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Glyphosate Efficacy on Velvetleaf (*Abutilon theophrasti*) is Affected by Stress

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Greenhouse and growth chamber studies were conducted to determine the effect of drought, flooding, and cold stress on the efficacy of glyphosate for velvetleaf control, and the interaction between these stresses and adjuvant and posttreatment temperature. Glyphosate activity on velvetleaf decreased when plants were stressed with drought ≥ flooding > cold. Leaf blades of environmentally stressed velvetleaf angled downward, which increased tolerance to glyphosate but was not as great a cause of tolerance as the stress effects. Glyphosate applied to 6- and 12-leaf velvetleaf was two and eight times more phytotoxic on nonstressed compared with drought-stressed plants, respectively. Glyphosate was most effective on nonstressed plants, followed by plants recovering from stress, and least effective on plants still under stress. None of the adjuvants completely overcame the adverse affects of stress on glyphosate efficacy; use of a nonionic surfactant and ammonium sulfate resulted in a 9–13 percentage point improvement in control of stressed plants compared with glyphosate applied without an adjuvant. Low temperatures (5 or 12 C) maintained for 48 h after herbicide treatment enhanced glyphosate phytotoxicity to stressed and nonstressed velvetleaf. Glyphosate at a low rate stressed velvetleaf, which made them more tolerant to subsequent glyphosate application compared with velvetleaf not pretreated with glyphosate.

Nomenclature: Glyphosate; velvetleaf, *Abutilon theophrasti* Medik. ABUTH.

Key words: Adjuvant, drought, flooding, glyphosate efficacy.

Velvetleaf, a summer annual, is a member of the mallow family. It is a major weed problem in the northern United States and eastern Canada and can reduce yield in many crops, especially soybean [*Glycine max* (L.) Merr.] (Oliver 1979), corn (*Zea mays* L.) (Spencer 1984), and sugarbeet (*Beta vulgaris* L.) (Renner and Powell 1991). It is a fast-growing, late-season weed that thrives after standard weed control practices are completed (Bussan et al. 2001; Spencer 1984). This weed also has allelopathic effects on crop plants (Bhowmik and Doll 1982). Velvetleaf control has been difficult, because of seedling emergence throughout the growing season and plants tolerate many herbicides (Hartzler and Battles 2001; Spencer 1984).

Glyphosate is a nonselective herbicide that effectively controls many weed species. It is one of the most widely used herbicides in the world. Use of glyphosate in agriculture increased dramatically with introduction of glyphosate-resistant crops in the late 1990s (Owen and Zelaya 2005). Glyphosate products are approved for weed control in more than 100 crops and are registered in more than 130 countries (Monsanto 2007).

Velvetleaf has more tolerance to glyphosate than many other common weeds (Hartzler and Battles 2001; Owen and Zelaya 2005; Pratt et al. 2003). Widespread use of glyphosate-tolerant crops combined with velvetleaf's ability to emerge later in the season, suggest that velvetleaf may increase as a weed control problem for growers who rely on glyphosate as their primary weed management tool. Velvetleaf also has diurnal leaf movement, which has been associated with decreased efficacy of several herbicides applied near or after sunset (Andersen and Koukkari 1978; Mohr and Smeda 2001; Sellers et al. 2003; Waltz et al. 2004).

Glyphosate activity on a specific weed results from a complex interaction between the herbicide rate, weed condition, and environment. Glyphosate can become more effective with increasing temperature and relative humidity (Adkins et al. 1998; Tanpipat et al. 1997). Glyphosate phytotoxicity is enhanced by ammonium sulfate and surfactants, whereas oil adjuvants are generally antagonistic to glyphosate (Leaper and Holloway 2000; Nalewaja and Matysiak 1993).

Postemergence herbicides are generally less effective for control of weeds that are subjected to high temperature or drought. These stresses generally reduce absorption, translocation, and metabolism of herbicides (Zimdahl 1999). With increasing reliance on glyphosate for weed control, there is a need to determine the efficacy of glyphosate for control of weeds subject to environmental stresses. This knowledge may help in developing effective weed control programs. The objective of this research was to determine the effect of drought, flooding, and cold stress on efficacy of glyphosate for velvetleaf control and the interaction between these stresses and adjuvants used with glyphosate.

Materials and Methods

General Procedure. Velvetleaf seed was collected and pooled from multiple plants near Fargo (46°87′N, 96°78′W), ND. Seed was planted 1 cm deep in a greenhouse potting mixture of one part commercial peat-based material[1] and one part Fargo-Ryan silty clay (fine, smectitic, frigid Typic Natraquert), contained in 11-cm-diam by 14-cm-deep plastic pots. Plants were grown in the greenhouse with 21 ± 3 C at night and 25 ± 5 C during the day. Natural daylight was extended to 16 h with metal halide lamps giving an intensity of 440 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) at plant level. Velvetleaf was thinned to two plants per pot within 1 wk after emergence. Plants were watered daily and fertilized weekly except when drought was a stress treatment.

Stress was induced when plants were at the six-leaf stage, except for one experiment when plants were drought-stressed at five stages of 4, 6, 8, 10, and 12 leaves. To obtain the five stages, velvetleaf was seeded several times. Drought and flooding treatments were conducted in the greenhouse. Drought-stressed plants were obtained by withholding water until visible wilting occurred and then maintaining the plants with wilting leaves during three consecutive days by providing limited water (50 ml d^{-1}). Flood stress was achieved by

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placing potted plants inside plastic buckets and flooding with water 0.5 cm above the soil surface for 3 d. Cold-stressed plants were obtained by placing plants in a growth chamber for 3 d at 5 C with a 16-h photoperiod and light irradiance at 320 PPF.

Glyphosate[2] at 400 g ae ha^{-1} was applied in the greenhouse with a moving-nozzle sprayer that delivered 160 L ha^{-1} at 280 kPa from an 8001 flat-fan nozzle.[3] Unless specified otherwise, plants were maintained in the greenhouse after herbicide treatment. The spray carrier was deionized water.

Effect of Stress on Glyphosate Efficacy. Four experiments were conducted to evaluate the efficacy of glyphosate on stressed velvetleaf. Treatments in the first experiment consisted of four glyphosate rates of 200, 400, 600, or 800 g ha^{-1} and four plant conditions, including nonstressed, cold-stressed, drought-stressed, and flood-stressed. In the second experiment, glyphosate at 400 g ha^{-1} was applied to nonstressed and drought-stressed plants at the 4-, 6-, 8-, 10-, and 12-leaf stages. The third experiment was conducted to determine the efficacy of glyphosate on velvetleaf that was recovering from drought stress. Plants were subjected to drought stress for 3 d consecutively, and then grown under favorable growing conditions (watered daily and fertilized weekly) for 4 d prior to glyphosate treatment.

The fourth experiment was conducted to determine the efficacy of glyphosate on velvetleaf subjected to stress from a glyphosate pretreatment. Glyphosate-stressed plants were prepared by applying glyphosate at 75 g ha^{-1} (sublethal rate) to six-leaf plants, and the second treatment was applied with glyphosate at 400 g ha^{-1} at 4 d after the first treatment. Plants without the first glyphosate treatment were used as controls. All plants were provided with favorable growing conditions immediately after herbicide treatments.

A separate experiment was conducted to determine glyphosate phytotoxicity to stressed and nonstressed velvetleaf as influenced by adjuvants. Velvetleaf was treated with glyphosate at 400 g ha^{-1} applied with or without ammonium nitrate at 2% w/v, ammonium sulfate (AMS) at 2 w/v, nonionic surfactant[4] (NIS) at 0.5% v/v, methylated seed oil[5] (MSO) at 2.3 L ha^{-1}, petroleum oil concentrate[4] at 2.3 L ha^{-1}, NIS at 0.5% plus AMS at 2 w/w, and MSO at 2.3 L ha^{-1} plus AMS at 2 w/w.

Posttreatment Low Temperature. Low temperature treatments were conducted in growth chambers with a 16-h photoperiod, light irradiance at 320 PPF, 60% relative humidity, and constant temperatures of 5, 12, and 25 C. Nonstressed and stressed velvetleaf were treated with glyphosate at 400 g ha^{-1} and immediately transferred to growth chambers with different temperatures for 48 h. Plants were then moved to the greenhouse for the remaining duration of the experiment. Although temperature treatments were conducted in different growth chambers and could not be completely randomized, the experiment was analyzed as if they were randomized.

Leaf Blade Angle. Any type of stress in preliminary experiments caused the velvetleaf leaf blades to tilt downward. To determine the effect of leaf blade angle on glyphosate phytotoxicity to velvetleaf, two leaf blade orientations, horizontal (0°) and downward at 70° below horizontal were used in this experiment, which represented the natural leaf blade orientation of nonstressed and drought-stressed velvetleaf plants, respectively. Horizontal leaf blades on drought-stressed plants were obtained by adaxially supporting them with circular loops of copper wire. Downward orientation of leaf blades on nonstressed plants were set by abaxially bending them with hooks of copper wire. Physical manipulation of leaf blade angle was removed 1 h after herbicide application.

Data Collection and Analysis. Plants were cut approximately 0.5 cm above the soil surface 14 d after herbicide treatment and percentage of fresh weight reduction was calculated based on the fresh weight of untreated plants to estimate treatment effects on glyphosate phytotoxicity.

Each study was conducted twice in a completely random design with four replicates. Data were combined when error mean squares were homogenous between repetitions. Studies that examined the effects of glyphosate rate and leaf blade angle on glyphosate efficacy were analyzed in a factorial arrangement. The factors in the glyphosate rate study were stress source and glyphosate rate and in the leaf blade angle study were leaf blade angle and stress source. Data were subjected to ANOVA, and means were separated using Fisher's Protected LSD test at the 0.05 level of significance. Regression analysis was used to determine the effects of plant stage on glyphosate phytotoxicity to velvetleaf.

Results and Discussion

Stressed Plant Characteristics. Typical symptoms of stressed velvetleaf included yellowing, stunting, inhibition of leaf size and number, and senescence of the leaf blade and petiole. Flooding caused the most severe visible symptoms and cold caused the fewest symptoms. As previously reported (Andersen and Koukkari 1978; Sellers et al. 2003), velvetleaf plants exhibited diurnal leaf movement, where leaf blades were oriented horizontally (0°) during daytime to nearly 90° below horizontal at night. However, the leaf blades of stressed velvetleaf tilted down even during daytime, and leaf blade angle varied with different stresses (data not shown). The leaf blades were nearly 90° below horizontal with flood stress and about 70° below horizontal with drought or cold stress.

When drought-stressed plants were provided with favorable growing conditions for 4 d, the stress symptoms on developed plant growth partially disappeared, and new growth that developed after the stress did not show any stress symptoms. Plants recovering from drought stress were shorter and had one less leaf than nonstressed plants. Leaf blade orientation on plants recovering from drought was near 0° (horizontal) during the daytime, except some old leaves remained at a downward angle of 20° to 30°.

Effect of Stress on Glyphosate Efficacy. There was no interaction between glyphosate rate and stress type. Control of velvetleaf increased as the rate of glyphosate increased, regardless of velvetleaf condition (data not shown).

Stress caused a substantial increase in tolerance of velvetleaf to glyphosate (Table 1). Generally, the adverse effect of stress on velvetleaf tolerance to glyphosate was drought $\geq$ flooding $>$ cold, although the relative visible injury symptoms were flooding $\geq$ drought $>$ cold. Glyphosate at 400 g ha^{-1}

Table 1. Effect of adjuvants on fresh weight reduction of environmentally stressed velvetleaf by glyphosate at 400 g ae ha^{-1}.

		Source of stress			
Adjuvant[a]	Rate	None	Cold	Drought	Flooding
		% fresh wt reduction			
None	0	84	68	46	50
AMS	2% w/v	90	77	58	60
AMN	2% w/v	85	69	50	48
NIS	0.5% v/v	89	75	60	62
MSO	2.3 L ha^{-1}	84	66	45	50
POC	2.3 L ha^{-1}	83	69	50	52
NIS + AMS	0.5% v/v + 2% w/v	93	80	67	68
MSO + AMS	2.3 L ha^{-1} + 2% w/v	90	78	59	64
LSD (0.05)		5	7	5	6

[a] AMN, ammonium nitrate; AMS, ammonium sulfate; MSO, methylated seed oil; NIS, nonionic surfactant; POC, petroleum oil concentrate.

resulted in an 84% reduction in fresh weight for nonstressed velvetleaf. But the fresh weight reductions were 46, 50, and 68% for drought-, flood-, and cold-stressed plants, respectively. One explanation for this finding is that drought stress decreased not only herbicide translocation in plants, which also occurs with other types of stress due to decreasing sugar production and movement, but also decreased herbicide absorption due to producing thick leaf cuticles (Parker and Boydston 2005).

Velvetleaf tolerance to glyphosate increased with age and at a faster rate in drought-stressed plants compared with nonstressed plants (Figure 1). Glyphosate applied to 6- and 12-leaf velvetleaf was about two and eight times more phytotoxic to nonstressed than to drought-stressed plants, respectively. Glyphosate at 400 g ha^{-1} applied to 10-leaf velvetleaf caused 70% fresh weight reduction for nonstressed plants, 20% for drought-stressed plants, and 55% for plants recovering from drought stress (data not shown). These data indicate that velvetleaf tolerance to glyphosate is not completely alleviated even when plants are exposed to favorable conditions following herbicide treatment.

Adjuvants Effects. The addition of AMS improved glyphosate activity on stressed velvetleaf more than on nonstressed plants, although the total control of stressed velvetleaf still was less than to nonstressed plants (Table 1). AMS at 2% w/v increased the efficacy of glyphosate at 400 g ha^{-1} on nonstressed, cold-stressed, drought-stressed, and flood-stressed velvetleaf by 6, 9, 12, and 10 percentage points, respectively.

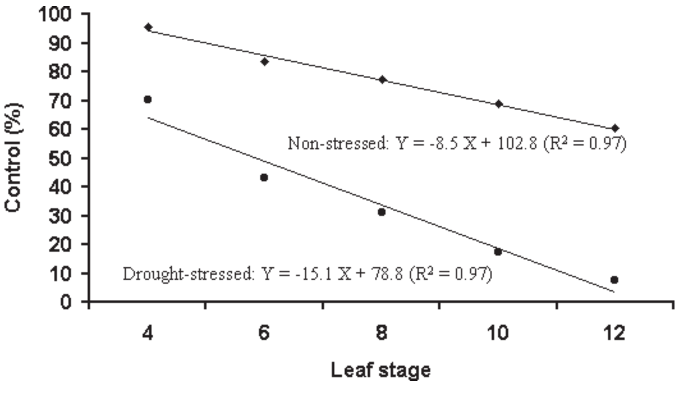

Figure 1. Effect of plant leaf stage on phytotoxicity of glyphosate at 400 g ae ha^{-1} to nonstressed and drought-stressed velvetleaf.

Table 2. The effect of leaf blade angle on fresh weight reduction of nonstressed and drought-stressed velvetleaf by glyphosate at 400 g ae ha^{-1}.

Leaf blade angle[a]	Nonstressed	Drought stressed
	% fresh wt reduction	
0° (horizontal)	84	52
70° (below horizontal)	72	47
LSD (0.05)	7	

[a] Horizontal leaf blades on drought-stressed velvetleaf were obtained by adaxially supporting them with circular loops of copper wire. Leaf blades that were 70° below horizontal on nonstressed plants were set by abaxially bending them with hooks of copper wire.

Deionized water was the spray carrier so the AMS was not functioning to overcome antagonism by cations in the spray water. Velvetleaf has calcium in leaf tissue, and AMS overcomes the calcium antagonism of glyphosate in a similar manner as reported for sunflower (*Helianthus annuus* L.) (Nalewaja and Matysiak 1992). Ammonium nitrate did not influence glyphosate efficacy on stressed and nonstressed velvetleaf (Table 1), which supports the concept that the sulfate ion from AMS rather than the ammonium ion is important for enhancing glyphosate phytotoxicity in the presence of calcium in leaf tissue (Hall et al. 2000; Pratt et al. 2003). The sulfate ion readily reacts with calcium to form calcium sulfate, an insoluble salt, which eliminates formation of glyphosate–calcium, an ineffective salt of glyphosate (Nalewaja and Matysiak 1993).

Glyphosate activity on stressed velvetleaf increased with the addition of NIS, especially to drought-stressed and flood-stressed plants (Table 1). The oil adjuvants, MSO and petroleum oil concentrate, did not improve control of velvetleaf with glyphosate. The addition of AMS plus NIS was the most effective adjuvant combination for enhancing glyphosate efficacy on stressed velvetleaf. Inclusion of MSO with AMS did not enhance glyphosate efficacy beyond that of AMS alone. Glyphosate at 400 g ha^{-1} with NIS at 0.5% v/v plus AMS at 2% w/v increased control of drought- and flood-stressed velvetleaf by 21 and 18 percentage points, compared with by 12 and 10 percentage points with AMS alone or 14 and 12 percentage points with NIS alone, respectively.

These results indicate the importance of the addition of AMS to enhance glyphosate activity on velvetleaf, even when water carriers are free of antagonistic cations. Also, the addition of NIS, but not oil adjuvants, is important for velvetleaf control with glyphosate. The glyphosate formulation used in the experiment contained surfactant and, with glyphosate 400 g ha^{-1} in 160 L ha^{-1}, should have provided enough surfactant for spray retention and droplet contact with the leaf, but perhaps insufficient surfactant for optimum glyphosate absorption.

Leaf Blade Angle. Glyphosate at 400 g ha^{-1} reduced the fresh weight of nonstressed velvetleaf by 84% with natural horizontal leaf blades and 72% when leaf blades were mechanically held at 70° below horizontal (Table 2). These results support previous reports that velvetleaf leaf blade orientation was one important reason accounting for differences in efficacy when herbicides were applied at different times of day (Andersen and Koukkari 1978; Sellers et al. 2003; Waltz et al. 2004).

Statistically, glyphosate efficacy on drought-stressed velvetleaf was similar with natural leaf blade orientation 70° below

Table 3. Effect of posttreatment temperature on fresh weight reduction of environmentally stressed velvetleaf by glyphosate at 400 g ae ha^{-1}.

Posttreatment temperature[a]	Source of stress			
	None	Cold	Drought	Flooding
C	% fresh wt reduction			
5	100	78	66	70
12	92	68	63	63
25	85	70	47	48
Control	84	72	45	50
LSD (0.05)	8	NA	5	6

[a] Plants were maintained at the posttreatment temperatures for 48 h before they were returned to the greenhouse while the control plants remained in the greenhouse after herbicide treatment.

horizontal and with mechanically supported horizontal leaf blades, although the trend was toward enhanced control of drought-stressed velvetleaf that had mechanically supported horizontal leaf blades (Table 2). These results suggest that leaf blade orientation below horizontal, which would reduce the spray target area of velvetleaf, does contribute to velvetleaf tolerance to glyphosate but has less effect than stress on tolerance. Velvetleaf leaf blades that tilt downward are a visible indicator of plant stress that results in substantially reduced velvetleaf control.

Posttreatment Low Temperature. Low temperatures of 5 and 12 C for 48 h after herbicide treatment enhanced glyphosate phytotoxicity to flood-, drought-, and nonstressed velvetleaf but did not affect control of cold-stressed plants (Table 3). These results are consistent with the previous report that maximum control of quackgrass [*Elytrigia repens* (L.) Nevski.] by glyphosate occurred when plants were grown in cool soils (Caseley 1972) but inconsistent with other studies where glyphosate efficacy to velvetleaf, wild oat (*Avena fatua* L.), and liverseedgrass (*Urochloa panicoides* Beauv.) increased as temperature increased (Adkins et al. 1998; Waltz et al. 2004). Reddy (2000) reported that glyphosate absorption and translocation by redvine [*Brunnichia ovata* (Walt.) Shinners] were highest in plants maintained at 35/30 C, followed by 15/10 C, and were lowest in plants maintained at 25/20 C. The variable results reported on the effect of temperature on glyphosate phytotoxicity probably are due to use of different bioassay species and varying temperature regimes in the experiments.

Repeat Glyphosate Treatment. Glyphosate at 75 g ha^{-1}, which is a sublethal rate for control of velvetleaf, caused visible injury to velvetleaf plants at 4 d after treatment. The injury symptoms were similar to the effect of drought and floodstress with leaf blades tilted downward about 45° from horizontal, which was followed by foliar yellowing. Glyphosate at 400 g ha^{-1} applied 4 d after the initial treatment reduced the fresh weight of glyphosate-stressed velvetleaf by 65% and nonstressed plants by 80%, respectively (data not shown). Apparently, a low rate of glyphosate stressed velvetleaf thereby making them more tolerant to subsequent glyphosate treatments, at least when treatments were separated by only 4 d for this research.

Glyphosate, which has no soil residual, frequently is applied twice per season to obtain near weed-free fields, especially in glyphosate-resistant crops. Often split-applied herbicide treatments are applied at low rates to small weeds with dual objectives of reducing early-season weed competition and reducing the total rate of herbicide applied (Norris 1991; Ramsdale and Messersmith 2002). The results with velvetleaf suggest that to adopt a split-applied concept, the first treatment should be applied to small velvetleaf (Figure 1); the glyphosate rate needs to be high enough to achieve acceptable control with the initial treatment; and the time interval between glyphosate treatments needs to be long enough that any surviving velvetleaf is visibly recovered from injury caused by the first treatment. Also, other research has shown that velvetleaf grown under nitrogen stress was not controlled by glyphosate as well as plants grown under high nitrogen conditions (Mithila et al. 2006).

In general, glyphosate phytotoxicity to velvetleaf decreased when the plants were stressed by cold, drought, or flooding prior to treatment. The addition of NIS or AMS partially overcame the stress-caused tolerance of velvetleaf to glyphosate, but the combination of AMS plus NIS was the best adjuvant to improve velvetleaf control. Posttreatment low temperature enhanced control of both nonstressed and stressed velvetleaf by glyphosate, although cold stress prior to treatment adversely affected glyphosate efficacy.

Velvetleaf leaf blades that tilted down from horizontal indicated a stress condition and tolerance to glyphosate, and stress was more important than leaf orientation for reducing the plant response to glyphosate. Velvetleaf that survived a sublethal glyphosate treatment was more tolerant to a subsequent glyphosate treatment than previously untreated plants.

The health of plants should be considered when using glyphosate for velvetleaf control. Velvetleaf under stress from low soil fertility, cold, drought, excess moisture, or previous glyphosate treatment has increased tolerance to glyphosate compared with nonstressed plants. Leaf orientation downward from horizontal is a visible indicator of stress to velvetleaf and tolerance to glyphosate. Effective control of velvetleaf by glyphosate, and probably by other herbicides, relies upon several factors including applying treatments to small plants and plants that are not stressed by environment or a prior herbicide treatment and treatment factors such as using an effective glyphosate rate and including AMS adjuvant.

Sources of Materials

[1] Sunshine Mix No. 1, Sun Gro Horticulture Inc., Bellevue, WA 98008.

[2] Roundup UltraMAX® formulation, Monsanto Company, St. Louis, MO 63167.

[3] Spraying Systems Inc., Wheaton, IL 60189.

[4] Activator 90®, nonionic surfactant, alkylpolyoxyethylene ethers and free fatty acids, and Herbimax, petroleum oil concentrate, paraffinic oil plus emulsifiers plus surfactant, from Loveland Industries, Inc., Greeley, CO 80632.

[5] Scoil®, methylated seed oil plus surfactants, AGSCO, Inc., Grand Forks, ND 58203.

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