomass reduction (%)^y		
Untreated control	—	0 k	0 k	0 k	0 k
Glyphosate	3	9 k	81 a–e	75 d–g	88 a–d
Glyphosate	6	23 j	84 a–d	84 a–d	91 abc
Glyphosate	12	55 hi	84 efg	84 a–d	93 ab
Glyphosate	24	63 gh	84 a–d	88 a–d	95 a
Glyphosate + NIS^x	3	3 k	78 b–e	66 fgh	76 c–f
Glyphosate + NIS	6	22 j	88 a–d	88 a–d	93 ab
Glyphosate + NIS	12	51 i	80 a–e	88 a–d	94 a
Glyphosate + NIS	24	63 gh	89 a–d	88 a–d	95 a

^{z}1 oz/acre = 70.0532 g·ha^{-1}.
^{y}Treatment comparison within weed species or weed species comparison within treatment. Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference test at $P < 0.05$.
^{x}Ortho X-77 (Chevron Chemical Co., Richmond, CA) at 0.25% v/v.

Table 3. Effects of application rates of glyphosate with and without nonionic surfactant (NIS) on carbon-14-labeled glyphosate (^{14}C-glyphosate) absorption in four weed species.

Treatment	Rate (oz/acre)^z	Ivyleaf morningglory	Florida beggarweed	Spanishneedles	Johnsongrass
			^{14}C-glyphosate absorption (% of applied)^y		
Glyphosate	6	63 g	87 ab	71 f	72 f
Glyphosate	12	75 ef	87 ab	80 ec	76 def
Glyphosate + NIS^x	6	73 ef	87 ab	78 de	76 def
Glyphosate + NIS	12	79 cd	88 a	83 bc	83 bc

^{z}1 oz/acre = 70.0532 g·ha^{-1}.
^{y}Treatment comparison within weed species or weed species comparison within treatment. Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference test at $P < 0.05$.
^{x}Ortho X-77 (Chevron Chemical Co., Richmond, CA) at 0.25% v/v.

tolerance level is relative and, in this case, glyphosate still reduced the biomass of ivyleaf morningglory in the range of 3% to 63% (Table 2). Similar results were reported by Starke and Oliver (1998) and Norsworthy et al. (2001) showing minimal glyphosate absorption by pitted morningglory.

The effect of the addition of NIS on ^{14}C-glyphosate absorption was species dependent. For example, the addition of NIS improved the absorption of ^{14}C-glyphosate in ivyleaf morningglory and spanishneedles; however, there was no effect of NIS on the absorption of ^{14}C-glyphosate in florida beggarweed at any rate of glyphosate and in johnsongrass at lower rate (Table 3). A study by Sherrick et al. (1986) revealed that absorption was increased 2- to 3-fold in field bindweed (*Convolvulus arvensis*) with addition of tallow amine (MON 0818, Monsanto) compared with glyphosate applied alone. In a similar study, Norsworthy et al. (2001) reported that the addition of NIS to glyphosate did not affect ^{14}C-glyphosate absorption in prickly sida and pitted morningglory, whereas absorption was reduced by the addition of NIS in hemp sesbania and barnyardgrass.

Florida beggarweed showed the highest ^{14}C-glyphosate absorption (>85%), and it did not vary with application rates or addition of NIS

(Table 3). This was consistent with the results of the glyphosate efficacy study, which indicated that florida beggarweed was the most sensitive (Table 1). Except in florida beggarweed, ^{14}C-glyphosate absorption increased with increasing glyphosate rates. A study by Koger and Reddy (2005) suggested that glyphosate absorption and translocation was more affected by application rate than the degree of plant exposure to glyphosate in pitted morningglory.

The results of the ^{14}C-glyphosate-translocation in the tested weed species revealed that johnsongrass showed the highest ^{14}C-glyphosate translocation (52% to 58%) regardless of application rate and inclusion of NIS. In other species, ^{14}C-glyphosate-translocation was variable [spanishneedles (32% to 52%), florida beggarweed (34% to 51%), and ivyleaf morningglory (16% to 39%)] based on rate and NIS (Table 4). For example, the ^{14}C-glyphosate translocation was increased with increasing rate of glyphosate from 6 to 12 oz/acre in ivyleaf morningglory and spanishneedles. Addition of NIS to glyphosate further improved the translocation of ^{14}C-glyphosate in ivyleaf morningglory and florida beggarweed. Usually similar trend was observed when translocation was calculated as percent of absorbed (Table 5). A study by Westwood et al. (1997) reported

that neither RH nor uptake and gross translocation processes accounted for differential glyphosate sensitivity in different field bindweed biotypes. The tolerance of ivyleaf morningglory to glyphosate observed in this study could be due to several factors including elevated levels of the target enzyme EPSPS, glyphosate metabolism, or sequestration away from the site of action. Most weed species do not metabolize glyphosate enough to avoid its toxic effects; however, in field horsetail (*Equisetum arvense*), it has been reported that low activity was due to ability to metabolize the herbicide (Marshall et al., 1987).

LEAF WAX CONTENT. The four weed species differed in leaf wax content and showed variable relationships between ^{14}C-glyphosate absorption and leaf wax content (data not shown). Ivyleaf morningglory that had the lowest leaf wax content (10.8 µg·cm^{-2} of leaf surface) showed relatively lower ^{14}C-glyphosate absorption and translocation (percent applied and percent absorbed) compared with other weed species. However, spanishneedles had lower wax content (15.7 µg·cm^{-2}) compared with florida beggarweed (24.7 µg·cm^{-2}) and johnsongrass (34.6 µg·cm^{-2}), but it showed the same ^{14}C-glyphosate absorption as johnsongrass and the same ^{14}C-glyphosate translocation as florida beggarweed

Table 4. Effects of application rates of glyphosate with and without nonionic surfactant (NIS) on carbon-14-labeled glyphosate (^{14}C-glyphosate) translocation in four weed species.

Treatment	Rate (oz/acre)[z]	Ivyleaf morningglory	Florida beggarweed	Spanishneedles	Johnsongrass
		^{14}C-glyphosate translocation (% of applied)[y]			
Glyphosate	6	16 e	34 cd	32 cd	53 ab
Glyphosate	12	27 d	38 c	46 b	52 ab
Glyphosate + NIS[x]	6	35 cd	45 b	37 c	57 a
Glyphosate + NIS	12	39 c	51 ab	52 ab	58 a

[z]1 oz/acre = 70.0532 g·ha^{-1}.
[y]Treatment comparison within weed species or weed species comparison within treatment. Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference test at $P < 0.05$.
[x]Ortho X-77 (Chevron Chemical Co., Richmond, CA) at 0.25% v/v.

Table 5. Effects of application rates of glyphosate with and without nonionic surfactant (NIS) on carbon-14-labeled glyphosate (^{14}C-glyphosate) translocation in four weed species.

Treatment	Rate (oz/acre)[z]	Ivyleaf morningglory	Florida beggarweed	Spanishneedles	Johnsongrass
		^{14}C-glyphosate translocation (% of absorbed)[y]			
Glyphosate	6	21 h	40 g	42 fg	70 b
Glyphosate	12	44 efg	43 efg	58 cd	63 bc
Glyphosate + NIS[x]	6	48 d–g	52 de	51 def	79 a
Glyphosate + NIS	12	49 d–g	58 cd	63 bc	77 a

[z]1 oz/acre = 70.0532 g·ha^{-1}.
[y]Treatment comparison within weed species or weed species comparison within treatment. Least square means within columns with no common letters are significantly different according to Fisher's protected least significant difference test at $P < 0.05$.
[x]Ortho X-77 (Chevron Chemical Co., Richmond, CA) at 0.25% v/v.

(Tables 3–5). Similarly, Norsworthy et al. (2001) reported that glyphosate absorption was not correlated with the quantity of chloroform leaf wax or leaf wettability in tested weed species.

As reported in previous study (Elmore et al., 1998), this study has shown that leaf wax content was less effective in the penetration of the herbicide through the cuticle. Lipoidal nature of the leaf surface minimizes the loss of water from the plant and may act as a barrier in the absorption of herbicides (Holloway, 1993). Compared with spanishneedles, johnsongrass had higher leaf wax content; however, the ^{14}C-glyphosate absorption was the same in both the species at all the rates of glyphosate (Table 3). Harr et al. (1991) performed the qualitative analysis of leaf wax in johnsongrass and reported that 93% of the total leaf waxes to be of polar nature which absorbed water soluble chemicals easily. In this study, ivyleaf morningglory and spanishneedles had lower leaf wax contents (data not shown); however, their percent nonpolar and polar waxes might be different, which might have affected the absorption of glyphosate. Therefore, further research is required for qualitative wax analysis in florida beggarweed, spanishneedles, and ivyleaf morningglory. Results of this study will contribute to the limited understanding of differential glyphosate tolerance in these weed species and may aid in developing alternative management options.

Combining agronomic practices with judicious herbicide use can lead to successful weed management and reduce the selection pressure for herbicide resistant weeds (Beckie et al., 2011). Many studies reported that cropping system diversity is the long-term solution for proactive herbicide-resistant weed management in annual crops (Harker et al., 2005; Owen, 2001); however, this option is less effective in perennial crops such as citrus. Therefore, integrated weed management practices, which might include sanitation, mulching, soil solarization, preplant fumigation, use of herbicides with different mode of action, tank mix of herbicides, and monitoring of weeds for herbicide-tolerance is required for effective management of weeds in Florida citrus.

Literature cited

Baylis, A.D. 2000. Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. Pest Mgt. Sci. 56:299–308.

Beckie, H.J. 2006. Herbicide-resistant weeds: Management tactics and practices. Weed Technol. 20:793–814.

Beckie, H.J., K.N. Harker, L.M. Hall, F.A. Holm, and R.H. Gulden. 2011. Risk assessment of glyphosate resistance in western Canada. Weed Technol. 25:159–164.

Blackshaw, R.E. and K.N. Harker. 2002. Selective weed control with glyphosate in glyphosate-resistant spring wheat (*Triticum aestivum* L.). Weed Technol. 16:885–892.

Bukovac, M.J., J.A. Flore, and E.A. Baker. 1979. Peach leaf surfaces: Changes in wettability, retention, cuticular permeability and epicuticular wax chemistry during expansion with special reference to spray application. J. Amer. Soc. Hort. Sci. 104:611–617.

Dill, G.M. 2005. Glyphosate-resistant crops: History, status and future. Pest Mgt. Sci. 61:219–224.

Duke, S.O. and S.B. Powles. 2008. Glyphosate: A once in a century herbicide. Pest Mgt. Sci. 64:319–325.

Elmore, C.D., T. Tonos, and M. Steele. 1998. Epicuticular wax composition of soybean leaves differing in pubescence, p. 49–54. In P. McMullan (ed.). Proc. 5th Intl. Symp. on Adjuvants for Agrochemicals. Memphis, TN.

Fernandez-Cornejo, J. and W.D. McBride. 2000. Genetically engineered crops for pest management in US agriculture. U.S. Dept. Agr., Econ. Res. Serv., Agr. Econ. Rpt. 786.

Frans, R., R. Talbert, and D. Marx. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices, p. 29–46. In: N.D. Camper (ed.). Research methods in weed science. Southern Weed Sci. Soc., Champaign, IL.

Franz, J.E. 1985. Chemistry of glyphosate, p. 3–17. In: E. Grossbard and D. Atkinson (eds.). The herbicide glyphosate. Butterworths, London.

Franz, J.E., M.K. Mao, and J.A. Sikorski. 1996. Glyphosate: A unique global pesticide. Amer. Chem. Soc., Washington, DC.

Green, J.M. 2009. Evolution of glyphosate-resistant crop technology. Weed Sci. 57:108–117.

Harker, K.N., G.W. Clayton, R.E. Blackshaw, J.T. O'Donovan, N.Z. Lupwayi, E.N. Johnson, Y. Gan, R.P. Zentner, G.P. Lafond, and R.B. Irvine. 2005. Glyphosate-resistant spring wheat production system effects on weed communities. Weed Sci. 53:451–464.

Harr, J., R. Guggenheim, G. Schulke, and R.H. Falk. 1991. The leaf surface of major weeds. Sandoz Agro, Basel, Switzerland.

Heap, I. 2011. International Survey of Herbicide Resistant Weeds. 5 May 2011. <http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go>.

Holloway, P.J. 1993. Sources and chemistry of plant cuticles. Pestic. Sci. 37:203–206.

Kirkwood, R.C. 1991. Pathways and mechanisms of absorption of foliage applied herbicides with particular reference to the role of surfactants, p. 219–243. In: R.C. Kirkwood (ed.). Target sites for herbicide action. Plenum Press, London.

Kirkwood, R.C. 1993. Use and mode of action of adjuvants for herbicides: A review of some current work. Pestic. Sci. 38:93–102.

Koger, C.H. and K.N. Reddy. 2005. Glyphosate efficacy, absorption and translocation in pitted morningglory (*Ipomoea lacunosa*). Weed Sci. 53:277–283.

Marshall, G., R.C. Kirkwood, and D.J. Martin. 1987. Studies on the mode of action of asulam, aminotriazone, and glyphosate in field horsetail (*Eqisetum arvensi* L.). II. The metabolism of [^{14}C] asulam, [^{14}C] aminotriazole, and [^{14}C] glyphosate. Pestic. Sci. 18:65–77.

Norsworthy, J.K., N.R. Burgos, and L.R. Oliver. 2001. Differences in weed tolerance to glyphosate involve different mechanisms. Weed Technol. 15:725–731.

Orson, J.H. 1999. The cost to the farmer of herbicide resistance. Weed Technol. 13:607–611.

Owen, M.D.K. 2001. World maize/soybean and herbicide resistance, p. 101–163. In: S.B. Powles and D.L. Shaner (eds.). Herbicide resistance and world grains. CRC Press, New York.

Powles, S.B. 2008. Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. Pest Mgt. Sci. 64:360–365.

Powles, S.B. and C. Preston. 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. Weed Technol. 20:282–289.

Reddy, K.N. and J.K. Norsworthy. 2010. Glyphosate resistant crop production systems: Impact on weed species shifts, p. 165–184. In V.K. Nandula (ed.). Glyphosate resistance in crops and weeds: History, development and management. Wiley, Hoboken, NJ.

Riechers, D.E., L.M. Wax, R.A. Liebl, and D.R. Bush. 1994. Surfactant-increased glyphosate absorption into plasma membrane vesicles isolated from common

lambsquarters leaves. Plant Physiol. 105: 1419–1425.

Schönburnn, E., S. Eschenburg, W.A. Shuttleworth, J.V. Schloss, N. Amrhein, J.N.S. Evans, and W. Kabsch. 2001. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate-3-phosphate synthase in atomic detail. Proc. Natl. Acad. Sci. USA 98:1376–1380.

Sharma, S.D., R.C. Kirkwood, and T.L. Whateley. 1996. Effect of nonionic nonylphenol surfactants on surface physicochemical properties, absorption and distribution of asulam and diflufenican. Weed Res. 36: 227–239.

Sharma, S.D. and M. Singh. 1999. Effect of two adjuvant types on the distribution of ^{14}C-glyphosate applied to model weed species, p. 729–734. Proc. Brighton Crop Protection Conf.—Weeds. Brighton, UK.

Sharma, S.D. and M. Singh. 2007. Effect of timing and rates of application of glyphosate and carfentrazone herbicides and their mixtures on the control of some broadleaf weeds. HortScience 42:1221–1226.

Shaw, D.R. and J.C. Arnold. 2002. Weed control from herbicide combinations with glyphosate. Weed Technol. 16:1–6.

Sherrick, S.L., H.A. Holt, and F.D. Hess. 1986. Effects of adjuvants and environment during plant development on glyphosate absorption and translocation in flied bindweed (*Convolvulus arvensis*). Weed Sci. 34:811–816.

Starke, R.J. and L.R. Oliver. 1998. Interaction of glyphosate with chlorimuron, fomesafen, imazethapyr, and sulfentrazone. Weed Sci. 46:652–660.

U.S. Department of Agriculture. 2010a. Citrus: Commercial Citrus Inventory. 5 May 2011. <http://www.nass.usda.gov/ Statistics_by_State/Florida/Publications/ Citrus/ccipre/ccipr10.pdf>.

U.S. Department of Agriculture. 2010b. Citrus: 2009–2010 Citrus Summary: Production, Practices and Value. 5 May 2011. <http://www.nass.usda.gov/Statistics_ by_State/Florida/Publications/Citrus/ cspre/cit92310.pdf>.

Vencill, W.K. 2002. Herbicide handbook. 8th ed. Weed Sci. Soc. Amer., Lawrence, KS.

Westwood, J.H., C.N. Yerkes, F.P. DeGennaro, and S.C. Weller. 1997. Absorption and translocation of glyphosate in tolerant and susceptible biotypes of field bindweed (*Convolvulus arvensis*). Weed Sci. 45:658–663.

Wyrill, J.B. and O.C. Burnside. 1976. Absorption, translocation and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. Weed Sci. 24:557–566.