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Environmental Effects on Velvetleaf (*Abutilon theophrasti*) Epicuticular Wax Deposition and Herbicide Absorption

H. Hatterman-Valenti, A. Pitty, and M. Owen*

Controlled environment experiments showed that velvetleaf plants grown under drought stress or low temperature (LT) treatments had greater leaf epicuticular wax (ECW) deposition compared to plants grown in soil with moisture at field capacity (FC) or a high temperature (HT) regime. Light intensity did not affect ECW deposition; however, increasing light intensity decreased the leaf ECW ester content and increased the secondary alcohol content. Plants grown at an LT regime or under FC had leaf ECW with fewer hydrocarbons and more esters than those grown at an HT or drought stress regime. Velvetleaf absorption of acifluorfen increased as light intensity decreased for plants grown in adequate soil water content, while the opposite was true for drought-stressed plants. Velvetleaf absorption of acifluorfen was approximately 3 and 10 times greater, respectively, with the addition of 28% urea ammonium nitrate (UAN) in comparison to crop oil concentrate (COC) or no adjuvant, regardless of the environmental treatments. Plants absorbed more acifluorfen when subjected to the LT regime in comparison to the HT regime when UAN was the adjuvant, while the opposite was true when COC was the adjuvant. Velvetleaf absorption of acifluorfen was not affected by drought stress when COC or no adjuvant was used and varied between studies when UAN was used. Velvetleaf absorption of bentazon was greatest for plants grown under HT/FC or high light/FC treatments and least with plants grown under HT/drought stress or low light/drought stress treatments, regardless of the adjuvant. However, bentazon absorption was higher with the addition of an adjuvant and for plants grown at a high light intensity or FC condition compared with medium to low light intensity or drought stress treatments.

Nomenclature: Acifluorfen; bentazon; velvetleaf *Abutilon theophrasti* Medic. ABUTH.
Key words: Drought stress, temperature, light intensity, herbicide uptake, epicuticular wax quality.

Velvetleaf is a highly competitive and problematic weed in many crop systems in the northern and eastern United States (Akey et al. 1991; Renner and Powell 1991; Spencer 1984). It is difficult to manage because of extended seedling emergence throughout the growing season, fast growth rate, high seed production, and dormancy mechanisms that result in persistent seeds (Warwick and Black 1988). Late-season plants often thrive after standard weed control practices are completed because plants tolerate many herbicides (Bussan et al. 2001; Hartzler and Battles 2001). Notably, velvetleaf is very difficult to control with glyphosate, which, given the current dominance of glyphosate-based production systems, is particularly problematic (Owen 2008).

Several researchers have reported the competitive attributes of velvetleaf and have remarked on how these attributes are most pronounced during times of significant environmental stress (McDonald et al. 2004; Munger et al. 1987; Patterson and Highsmith 1989). Similarly, environmental stresses, especially moisture stress, reduce herbicide performance on velvetleaf (Hinz and Owen 1994; Zhou et al. 2007). However, little has been reported on velvetleaf response to more than one environmental stress or how herbicide absorption is impacted when velvetleaf have been subjected to more than one environmental stress.

The cuticle acts as a protective layer on all aerial parts of higher plants and is the primary barrier to POST herbicide penetration (Kolattukudy 1970). It is composed of two layers, the inner layer of cutin and the outer ECW layer. Cuticular wax is mainly composed of long-chain aliphatic compounds derived from very long chain fatty acids (Kunst and Samuels 2003). The morphology and development of ECW is influenced by many environmental factors including temperature, light, relative humidity (RH), and soil water content, all of which consequently affect the foliar absorption of POST herbicides (Garcia et al. 2002; Levene and Owen 1995; Stevens and Baker 1987).

The importance of adjuvants for improving the activity of a POST herbicide has been recognized since the late 1940s (Staniforth and Loomis 1949). Research using adjuvants with acifluorfen and bentazon indicated that an oil adjuvant provided the greatest increase in weed control (Nalewaja et al. 1975; Willingham and Graham 1986). However, when fluid fertilizers were used as adjuvants for these herbicides, better weed control (in particular with velvetleaf) and less soybean injury occurred compared to oil adjuvants (Koppatschek et. al. 1986).

Little has been reported about the ability of an adjuvant to overcome reduced herbicide efficacy in response to environmental stresses, especially when using acifluorfen or bentazon. Willingham and Graham (1988) showed that the acifluorfen activity was greater when diammonium phosphate (DAP) was added compared to a nonionic surfactant and that an application with DAP nearly overcame the reduced herbicide penetration into velvetleaf from a low RH treatment. Hinz and Owen (1994) showed that velvetleaf absorption of bentazon was greater when COC was the adjuvant compared to 28% UAN under drought-stressed conditions (−0.4 mPa). However, dry weights were similar, which led them to conclude that UAN could not improve bentazon performance with drought-stressed velvetleaf. Somewhat contradictorily, Levene and Owen (1995) found that velvetleaf absorption of bentazon was similar when COC was compared to UAN under drought-stressed conditions (−0.4 mPa). Given the changes in weed communities attributable to the unprecedented adoption of glyphosate-resistant crop technologies and recurrent use of glyphosate as the primary tactic for weed control, specifically the evolved resistance in a number of important weeds in soybean production areas, herbicides such as bentazon and acifluorfen are likely to assume a more important role in soybean production (Herbicide Resistance Action Committee [HRAC] 2010; Owen and Zelaya 2005).

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Understanding how these herbicides and weeds respond to environmental conditions will result in more effective use of the products. Thus the objectives of this study were to determine the effects of temperature, drought stress, and light intensity on the quantity and chemical composition of leaf ECW for velvetleaf and to evaluate the effects of UAN and COC adjuvants and temperature, drought stress, and light intensity on velvetleaf absorption of acifluorfen and bentazon.

Materials and Methods

Plant Material. Velvetleaf seeds were placed in three evenly spaced holes in containers (capacity 225 ml) filled with 165 g of pasteurized, air-dried mix of Canisteo clay loam (fine-loamy mixed [calcareous], mesic Typic Haplaquolls), sand, and peat (3 : 1 : 1 by vol) that was watered to FC. After germination, plants were thinned to three plants per container and subjected to environmental treatments until the third- to fourth-leaf stage when herbicides were applied or ECW was removed. For the first study, plants were grown in growth chambers and subjected to factorial combinations of two temperatures, two soil water contents, and a maximum light intensity (812 micromoles [μmol] m^{-2} s^{-1} photosynthetic photon flux (PPF) at plant canopy level). Temperatures were designated as LT and HT with 20/15 C and 32/22 C day/night temperatures, respectively. A soil water content release curve was developed from procedures modified from Oyarzabal (1991) for the soil–sand–peat mixture in order to maintain plants at a soil water content of FC (−0.3 kPa) or drought stressed (DS) (−5 to −10 kPa). The soil moisture curve was determined using a tension table in the low suction range (0.0001 to 0.1 mPa) and with a ceramic pressure plate apparatus in the high suction range (0.1 to 1.5 mPa) and applied to the van Genuchten equation, thus describing the relationship between matric potential and the volumetric soil water content (van Genuchten 1980). Soil water content for the soil water treatments was monitored daily with a soil probe, and the containers were watered as needed to maintain the designated soil water contents.

In the second study, plants were grown in growth chambers and subjected to factorial combinations of the same two soil water content treatments as previously described and three light intensities, referred to as high light intensity, medium light intensity, and low light intensity, with maximum radiation levels of 812, 390, and 145 μmol m^{-2} s^{-1} PPF, respectively, using metal-halide high intensity discharge lamps. Light intensity was measured at the top of the plant canopy. Growth chambers were programmed for a 15.5-h photoperiod increasing proportionally with time from 90 μmol m^{-2} s^{-1} PPF to the maximum level for the first 7 h and then decreasing to 90 μmol m^{-2} s^{-1} PPF for the last 8.5 h of the photoperiod.

Growth chamber RH was maintained at 65/85% (day/night) for both studies. Soil water treatments were monitored as described previously. Containers assigned to the FC regime were watered once daily to bring the soil water content to −0.3 kPa. Containers for the DS treatment were watered as needed to bring the soil water content to −5 kPa.

Epicuticular Wax Quantity. The amount of ECW deposited on leaves was determined by a colorimetric method (Ebercon et al. 1977). The youngest fully expanded leaf was sampled, and the leaf area was determined using a leaf area meter.[1] Samples were immersed in 20 ml of chloroform for 15 s without immersing the cut end. The chloroform-ECW mixture was then filtered and combined with 5 ml of chloroform from the funnel rinse before evaporating in an 85 C water bath.

After chloroform evaporation, each filtrate sample was diluted with 5 ml sulfuric acid/potassium bichromate reagent, placed in boiling water for 30 min, cooled, and diluted with 12 ml of deionized water. Absorbance at 590 nm was measured[2] after a 3-h interval. The relative absorbance of a sample was determined by comparison with 20 ml chloroform "blank" that was subjected to all the procedures described in the previous paragraph but without a leaf.

A standard curve for ECW quantity was developed using ECW from mature field-grown plants. The ECW stock solution was made gravimetrically in Chloroform at concentrations of 0, 100, 200, 400, 800, 1,200, and 1,600 micrograms (μg) ECW and absorbance measured at 590 nm. The procedure was repeated six times with regression analysis conducted on the average measured absorbance. The standard curve was linear throughout the range of ECW concentrations and gave a regression equation of:

$$y = 131.6x \qquad [1]$$

where y = absorbance at 590 nm and x = amount of ECW in micrograms.

Epicuticular Wax Chemical Composition. The ECW was labeled with $^{14}CO_2$ using a procedure described by Wilkinson and Mayeux Jr. (1987), and the chemical components were separated using thin layer chromatography (TLC) as described by Holloway and Challen (1966). A total of 16.7 kilobecquerel (kBq) of ^{14}C-sodium carbonate[3] (specific activity of 170.1 kBq $mmol^{-1}$) was diluted with 1,100 μl of water to make a stock solution of 1.67 by 10^4 Bq μl^{-1}. Plants were enclosed in a sealed plexiglass chamber and labeled with 30 μl (3 by 10^7 disintegrations per minute) of the stock solution. The plexiglass chamber was placed inside a growth chamber programmed as described in the plant material section. Radiolabeled CO_2 was evolved by injecting 3 ml of hydrochloric acid (36.5 to 38.0%) through a rubber stopper into the container with ^{14}C-sodium carbonate. After 24 h, the ECW was removed with chloroform as previously described and allowed to air dry until approximately 1 ml of chloroform/ECW solution remained. The remaining sample was sealed and stored until the ECW components were separated by one direction TLC.

The 250-micrometer silica gel TLC plates[4] were washed with purified grade, double-distilled benzene and dried at 110 C. A 40-μl aliquot of ^{14}C-labeled ECW was spotted and developed to 15 cm in benzene. ECW fractions were identified by including the known standard in separate channels of the TLC plate and then comparing the front values (R_f) of the standard and the separated components. Spots containing ^{14}C-ECW components were detected by attaching a sheet of X-ray film[5] to the TLC plate and storing for 2 wk. Separated components were located by spraying the plates with 5% sulfuric acid and charring at 160 C. Identified bands were removed, mixed with 15 ml scintillation cocktail, and assayed by liquid scintillation spectrometry[6] (LSS). The amount of radioactivity in each fraction was expressed as the percentage of the total radioactivity recovered from all fractions. Recovery of ^{14}C in isolated fractions ranged from 96 to 99%.

Herbicide Absorption. Plants at the three- to four-leaf stage were treated on the adaxial surface of the newest fully expanded leaf between major veins with five 1-µl droplets of ^{14}C-labeled acifluorfen[7] with a specific activity 0.23 kBq mol^{-1} or ^{14}C-labeled bentazon[8] with a specific activity 0.39 kBq mol^{-1}. A single 1-µl droplet contained 33 Bq of ^{14}C-acifluorfen or 267 Bq ^{14}C-bentazon. Unlabeled herbicide was added to the radioactive solution to obtain an acifluorfen spray solution containing 0.42 kg ai ha^{-1} at a spray volume of 187 L ha^{-1} or a bentazon spray solution containing 1.12 kg ai ha^{-1} at a spray volume of 280 L ha^{-1}. All plants were watered to FC the day before the herbicide application and kept at FC thereafter. Herbicide mixtures were applied with no adjuvant, water plus 28% UAN (9.4 L ha^{-1}), or water plus COC[9] (0.5 L ha^{-1} for acifluorfen and 2.3 L ha^{-1} for bentazon). Plants were returned to a growth chamber with 32/22 C day/night temperatures, 90 to 812 µmol m^{-2} s^{-1} PPF, 65/85% day/night RH, and 15.5-h photoperiod after the droplets dried.

Treated plants were sampled 12, 24, and 48 h after a herbicide application by detaching the treated leaf and immersing immediately for 5 min in 4 ml of scintillation cocktail (3 : 1 [v/v] mixture of xylene[3] and octylphenoxypolyethoxyethanol[3] with 4 gram L^{-1} 2,5-diphenyloxazole[3]) (White et al. 2002). The leaf was then rinsed with 15 ml of scintillation cocktail, which was combined with the original 4-ml solution and assayed by LSS. The remaining plant was separated from the soil and rinsed. Both plant samples were frozen and stored at −60 C until combusted. Frozen plant parts were combusted to $^{14}CO_2$ in a sample oxidizer[10] using 10 : 9 Permafluor V–Carbosorb II as scintillation solution and then assayed by LSS. The combined radioactivity in the treated leaf plus that recovered in the shoots and roots was designated as the total amount of herbicide taken up by the plant.

Each study was conducted in a randomized complete block design with four replications, and was repeated. Data presented are from two separate studies except when Bartlett's test for homogeneity of variance allowed a combined analysis of variance (Peterson 1994). The ^{14}C-herbicide absorption percentages were analyzed as a split–split–split plot with either temperature or radiation as the main factor, soil water content as the subfactor, and adjuvant as the sub subfactor. Treatment differences were determined by Fisher's Protected LSD test at the 5% level of probability.

Results and Discussion

Epicuticular Wax Deposition. Soil water content and temperature affected the amount of ECW deposited on velvetleaf leaves. Plants grown under drought stress in studies I and II had leaves with 81 and 50% more ECW, respectively, than leaves from plants with adequate moisture (Table 1). Likewise, leaves from plants subjected to LT had 70% more ECW than leaves from plants grown under HT. Light intensity did not affect ECW quantity with a range of 5.3 to 5.9 µg cm^{-2} ECW for the three light levels used in this experiment. Plants subjected to the combination of LT and drought-stress treatments had the greatest leaf ECW (12.5 µg cm^{-2}), while plants subjected to the HT/drought stress, LT/FC, and HT/FC regimes had leaf ECW of 6.3, 5.9, and 4.5 µg cm^{-2}, respectively (data not shown).

Table 1. Effect of temperature or soil water content on velvetleaf epicuticular wax deposition.

Parameter	Treatment	Epicuticular wax
		µg cm^{-2}
Study I		
Temperature[a]	Low	9.2
	High	5.4
	LSD (0.05)	0.9
Soil water content[b]	Field capacity	5.2
	Drought stress	9.4
	LSD (0.05)	0.9
Study II		
Soil water content[b]	Field capacity	4.4
	Drought stress	6.6
	LSD (0.05)	0.7
Light intensity[c]	High	5.4
	Medium	5.9
	Low	5.3
	LSD (0.05)	NS

[a] Low temperature = 15/20 C day/night temperatures; and high temperature = 22/32 C day/night temperatures.

[b] Field capacity = soil water content at field capacity (−0.33 kPa); and drought stress = soil water content from −5 to −10 kPa.

[c] High light = 812; medium light = 390; and low light = 145 µmol m^{-2} s^{-1} maximum PPF.

Increased ECW production on leaves of plants grown under drought stress has been observed with several species (Bondada et al. 1996; McWhorter 1993; Premachandra et al. 1992; Thankamani and Ashokan 2002). An increase in ECW also has been shown to reduce the net radiation load of the canopy as well as cuticular transpiration thus improving stomata control over transpiration and water-use efficiency (Blum 1979; Jefferson et al. 1989; Sanchez et al. 2001).

LTs have been shown to increase ECW production and were mainly attributed to different leaf expansion rates (Reed and Tukey 1982; Wilkinson and Kasperbauer 1980). Leaves from velvetleaf grown under LT did require an additional week to reach full expansion compared with the HT regime. Furthermore, Levene and Owen (1995) showed that the deposition of ECW cm^{-2} decreased as velvetleaf leaf size increased.

Epicuticular Wax Chemical Composition. Velvetleaf ECW had five major chemical components: fatty acids, primary alcohols, secondary alcohols, esters, and hydrocarbons with the respective R_f values of 0.00 to 0.04, 0.11 to 0.16, 0.26 to 0.33, 0.62 to 0.71, and 0.86 to 0.93. The DS treatment decreased the ester content in the ECW by approximately 18% and increased the hydrocarbon content by approximately 24%, when compared to the FC treatment regardless of the temperature (Table 2). Likewise, the HT treatment decreased the ester content in the ECW by approximately 25% and increased the hydrocarbon content by approximately 11%, when compared to the LT treatment regardless of the soil water content.

High light intensity had an opposite effect on ester content in ECW with 40% less synthesized under high light intensity compared to the low light regime. Secondary alcohol content also was affected by light intensity with the high light intensity increasing this alcohol content by approximately 41%. Macey

Table 2. Effect of temperature, soil water content, or light intensity on velvetleaf epicuticular wax chemical composition.

Treatments[a]	Epicuticular wax components				
	Fatty acid	Primary alcohol	Secondary alcohol	Ester	Hydrocarbon
	% of total				
Study I					
Low temperature	13.8	29.7	22.1	11.5	17.8
High temperature	10.9	31.2	24.0	8.7	20.3
LSD (0.05)	NS	NS	NS	1.9	2.0
Field capacity	13.1	31.1	22.0	11.2	17.3
Drought stress	11.6	29.8	24.1	8.9	20.8
LSD (0.05)	NS	NS	NS	1.8	2.1
Study II					
High light	10.9	31.2	24.0	8.7	20.3
Medium light	10.1	32.2	21.2	11.2	20.2
Low light	13.9	27.3	17.4	15.3	20.5
LSD (0.05)	NS	NS	3.5	3.4	NS
Field capacity	12	29	21	13	19
Drought stress	11	31	21	10	21
LSD (0.05)	NS	NS	NS	NS	NS

[a] Low temperature = 20/15 C day/night temperatures; high temperature = 32/22 C day/night temperatures; field capacity = soil water content at field capacity (−0.33 kPa); drought stress = soil water content from −5 to −10 kPa; high light = 812, medium = 390, and low = 145 μmol m^{-2} s^{-1} maximum PPF.

(1970) reported a similar ester content decrease and secondary alcohol increase in cabbage (*Brassica oleracea* L.) grown under high light intensity.

Conditions such as HT and drought stress may induce an increase in hydrocarbon content as a defense mechanism to conserve water in the plant. Hydrocarbons are considered highly hydrophobic, whereas alcohols and acids are relatively hydrophilic (Baker 1982). Bondada et al. (1996) reported a drastic increase in hydrocarbon content in cotton (*Gossypium hirsutum* L.) leaf ECW under water-stressed conditions and suggested it was an adaptation protecting the plants from adverse environmental conditions. An increase in hydrocarbon content may also help reduce cuticular transpiration. Kolattukudy (1970) reported that hydrocarbons were more effective in reducing transpiration than any other wax component and the least wettable of all other wax components.

Herbicide Absorption. Herbicide losses from photodecomposition or volatilization, or both, were minimal and did not affect the results (data not shown). Total ^{14}C recovery, regardless of the herbicide or adjuvant, was greater than 95% and similar to recoveries in other studies with acifluorfen or bentazon (Hinz and Owen 1994; Levene and Owen 1994, 1995; Wills and McWhorter 1981).

Bentazon absorption varied between soil water content and temperature, and between soil water content and light intensity. Bentazon absorption was lowest when plants were grown under DS and HT regimes with absorption of only 6.4% of the applied herbicide (Figure 1). In contrast, bentazon absorption was the greatest (10% of applied) when plants were grown under FC and HT treatments. Moisture stress has been shown to reduce bentazon absorption in velvetleaf (Hinz and Owen 1994; Levene and Owen 1995). Koukkari and Johnson (1979) also reported that the leaves of velvetleaf were less injured by bentazon under low soil moisture and RH compared to high RH conditions. However, higher absorption when plants were grown under FC and LT regimes or DS and LT regimes compared to the DS and HT regimes suggests that ECW composition may influence herbicide absorption. The ECW hydrocarbon content was greater under DS and HT regimes. Chachalis et

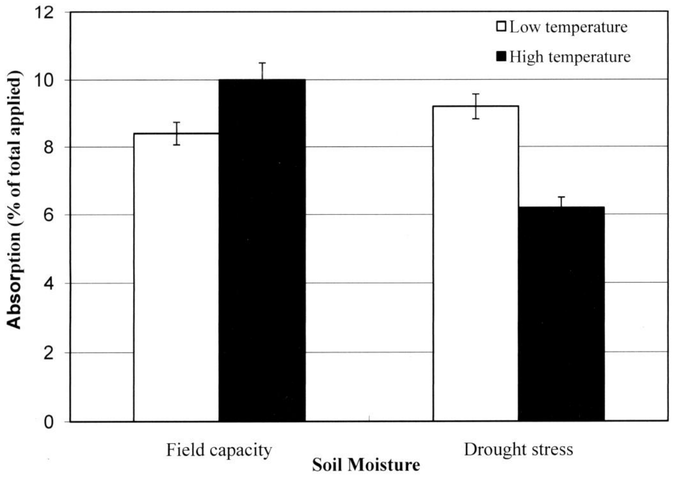

Figure 1. Temperature and soil water content affect on ^{14}C-bentazon absorption by velvetleaf. LSD (0.05) = 0.5. Vertical bars represent standard errors of the means.

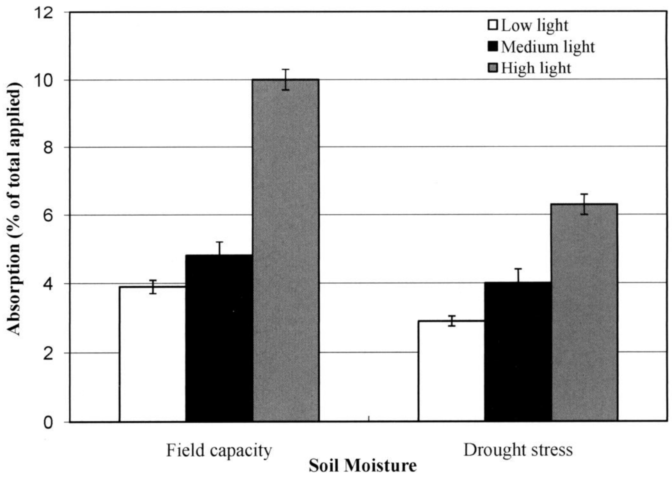

Figure 2. Light intensity and soil water content affect on ^{14}C-bentazon absorption by velvetleaf. LSD (0.05) = 0.6. Vertical bars represent standard errors of the means.

Table 3. Effect of environmental factors (temperature, soil water content, or light intensity) prior to herbicide application and spray adjuvants on velvetleaf absorption of ^{14}C-acifluorfen.

Treatments[a]	None[b]	UAN	COC
		% total absorption[c]	
Study I			
Low temperature	3.2	46.9	7.6
High temperature	4.0	41.2	15.1
LSD (0.05)		3.2	
Field capacity	3.5	38.5	11.3
Drought stress	3.9	48.9	11.2
LSD (0.05)		4.8	
Study II			
High light	4.2	41.4	15.0
Medium light	3.6	46.1	8.3
Low light	5.3	40.8	13.8
LSD (0.05)		4.3	
Field capacity	4.9	45.8	12.6
Drought stress	4.0	39.9	12.3
LSD (0.05)		3.9	

[a] Low temperature = 20/15 C day/night temperatures; high temperature = 32/22 C day/night temperatures; field capacity = soil water content at field capacity (−0.33 kPa); drought stress = drought stress (−5 to −10 kPa); high light = 812, medium = 390, and low = 145 μmol s^{-1} m^{-2} maximum PPF.

[b] None was herbicide alone; urea ammonium nitrate (UAN) was 28% N added at 9.4 L ha^{-1}; crop oil concentrate (COC) was added at 0.5 L ha^{-1}.

[c] Percentages are averages of repeated trials averaged over time.

Table 4. Effect of environmental factors (temperature, soil water content, or light intensity) prior to herbicide application and spray adjuvants on velvetleaf absorption of ^{14}C-bentazon.

Treatments[a]	None[b]	UAN	COC
		% total absorption[c]	
Study I			
Low temperature	4.3	14.0	8.3
High temperature	4.9	8.0	12.1
LSD (0.05)		1.9	
Field capacity	4.8	11.1	11.9
Drought stress	4.2	10.4	8.7
LSD (0.05)		1.7	
Study II			
High light	4.6	8.0	12.1
Medium light	1.9	4.8	6.6
Low light	1.1	3.8	5.1
LSD (0.05)		4.1	
Field capacity	2.8	6.1	9.6
Drought stress	2.7	4.7	6.3
LSD (0.05)		2.4	

[a] Low temperature = 20/15 C day/night temperatures; high temperature = 32/22 C day/night temperatures; field capacity = soil water content at field capacity (−0.33 kPa); drought stress = soil water content from −5 to −10 kPa; high light = 812, medium light = 390, and low light = 145 μmol s^{-1} m^{-2} maximum PPF.

[b] None was herbicide alone; urea ammonium nitrate (UAN) was 28% N added at 9.4 L ha^{-1}; crop oil concentrate (COC) was added at 2.3 L ha^{-1}.

[c] Percentages are averages of repeated trials averaged over time.

al. (2001) showed that ECW composition of redvine [*Brunnichia ovata* (Walt.) Shinners] leaves became more hydrophobic as they matured and concluded that the lower glyphosate efficacy was related to the more hydrophobic nature of redvine ECW compared to that of trumpetcreeper [*Campsis radicans* (L.) Seem ex. Bureau].

Bentazon absorption increased with increasing light intensity with the greatest ^{14}C in plants grown under the high light and FC condition (Figure 2). Less bentazon absorption under DS was most obvious with the high light regime, which had 36% less total absorption compared to the high light and FC treatment. Retzlaff (1983) showed that bentazon absorption increased with increasing light intensity in mustard (*Sinapis alba* L.) suggesting that bentazon absorption may be dependent upon the supply of adenosine triphosphate available in the plants. The results of this study reinforce the results by Retzlaff (1983), but also suggest that the quantity of ECW may affect bentazon absorption as the quantity of ECW was greater under the DS condition. Oosterhuis et al. (1991) concluded that an increase in leaf ECW production for cotton was associated with decreased foliar-applied chemical penetration.

Acifluorfen absorption also varied with variation in soil water content and light intensity. When plants had adequate soil water (FC), acifluorfen absorption decreased with increasing light intensity, whereas the opposite was true under the DS condition (Figure 3). Kowalczyk et al. (1983) determined that differences in difenzoquat activity could not be attributed to leaf anatomical changes but were attributed to physiological changes that affected tissue susceptibility to the

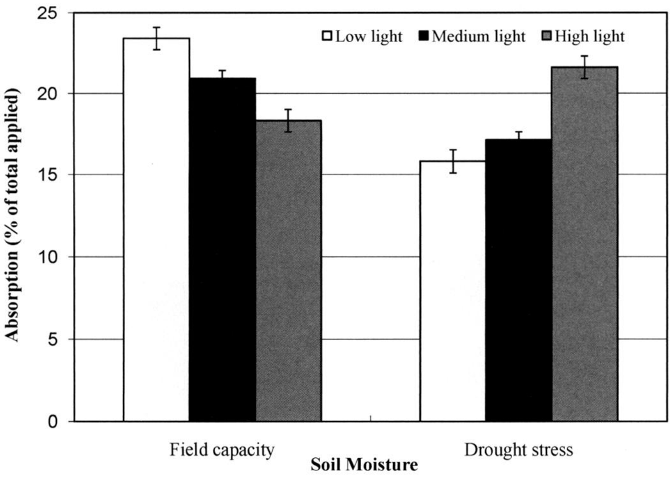

Figure 3. Light intensity and soil water content affect on ^{14}C-acifluorfen absorption by velvetleaf. LSD (0.05) = 4.1. Vertical bars represent standard errors of the means.

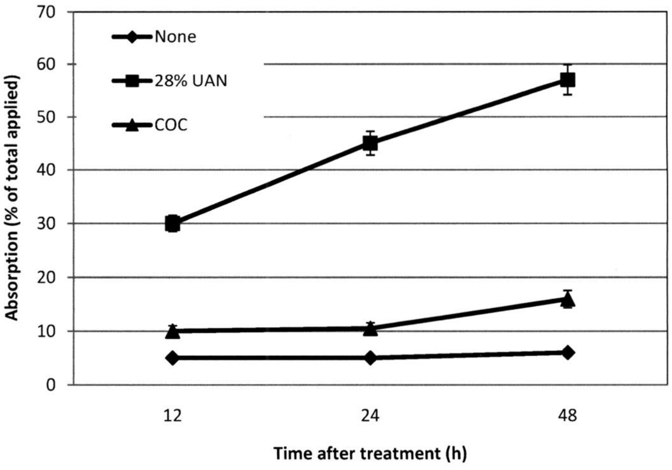

Figure 4. Herbicide adjuvants (28% urea ammonium nitrate [UAN] and crop oil concentrate [COC]) and time after treatment affect on ^{14}C-acifluorfen absorption by velvetleaf. LSD (0.05) = 4.3. Vertical bars represent standard errors of the means.

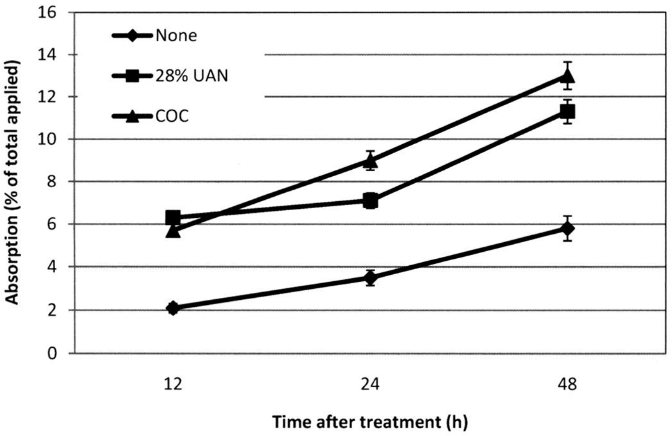

Figure 5. Herbicide adjuvants (28% urea ammonium nitrate [UAN] and crop oil concentrate [COC]) and time after treatment affect on ^{14}C-bentazon absorption by velvetleaf. LSD (0.05) = 2.5. Vertical bars represent standard errors of the means.

herbicide. Acifluorfen is a protoporphyrinogen oxidase (Enzyme Commission [EC] 1.3.3.4) inhibitor herbicide, which, in the presence of light, causes rapid cell membrane disruption (Wright et al. 1995). Consequently, under optimum moisture and high light intensity, acifluorfen penetration may have been reduced by rapid cell membrane destruction, which is consistent with herbicides targeting this site of action.

Acifluorfen and bentazon absorption varied with environmental condition (temperature, moisture, or light intensity) and spray adjuvants, and between spray adjuvant and time. Interactions of light intensity by time and soil water content by time also influenced bentazon absorption. However, the interaction of light intensity by time for bentazon absorption was due to magnitude difference with high light in comparison to low and medium light. Approximately 40% of the applied acifluorfen was taken into the plant when UAN was used as the adjuvant, regardless of the environmental treatment, which was almost 3 and 10 times greater absorption than when COC or no adjuvant was used, respectively (Table 3). When UAN was the additive, 30% of the applied acifluorfen was absorbed by 12 h after applications, increasing to 56% by 48 h after application (Figure 4).

Velvetleaf absorption of bentazon was low with less than 15% taken into the plant regardless of the adjuvant or environmental treatment (Table 4). Bentazon absorption was greatest when UAN was the adjuvant and plants were grown under a LT regime. In general, the addition of COC or UAN increased bentazon absorption at least twice what was absorbed when no adjuvant was used. Increasing light intensity tended to increase bentazon absorption. Velvetleaf absorption of bentazon was 12.1% when COC was the adjuvant and plants were grown under the highest light intensity.

The addition of COC to bentazon increased the rate of bentazon absorption from 12 to 24 h after treatment compared to UAN (Figure 5). However, from 24 to 48 h after treatment, the rate of bentazon absorption was similar for both adjuvants.

Plants subjected to a HT regime absorbed 12% less acifluorfen and 43% less bentazon when UAN was added compared with the LT regime even though leaf ECW was greater with the LT regime (Tables 3 and 4). However, when COC was used, acifluorfen and bentazon absorption increased 99 and 46%, respectively, for plants grown under the HT regime compared with the LT regime. Bentazon absorption has been shown to be greater at higher temperatures when applied with no adjuvant (Retzlaff 1983), while acifluorfen absorption has been shown to be greater at lower temperatures when averaged over three adjuvants including DAP (Willingham and Graham 1988).

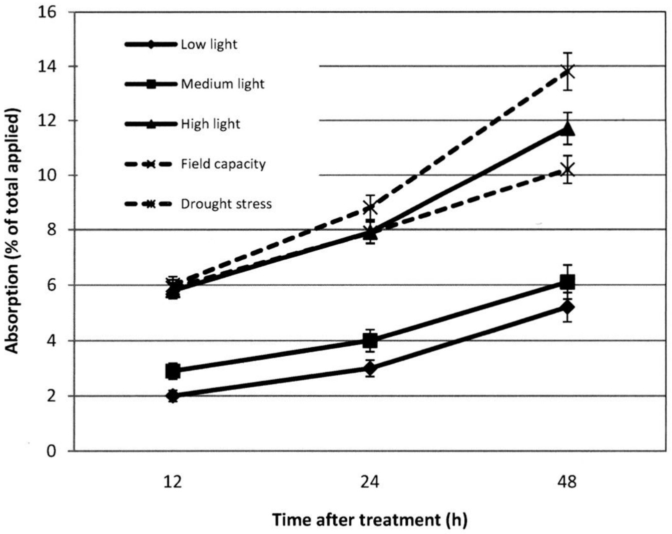

Figure 6. Light intensity or soil water content and time after treatment affect on ^{14}C-bentazon absorption by velvetleaf. LSD (0.05) = 2.7 and 1.3, respectively. Vertical bars represent standard errors of the means.

The results of this study showed that the effect of temperature on acifluorfen or bentazon absorption was dependent on the herbicide adjuvant. Reduced herbicide absorption with the addition of COC for plants subjected to the LT regime may have been influenced by the higher amount of leaf ECW. Likewise, the higher leaf ECW hydrocarbon content may have contributed to the reduced herbicide absorption with the addition of UAN for plants subjected to the HT regime. However, it is evident that ECW quantity and composition changes in response to temperature are not solely responsible for the differences in herbicide absorption.

Soil water content did not affect acifluorfen absorption when no adjuvant or COC was used, while UAN resulted in a marked increase of acifluorfen absorption under drought stress compared with FC treatments (Table 3). However, DS reduced total bentazon absorption when COC was used (Table 4). The rate of bentazon absorption also was less for plants subjected to the DS regime in comparison with the FC regime (Figure 6). Willingham and Graham (1988) reported that RH and temperature had a greater effect on acifluorfen penetration into velvetleaf than soil water content. Velvetleaf control has also been reported to be variable with the addition of COC or UAN to acifluorfen (Koppatschek et al. 1986; Mohan and Rathmann 1986). Therefore, consistent reduction in acifluorfen absorption in DS plants was not expected. Similar results have also been reported with bentazon. Hinz and Owen (1994) reported that DS reduced bentazon penetration when UAN was the adjuvant and increased bentazon penetration when COC was the adjuvant, while Levene and Owen (1995) reported that DS velvetleaf had greater leaf ECW and reduced bentazon penetration when either COC or UAN was used. The results of this study reinforce those of Levene and Owen (1995) in that DS plants had more leaf ECW, which may have helped reduce the rate of and total amount of bentazon absorbed for the DS plants, even though this was significant only when COC was the adjuvant.

Light intensity did not consistently affect acifluorfen absorption. Plants receiving medium light had greater acifluorfen absorption than high or low light intensities when UAN was the adjuvant, while the opposite was true when COC was the adjuvant (Table 3). High light intensity did, however, increase bentazon absorption in comparison to the low light intensity as long as an adjuvant was used (Table 4). The rate of bentazon absorption and overall absorption also was greater with high light intensity in comparison with medium or low light intensity (Figure 6). By 48 h after treatment, less than 6.5% of the applied bentazon was absorbed into the leaves of plants grown under medium or low light intensities, whereas almost 6% had been absorbed by 12 h after treatment increasing to 11% by 48 h after application for plants grown under the high light intensity. Retzlaff (1983) reported an increase in bentazon absorption as light intensity increased and a higher rate of bentazon absorption with the high light intensity. The implications of the reduced bentazon absorption is important for the control of velvetleaf that has emerged under the soybean canopy, which may be more difficult to control as a result of less light intensity.

The results from this study show that air temperature, soil water content, and light intensity changed the leaf ECW quantity and composition for velvetleaf. Conditions of DS, HT, and high light intensity may induce plant stress such that water conservation becomes a priority. Results also suggest that the complex interaction of environmental conditions on velvetleaf ECW chemical composition, ECW quantity, and anatomical and physiological characteristics of velvetleaf, along with the adjuvant, and herbicide properties, ultimately determine acifluorfen or bentazon absorption. Generally, this research suggests that the influence of soil moisture and temperature is greater on the response of velvetleaf to acifluorfen and bentazon than the interaction between soil moisture and light. However, the interaction with light cannot be discounted when considering the deposition and chemical composition of the ECW and resultant absorption of the herbicides. To what extent the differences in the velvetleaf ECW chemical composition has on the absorption of acifluorfen or bentazon is unclear. Results do suggest that the complex environmental interactions impact the overall physiology of velvetleaf and even when conditions place the plants in a more favorable growth condition, leaf ECW appears to be an effective barrier to acifluorfen or bentazon absorption. However, adjuvants such as COC and UAN will impact the relative absorption of acifluorfen or bentazon. Velvetleaf ECW quantity and composition may have a greater effect on acifluorfen or bentazon absorption when COC is the adjuvant as environmental conditions associated with less leaf ECW quantity and hydrophilic composition generally had the greatest herbicide absorption. Velvetleaf anatomical and physiological leaf characteristics may be more important for acifluorfen absorption when UAN is the adjuvant as herbicide absorption was almost three times greater than COC regardless of the environmental condition.

Given that herbicides, such as acifluorfen and bentazon, may be utilized more frequently as glyphosate resistance evolves in agronomical important weeds, and that velvetleaf populations are increasing in the Midwest, information about how these herbicides respond to environmental factors is useful and will help growers better manage weeds. Future research to better characterize the complex interactions described in this research is needed. The range of environmental stresses included in the interactions should be reviewed and a general understanding on the physiological "competency" of velvetleaf needs to be considered. Importantly, the research should consider whether or not these interactions are important under field conditions; it is suggested that one stressor may override the impact of other environmental conditions and thus may provide a better understanding of plant response as well as insight into the results reported above.

Sources of Materials

[1] Li-Cor LI-3000 leaf area meter, Li-Cor Lambda Instrument Corp., Lincoln, NE 68504.

[2] Beckman model DU spectrophotometer, Beckman Instruments, Fullerton, CA 92634.

[3] ^{14}C-sodium carbonate, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178-9916.

[4] Linear-K pre-adsorbent TLC plates, Whatman Inc., 9 Bridwell Place, Clifton, NJ 07014.

[5] X-Omat AR Kodak diagnostic film, Eastman Kodak Co., Rochester, NY 14650.

[6] Beckman model LS 3801 liquid scintillation system, Beckman Instruments, Fullerton, CA 92634.

[7] Ultra Blazer, United Phosporus, Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

[8] Basagran, BASF Ag Products, 26 Davis Drive, Research Triangle Park, NC 27709-3528.

[9] Prime Oil, Riverside/Terra Corp., Sioux City, IA 51101.

[10] Model 306A Packard tri-carb sample oxidizer, Packard Instrument Co., Downers Grove, IL 60515.

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