

# Characterization of leaf surface, wax composition, and control of redvine and trumpetcreeper with glyphosate

Demosthenis Chachalis

Southern Weed Science Research Unit, USDA-ARS, P.O. Box 350, Stoneville, MS 38776. Current address: Greek National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Volos, P.O. Box 303, Volos 380 01, Greece

Krishna N. Reddy

Corresponding author. Southern Weed Science Research Unit, USDA-ARS, P.O. Box 350, Stoneville, MS 38776; kreddy@ars.usda.gov

C. Dennis Elmore

Application and Production Technology Research Unit, USDA-ARS, P.O. Box 36, Stoneville, MS 38776.

Laboratory and greenhouse studies were conducted on redvine and trumpetcreeper to characterize leaf surface and wax composition, determine responses of these weeds to glyphosate, characterize the nature of interactions between glyphosate and several selective postemergence herbicides (e.g., acifluorfen, bentazon, chlorimuron, imazaquin, and pyriithiobac) used in soybean and cotton, and determine the effects of various adjuvants on glyphosate activity on both species. Trumpetcreeper was consistently more susceptible to glyphosate than redvine. Glyphosate spray solution droplets had lower contact angle in trumpetcreeper than in redvine. Micro-roughness of the trumpetcreeper adaxial leaf surface was greater due to trichomes and glands compared to that of redvine, which had no trichomes or glands. Stomata or crystal wax deposition on the adaxial leaf surface were not observed in either species. The wax mass per unit area (22 to 37  $\mu\text{g cm}^{-2}$ ) was similar regardless of the leaf age in both species. Epicuticular wax consisted of hydrocarbons, alcohols, acids, and triterpenes. Wax composition of young leaves of redvine was relatively hydrophilic (72% alcohols and acids, 24% hydrocarbons) compared to the hydrophobic components (23% alcohols and acids, 49% hydrocarbons) of old leaves. In contrast, wax of trumpetcreeper young leaves was relatively hydrophobic (9% alcohols and acids, 29% hydrocarbons), whereas old leaves had similar levels of hydrophilic and hydrophobic components (28% alcohols and acids, 31% hydrocarbons). Glyphosate mixed with selective postemergence herbicides were antagonistic when applied to redvine and trumpetcreeper, except acifluorfen. Various adjuvants did not increase glyphosate efficacy except ammonium sulfate, which increased glyphosate efficacy when applied alone to trumpetcreeper. These results showed that lower glyphosate efficacy was related to the more hydrophobic nature of redvine epicuticular waxes compared to that of trumpetcreeper.

**Nomenclature:** Acifluorfen; bentazon; chlorimuron; glyphosate; imazaquin; pyriithiobac; cotton, *Gossypium hirsutum* L.; redvine, *Brunnichia ovata* (Walt.) Shinnery BRVCI; soybean, *Glycine max* (L.) Merr.; trumpetcreeper, *Campsis radicans* (L.) Seem. ex Bureau CMIRA.

**Key words:** Adjuvant, contact angle, gas chromatography–mass spectrometry, leaf morphology, scanning electron microscopy, BRVCI, CMIRA.

Redvine and trumpetcreeper are common perennial vines found in Mississippi. Redvine has been the most common perennial weed found in more than 31% of soybean fields and in 43% of cotton fields in the Mississippi Delta (Elmore 1984).

Both species are among the 10 most troublesome weeds in cotton and soybean (Dowler 1998). These species are difficult to control because of their extensive deep root system (Elmore 1984). Propagation is mostly vegetative, but these species produce numerous seeds in noncultivated areas such as ditches, roadsides, and fence rows that have potential to spread to new areas (Chachalis and Reddy 2000; Shaw et al. 1991). Tillage practices to control these perennial weeds are ineffective because disturbance of the existing root system results in shoot production from root segments (Castillo et al. 1998; Shaw and Mack 1991). Both species are difficult to control with herbicides. Many herbicides kill only foliage, with little or no translocation to roots. A commonly suggested control method is to apply dicamba, imazapyr, or glyphosate to plants in the fall, either in a fallow field or after crop harvest (DeFelice and Oliver 1980; Elmore 1984; Hurst 1994, 1995; Shaw and Mack 1991).

The development of transgenic crops resistant to glyphosate means this herbicide can be used to control weeds without injuring the crop. Glyphosate is phytotoxic to redvine (DeFelice and Oliver 1980; Reddy 2000; Shaw and Mack 1991) and trumpetcreeper (DeFelice and Oliver 1980). Use of herbicide mixtures is a common weed control practice because mixtures may produce synergistic or additive interactions, reduce rates, and improve control of targeted weed species. Glyphosate mixed with several herbicides has been studied, and the nature of interactions varied depending upon herbicide chemistry and weed species (Hatzios and Penner 1985; Rao and Reddy 1999; Scott et al. 1998).

Leaf epicuticular waxes contain primarily a variety of long-chain hydrocarbons, acids, alcohols, aldehydes, and esters (Baker 1982). Wax is primarily nonpolar (hydrophobic) but varies among species with regard to the degree of hydrophobicity, primarily due to chemical composition of the wax. Hydrocarbons are highly hydrophobic, whereas alcohols and acids are less hydrophobic. Published information was not found on leaf surface micromorphology of redvine and trumpetcreeper.

The objectives of this study were to (1) characterize leaf surface and wax composition in redvine and trumpetcreeper, (2) determine responses of these weeds to glyphosate, (3) characterize the nature of interactions between glyphosate and several selective postemergence herbicides used in soybean and cotton, and (4) determine the effects of various adjuvants on glyphosate activity on redvine and trumpetcreeper.

## Materials and Methods

### Glyphosate Efficacy

Research was conducted from spring to winter 1999. Plants were grown from roots collected near the Southern Weed Science Research farm. One rootstock, about 7-cm long, was planted in a 9-cm-diam pot containing a mixture of soil (Bosket sandy loam, fine-loamy, mixed, thermic Mollic Hapludalfs) and potting soil<sup>1</sup> (1:1 v/v). Plants were grown in a greenhouse at 33/25 C ( $\pm$  3 C) day/night temperature. Natural light was supplemented with light from sodium vapor lamps to provide a 14-h photoperiod. Soil was surface irrigated as needed. Plants at the four- to seven-leaf stage (18- to 24-cm tall) were sprayed with a commercial formulation of glyphosate at 1.1 and 3.3 kg ai ha<sup>-1</sup> using an indoor spray chamber equipped with an air-pressurized system<sup>2</sup> at 190 L ha<sup>-1</sup> and 140 kPa. Herbicide efficacy was assessed at 3 wk after treatment (WAT). Shoots were clipped at the soil surface and oven dried and dry biomass was recorded. Data were expressed as percent shoot dry biomass reduction (% control) compared to untreated plants. Clipped plants were allowed to regrow for 5 wk, and the regrowth was harvested as described above to determine regrowth potential of the herbicide-treated plants. Treatments were arranged in split plot design, with the species as the main plots and the herbicide rate as a subplot. Treatments were replicated four times and the experiment was repeated. Nontransformed data are presented because transformations did not affect conclusions. Data were subjected to analysis of variance (ANOVA), and means were separated using Fisher's LSD test at  $P = 0.05$ .

Measurements of contact angle were taken on the adaxial surface of the fifth to seventh fully expanded leaf as described by Elmore and Paul (1998). The leaves were selected daily at random just before measurement. The mean of the contact angles of both sides of a 1- $\mu$ l droplet on a leaf surface was calculated approximately 1 min after droplet application using a goniometer.<sup>3</sup> Each value was the mean of the contact angles of both sides of the droplet. From each plant only one leaf was collected, replicated five times, and the experiment was repeated.

### Scanning Electron Microscopy of Leaf Surfaces

Leaves were collected from viney plants approximately 3 mo old. Young leaves (first or second leaf from the apical meristem) and old leaves (fifth to seventh leaf from the apical meristem) were collected from each plant. Two leaf segments (approximately 20 mm<sup>2</sup>) were taken from the adaxial and abaxial surface of the middle part of the leaf. Each segment was fixed for 12 h in 4% glutaraldehyde and rinsed three times with distilled water before dehydration in a graded ethanol series. Samples were critical point-dried<sup>4</sup> and

mounted on aluminum stubs. Samples were gold-coated using a sputter coater<sup>5</sup> and examined with a scanning electron microscope.<sup>6</sup> For counting purposes, all leaf surfaces were photographed at the same magnification. Numbers of stomata, glands, and trichomes were counted as described by Elmore and Paul (1998). Two photographs from each sample were examined, and the study was repeated four times.

### Wax Mass and Composition

The wax extraction and analysis procedure was previously described by Elmore et al. (1998). Young and old leaves were collected from field-grown plants. Wax was extracted from approximately 50 leaves by immersing leaves for 10 s in 1 L HPLC-grade chloroform at room temperature. The chloroform/wax solution was filtered through analytical-grade filter paper, and the volume was reduced to approximately 20 ml in a rotary evaporator. The reduced chloroform/wax solution was transferred to a preweighed 25-ml glass scintillation vial. Chloroform was evaporated to dryness under a hood, and the vials were kept in a desiccator with silica gel blue for 7 d before wax mass was recorded. Each vial was reweighed after an additional 7 d in a desiccator to ensure complete dryness of the wax sample. Wax mass was expressed as wax mass per unit leaf area. The total leaf area of leaf samples was determined with a photoelectric leaf area meter.<sup>7</sup> From each plant, two sets of young and old leaves were collected for a total number of approximately 50 leaves in each leaf age group. Treatments were two by two factorial arrangements with plant species and leaf age as two factors in randomized complete block design with three replications.

For wax analysis, each wax sample (approximately 100  $\mu$ g) was silylated for gas chromatography-mass spectrometry analysis by adding 50  $\mu$ l hexamethyldisilazane, 100  $\mu$ l heptane, and 10  $\mu$ l pyridine, and placing it in the oven at 80 C for 12 h. A gas chromatograph<sup>8</sup> with a 5971 mass-selective detector equipped with a HP 7673 autosampler interfaced with a HP ChemStation was used. A 12-m by 0.2-mm inner diameter fused silicon capillary column coated with a 0.33-mm film of methyl silicone inserted directly into the ion source was used. The ion source was maintained at constant pressure of 10<sup>-5</sup> Torr. Ultra purity helium was the carrier gas. Operating temperatures were as follows: ion source, 200 C; injection port, 300 C; transfer lines, 300 C. The temperature was ramped from 40 C to 125 C at a rate of 70 C min<sup>-1</sup> and from 125 C to 300 C at the rate of 4 C min<sup>-1</sup>. One microliter of the wax mixture was injected into the injection port set for a split ratio of approximately 62:1. Spectra were obtained at 70 eV, with mass range scanned from 50 to 650 daltons. The percent contribution of each major wax component to total wax was calculated using the peak area method (Kitson et al. 1996). Values represent the average of three chromatographs.

### Glyphosate Interactions with Selective Postemergence Herbicides

Efficacy of glyphosate mixtures with the selective post-emergence herbicides acifluorfen at 560 g ai ha<sup>-1</sup>, bentazon at 1,100 g ai ha<sup>-1</sup>, chlorimuron at 13 g ai ha<sup>-1</sup>, imazaquin at 140 g ai ha<sup>-1</sup>, and pyriithiobac at 70 g ai ha<sup>-1</sup> was studied. Acifluorfen, chlorimuron, and pyriithiobac solutions

TABLE 1. Control at 3 wk after treatment (WAT), regrowth reduction at 8 WAT, and spray droplet contact angle of redvine (*Brunnichia ovata* BRVCI) and trumpetcreeper (*Campsis radicans* CMIRA) plants treated with glyphosate (1.1 and 3.3 kg ai ha<sup>-1</sup>).

Treatment	Rate	Control 3 WAT		Regrowth reduction 8 WAT		Spray droplet contact angle <sup>a</sup>	
		BRVCI	CMIRA	BRVCI	CMIRA	BRVCI	CMIRA
	kg ha <sup>-1</sup>			%		degrees	
No herbicide	0.0	0	0	0	0	63	66
Glyphosate	1.1	54	62	85	100	45	41
	3.3	69	78	98	100	—	—
LSD (0.05)		6		9		3	

<sup>a</sup> Dash (—) indicates not determined.

were prepared using 0.25% (v/v) nonionic surfactant<sup>9</sup> because addition of a surfactant is required (Anonymous 2000). Expected response for herbicide combinations was calculated as described by Colby (1967). If the observed response of an herbicide combination was significantly (LSD,  $P = 0.05$ ) lower or higher than the expected value, the combination was declared antagonistic or synergistic, respectively. Combinations were considered to be additive (no interaction) when the observed and expected response were similar. Control 3 WAT and regrowth 8 WAT was determined as described above. Treatments were arranged in a split plot design with the plant species as the main plots and the herbicides as the subplots. Treatments were replicated four times and the experiment was repeated. Data were subjected to ANOVA, and means were separated using Fisher's LSD test at  $P = 0.05$ .

### Adjuvant Effect on Glyphosate Efficacy

Glyphosate at 1.1 kg ai ha<sup>-1</sup> was applied with several adjuvants: 0.25% and 0.5% (v/v) nonionic surfactant<sup>9,10</sup>; 0.25% (v/v) organosilicone surfactant<sup>11</sup>; 2% (wt/v) ammonium sulfate (AMS); 1% (v/v) methylated seed oil (MSO)<sup>12</sup>; 1% (v/v) crop oil concentrate<sup>13</sup>; and a 0.5% (v/v) blend of methylated seed oil with emulsifiers.<sup>14</sup> In addition, the effect of ammonium sulfate combined with selected surfactants was examined. Percent control and regrowth reduction was determined as described above. Treatments were arranged in a split plot design with the plant species as the main plots and the adjuvants as the subplots. Treatments were replicated four times, and the experiment was repeated. Data were subjected to ANOVA, and means were separated using Fisher's LSD test at  $P = 0.05$ .

## Results and Discussion

### Glyphosate Efficacy

Trumpetcreeper was consistently more susceptible than redvine to glyphosate (Table 1). In redvine, glyphosate at 1.1 and 3.3 kg ha<sup>-1</sup> provided 54 and 69% control, respectively, and 85 and 98% regrowth reduction, respectively. A similar level of redvine control with glyphosate has been reported (Reddy 2000). Trumpetcreeper control by glyphosate at 1.1 and 3.3 kg ha<sup>-1</sup> was 62 and 78%, respectively, and complete inhibition of shoot regrowth at either glyphosate rate was observed. Leaf contact angle of glyphosate spray solution was higher in redvine than in trumpetcreeper.

### Scanning Electron Microscopy of Leaf Surfaces

Leaf surfaces of redvine and trumpetcreeper were distinctly different (Figure 1). The adaxial leaf surface of redvine was relatively smooth with no trichomes or glands (Figure 1A), but glands and trichomes were present on trumpetcreeper (Figure 1C). In both species, little difference between young and old leaves in the adaxial leaf surface structure was observed (Table 2).

Both species lacked stomata on the adaxial leaf surface, whereas the number of stomata on the abaxial leaf surface of trumpetcreeper was twice that of redvine (Table 2). Little difference between young and old leaves in the number of stomata on the abaxial leaf surface was observed. Stomata in both species were raised in relation to the surrounding leaf surface, thus increasing the micro-roughness of the leaf surface (Figures 2B and 2D). Numbers of stomata measured in the adaxial leaf surface were less than reported for most weed species (Ormrod and Renney 1968). Several reports have indicated herbicide infiltration of stomata (Wanamarta and Penner 1989). Lack of stomata on the adaxial leaf surface in both species diminishes the stomatal infiltration of herbicides because the herbicide spray droplet is primarily intercepted by the adaxial leaf surface.

Redvine abaxial leaf surfaces of both young and old leaves had trichomes but no glands (Table 2; Figure 1B), whereas both leaves of trumpetcreeper had both glands and trichomes (Table 2; Figure 1D). Adaxial leaf surfaces of both young and old leaves of trumpetcreeper had glands and trichomes but were absent in both leaf surfaces of redvine. The presence of glands on both adaxial and abaxial leaf surfaces in trumpetcreeper would result in increased micro-roughness and, hence, lower spreading of herbicide droplets, although a modest decrease in the contact angle in trumpetcreeper was observed compared to redvine (Table 1). In addition, glands frequently collapse and discharge a mucilage type of secretion that may increase the rate of oil spreading but inhibit water spreading (McWhorter 1993). We observed a similar type of collapse, possibly due to aging in many glands in trumpetcreeper leaves.

Trichomes generally were distributed on veins in redvine (Figure 1B) but occurred uniformly over the entire leaf in trumpetcreeper (Figure 1D). Trichomes were heavily silicated, multicellular, and elongated in trumpetcreeper (Figure 2D) and were heavily silicated, unicellular, and short in redvine (Figure 2B). These heavily silicated structures would likely increase the rate of spread of oil but inhibit the spread of water droplets (McWhorter 1993). Trichomes act in a complex way in relation to spread of herbicide solution and

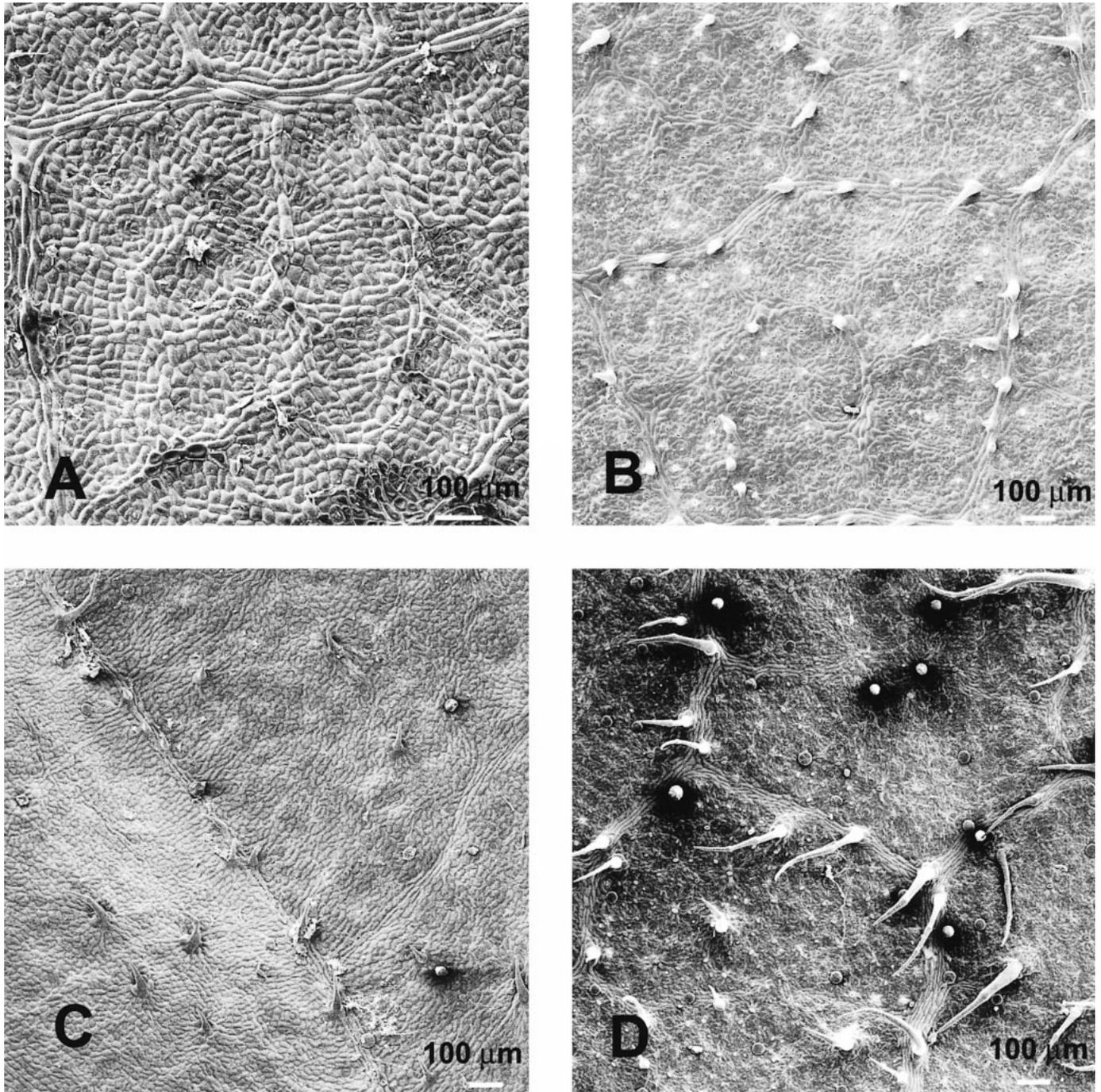


FIGURE 1. Scanning electron micrographs of old leaf (fifth to seventh leaf from the apical meristem) surface structure: (A) redvine (*Brunnichia ovata*) adaxial surface was smooth with no trichomes or glands; (B) redvine abaxial surface with numerous short, unicellular trichomes distributed on veins; (C) trumpet creeper (*Campsis radicans*) adaxial surface showing trichomes and glands; and (D) trumpet creeper abaxial surface showing elongated, multicellular trichomes and glands. Bar represents 100  $\mu\text{m}$  in each picture.

absorption of herbicide. Trichomes may hinder wetting and spreading of droplets (Hull et al. 1982), and droplets may shatter or bounce upon impact with trichomes (Hess et al. 1974). Conversely, trichomes may provide a site of entry for foliar-applied herbicides, as shown with fluorescent dyes (Benzing and Burt 1970). Also, the density of trichomes appears to play a role, because closely spaced trichomes may result in air pockets beneath the droplets that would prevent leaf surface contact (Hess et al. 1974).

Crystalline wax deposition was limited in both species

(Figures 1 and 2). Crystalline wax deposition has been observed in several weed species (Harr et al. 1991) and has provided micro-roughness that enhances the spread of oil (McWhorter 1993). The epidermal cells of trumpet creeper leaves had a very close and dense arrangement of cuticular folds (Figure 2C) that provided micro-roughness. In contrast, the epidermal cells of redvine leaves were smooth (Figure 2A). Intricate ridges or striations of the epidermal cells have been widely observed among species (Harr et al. 1991).

TABLE 2. Leaf surface characteristics of the adaxial and abaxial surface of redvine (*Brunnichia ovata*) and trumpetcreeper (*Campsis radicans*) young leaves (first or second leaf from the apical meristem) and old leaves (fifth to seventh leaf from the apical meristem) from field-grown plants.

Species	Leaf age	Leaf surface characteristics					
		Adaxial surface			Abaxial surface		
		Stomata	Glands	Trichomes	Stomata	Glands	Trichomes
		No. mm <sup>-2</sup>					
Redvine	Young	0	0	0	109	0	9
	Old	0	0	0	115	0	18
Trumpetcreeper	Young	0	5	3	220	9	9
	Old	0	7	4	255	15	5
LSD (0.05)		0	1	1	34	3	2

### Wax Mass and Composition

Wax mass per unit leaf area varied from 22 to 37  $\mu\text{g cm}^{-2}$  in redvine and trumpetcreeper (Table 3). The epicuticular wax mass in most species varies from 10 to 200  $\mu\text{g cm}^{-2}$  (McWhorter 1993), but wax mass above 300  $\mu\text{g cm}^{-2}$  has been reported (Baker 1982). In general, the amount of wax and the spray droplet coverage is inversely related. In other studies, the wax mass per unit area and total leaf surface area were inversely related (McWhorter 1993). This might be due to a more efficient wax production by young leaves or to dilution of wax per unit area as cell expansion continues.

Redvine and trumpetcreeper epicuticular wax consisted of hydrocarbons, alcohols, acids, and triterpenes, with a clear distinction between species in wax composition (Table 3). Esters were not detected in the wax. Esters were a major component of epicuticular waxes in honey mesquite (*Prosopis glandulosa* Torr.) (Mayeux and Wilkinson 1990) and *Fagus sylvatica* (Gülz et al. 1992).

Both species exhibited a clear difference between young and old leaves regarding the presence of major components (Table 3). The major components of redvine young leaf wax were the C<sub>26</sub> to C<sub>30</sub> alcohols, whereas the major components in old leaf wax were the C<sub>29</sub> and C<sub>31</sub> hydrocarbons.

The major components of trumpetcreeper young leaf wax were the C<sub>31</sub> hydrocarbons and triterpenes, but old trumpetcreeper leaves had additional C<sub>28</sub> and C<sub>30</sub> alcohols. Major components of wax in honey mesquite (Mayeux and Wilkinson 1990), *Clarkia elegans* (Hunt et al. 1976), and *F. sylvatica* (Mayeux and Wilkinson 1990) were the C<sub>29</sub> alkanes and C<sub>24</sub> to C<sub>32</sub> alcohols. Previous reports have shown that the composition of epicuticular wax changes as the leaf aged (McWhorter 1993). Alcohols and acids increased while alcohols decreased dramatically as the leaf aged (McWhorter 1993). Alcohols in *F. sylvatica* were at similar levels regardless of leaf age, but differences were observed in aldehydes, esters, and acids (Gülz et al. 1992).

Wax of young redvine leaves consisted primarily of alcohols (61%) and hydrocarbons (24%), whereas wax of old leaves consisted of hydrocarbons (49%) and triterpenes (28%) (Table 4). Wax of young trumpetcreeper leaves consisted primarily of triterpenes (62%) and hydrocarbons (29%), whereas wax of old leaves consisted of triterpenes (41%), hydrocarbons (31%), and alcohols (26%). Although triterpenes are generally considered hydrophobic substances (Croteau and Johnson 1980), we do not know how the specific triterpenes of redvine and trumpetcreeper influence the hydrophobic or hydrophilic characteristics of the leaves.

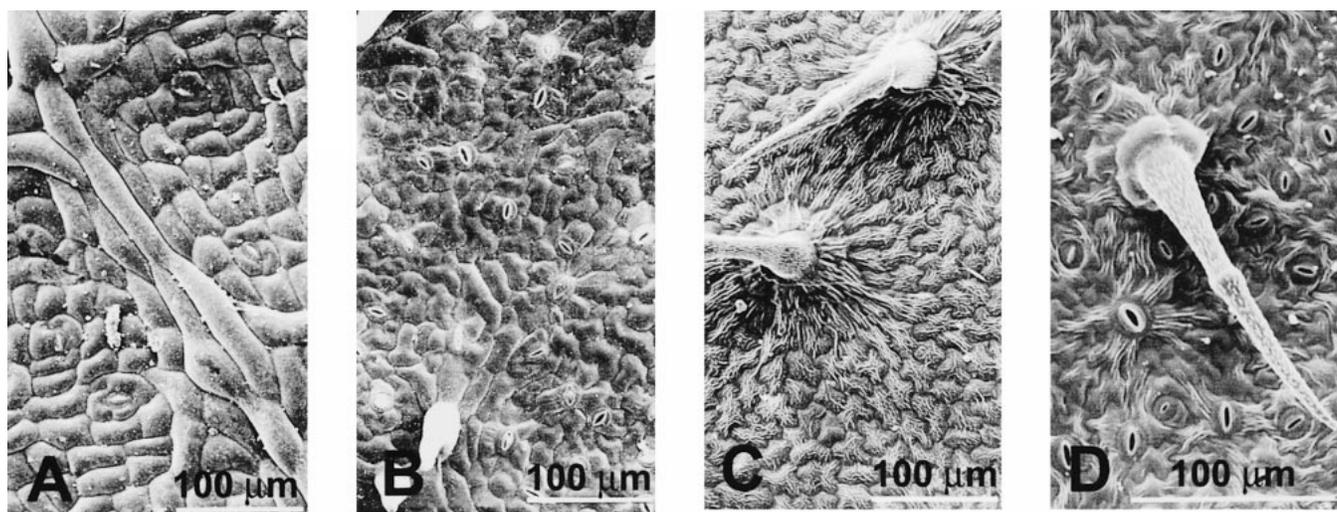


FIGURE 2. Scanning electron micrographs of old leaf (fifth to seventh leaf from the apical meristem) surface structure: (A) redvine (*Brunnichia ovata*) adaxial surface was smooth lacking stomata; (B) redvine abaxial surface showing trichomes and stomata; (C) trumpetcreeper (*Campsis radicans*) adaxial surface showing trichomes and intricate ridges of epidermal cells; and (D) trumpetcreeper abaxial surface showing stomata and heavily silicified trichome. Abaxial and adaxial leaf surfaces of both species lacked crystalline epicuticular wax deposition. Bar represents 100  $\mu\text{m}$  in each picture.

TABLE 3. Epicuticular wax mass and composition of redvine (*Brunnichia ovata*) and trumpetcreeper (*Campsis radicans*) young leaves (first or second leaf from the apical meristem) and old leaves (fifth to seventh leaf from the apical meristem).

Species	Leaf age	Wax mass <sup>a</sup> μg cm <sup>-2</sup>	Wax composition <sup>b</sup>			
			Hydrocarbons	Alcohols	Acids	Triterpenes
Redvine	Young	24	C <sub>31</sub>	C <sub>24</sub> , C <sub>26</sub> <sup>*</sup> , C <sub>28</sub> <sup>*</sup> , C <sub>30</sub> <sup>*</sup> , C <sub>32</sub>	C <sub>26</sub> , C <sub>28</sub> , C <sub>30</sub> , C <sub>32</sub>	+
	Old	37	C <sub>27</sub> , C <sub>29</sub> <sup>*</sup> , C <sub>31</sub> <sup>*</sup>	C <sub>24</sub> , C <sub>26</sub> , C <sub>28</sub> , C <sub>30</sub> , C <sub>32</sub>	C <sub>24</sub> , C <sub>26</sub> , C <sub>28</sub> , C <sub>30</sub> , C <sub>32</sub>	+*
Trumpetcreeper	Young	22	C <sub>31</sub> <sup>*</sup>	C <sub>26</sub> , C <sub>28</sub>	—	+*
	Old	35	C <sub>29</sub> , C <sub>31</sub> <sup>*</sup> , C <sub>33</sub>	C <sub>26</sub> , C <sub>28</sub> <sup>*</sup> , C <sub>30</sub> <sup>*</sup>	C <sub>28</sub> , C <sub>30</sub>	+*

<sup>a</sup> Means were not statistically different based on Fisher's protected LSD (0.05) test.

<sup>b</sup> The number of carbons is indicated by the subscript numeral, an asterisk indicates a major chromatograph peak, a minus (—) indicates absence, and a plus (+) indicates presence.

Identifying all specific triterpenes that occur in these species' leaves is a formidable task beyond the scope of this research. Long-chain hydrocarbons are highly hydrophobic, whereas alcohols and acids are less so because of the presence of carbonyl and carboxyl groups. Thus, young leaves of redvine were relatively hydrophilic (alcohols) and changed to hydrophobic (hydrocarbons) as the leaf aged. In contrast, wax in trumpetcreeper young leaves tended to be relatively hydrophobic (hydrocarbons), but wax of old leaves had similar proportions of hydrophilic (alcohols) and hydrophobic (hydrocarbons) components. In our experiments, plants consisted of two to four times more old leaves than young leaves. Thus at the whole-plant level, the epicuticular wax amount and composition of old leaves would likely have a greater effect on herbicide absorption than young leaves.

Glyphosate is highly hydrophilic (Ahrens 1994); therefore, it is more likely to be absorbed less in redvine than in trumpetcreeper and would be consistent with our control results (Table 1). Ester formulations of herbicides would penetrate the wax cuticle in redvine more readily than salts. Herbicide esters would be more soluble in an epicuticular wax high in alkanes than either inorganic or amine salts and would diffuse through them more readily than salts. Finally, we do not know how environmental conditions influenced the quantity, composition, and morphology of leaf epicuticular waxes of redvine and trumpetcreeper. However, studies conducted by other researchers under controlled conditions have documented the effects of light intensity, temperature

(Reed and Tuckey 1982), photoperiod (Wilkinson 1972), and water stress (Baker 1982).

### Glyphosate Interactions with Selective Postemergence Herbicides

Redvine control by mixtures of glyphosate and selective postemergence herbicides were antagonistic (Table 5). For trumpetcreeper, only the acifluorfen plus glyphosate mixture was additive, whereas all others were antagonistic. Contrary to our results, Brommer et al. (1998) have reported that glyphosate mixtures with chlorimuron and imazaquin increased control of redvine; however, their data were not tested to determine the nature of the interactions using Colby's method (Colby 1967). The nature of interactions produced by glyphosate mixtures with other herbicides is dependent on herbicide chemistry and weed species. In other species, similar glyphosate and postemergence herbicide mixtures exhibited either additive or antagonistic interactions (Hatzios and Penner 1985; Jordan et al. 1997; Rao and Reddy 1999).

### Adjuvant Effect on Glyphosate Efficacy

The addition of most surfactants to the spray solutions applied on redvine did not improve glyphosate activity compared to glyphosate alone (Table 6). In contrast, glyphosate plus MSO resulted in a lower control of redvine 3 WAT compared to glyphosate alone, but this reduction was not apparent on regrowth 8 WAT. In both species, addition of X-77 nonionic surfactant to glyphosate resulted in greater regrowth than glyphosate alone, which suggests that glyphosate accumulation was reduced in meristematic regions such as growing buds. The addition of AMS alone improved glyphosate efficacy in trumpetcreeper 3 WAT. However, glyphosate efficacy was not improved in either experiment by the combination of AMS with other surfactants. Previous reports have shown that glyphosate efficacy was enhanced by the addition of either AMS or organosilicone surfactants in velvetleaf (*Abutilon theophrasti* Medicus), common lambsquarters (*Chenopodium album* L.), and giant foxtail (*Setaria faberi* Herrm.) (Roggenbuck and Penner 1997).

Results of these studies indicated that glyphosate activity was greater on trumpetcreeper than redvine, and most glyphosate mixtures with selective postemergence herbicides resulted in antagonistic interactions in both species. The addition of adjuvants did not improve glyphosate efficacy, ex-

TABLE 4. Percent contribution of each major wax component to the total wax of redvine (*Brunnichia ovata*) and trumpetcreeper (*Campsis radicans*) young leaves (first or second leaf from the apical meristem) and old leaves (fifth to seventh leaf from the apical meristem). Values were determined using the peak area method (Kitson et al. 1996).

Composition	Wax component (leaf age)			
	Redvine		Trumpetcreeper	
	Young	Old	Young	Old
	%			
Hydrocarbons	24	49	29	31
Alcohols	61	16	9	26
Acids	11	7	0	2
Triterpenes	4	28	62	41
LSD (0.05)	9			

TABLE 5. Percent reduction (observed and expected) in shoot dry weight at 3 wk after treatment (WAT) in redvine (*Brunnichia ovata* BRVCI) and trumpetcreeper (*Campsis radicans* CMIRA) after treatment with glyphosate applied alone and in combination with other selective postemergence herbicides.

Treatment	Shoot dry weight reduction 3 WAT <sup>a,b</sup>			
	Observed		Expected	
	BRVCI	CMIRA	BRVCI	CMIRA
	----- % -----			
No herbicide	0	0		
Glyphosate	57	53		
Acifluorfen	34	51		
Glyphosate + acifluorfen	52 (-)	72	71	77
Bentazon	31	34		
Glyphosate + bentazon	49 (-)	49 (-)	70	70
Chlorimuron	29	22		
Glyphosate + chlorimuron	29 (-)	45 (-)	70	64
Pyriithiobac	21	45		
Glyphosate + pyriithiobac	44 (-)	50 (-)	66	75
Imazaquin	30	36		
Glyphosate + imazaquin	33 (-)	47 (-)	70	70
LSD (0.05)	----- 16 -----			

<sup>a</sup> A minus (-) represents antagonism based on Colby's (1967) method.

<sup>b</sup> Expected values from herbicide combinations as calculated by Colby's (1967) method.

cept AMS in trumpetcreeper. The adaxial leaf surface in trumpetcreeper had trichomes and glands but was smooth in redvine. Stomata or crystal wax deposition on the adaxial leaf surface were not observed in either species. The young leaf wax of redvine had components of a relative hydrophilic nature (alcohols) that changed to a relative hydrophobic nature (hydrocarbons) as the leaf aged. In contrast, the young leaf wax of trumpetcreeper tended to be relatively hydrophobic (hydrocarbons), whereas wax of old leaves had both hydrophilic (alcohols) and hydrophobic (hydrocarbons) components. Because glyphosate is highly hydrophilic, it appears that the higher herbicide efficacy was related to the more hydrophilic nature of trumpetcreeper epicuticular waxes compared to that of redvine.

### Sources of Materials

- <sup>1</sup> Jiffy mix, Jiffy Products of America Inc., Batavia, IL 60510.
- <sup>2</sup> Spray system with TeeJet 8002E nozzles, Spraying Systems Co., North Avenue at Schmale Road, Wheaton, IL 60189.
- <sup>3</sup> NRL C. A. goniometer, model 100-00-115, Rame-Hart Inc., Mountain Lakes, NJ 07046.
- <sup>4</sup> Balzers CPD 020, Balzers, 8 Sagamore Park Road, Hudson, NH 03051.
- <sup>5</sup> Hummer X, Anatech, Ltd., 5510 Vine Street, Alexandria, VA 22310.
- <sup>6</sup> JEOL-JSM 840 (USA), 11 Dearborn Road, Peabody, MA 10960.
- <sup>7</sup> Portable area meter, model LI-3000, Lamda Electronic Corp., 515 Broad Hollow Road, Melville, NY 11746.
- <sup>8</sup> Hewlett-Packard Model 5890, Hewlett-Packard Co., 9000 Executive Park Drive, Suite C-150, Knoxville, TN 37923.

TABLE 6. Effect of glyphosate and adjuvants on shoot dry weight reduction at 3 wk after treatment (WAT) and regrowth reduction at 8 WAT in redvine (*Brunnichia ovata* BRVCI) and trumpetcreeper (*Campsis radicans* CMIRA).

Treatment <sup>a</sup>	Control 3 WAT		Regrowth reduction 8 WAT	
	BRVCI	CMIRA	BRVCI	CMIRA
	----- % -----			
No herbicide	0	0	0	0
Glyphosate	58	64	87	90
Glyphosate + X-77	50	63	30	52
Glyphosate + Silwet L-77	57	67	84	88
Glyphosate + AMS	61	74	87	85
Glyphosate + MSO	45	57	81	86
Glyphosate + Agri-Dex	56	63	81	89
Glyphosate + Dyne-Amic	53	62	84	86
Glyphosate + Optima	48	61	84	89
Glyphosate + AMS + Silwet L-77	65	68	93	94
Glyphosate + AMS + MSO	63	67	94	95
Glyphosate + AMS + Agri-Dex	61	69	95	95
Glyphosate + AMS + Dyne-Amic	61	70	87	90
LSD (0.05)	----- 10 -----		----- 9 -----	

<sup>a</sup> AMS, ammonium sulfate; MSO, methylated seed oil.

<sup>9</sup> X-77 (a mixture of alkylaryl polyoxyethylene glycols, free fatty acids and isopropanol), Loveland Industries, Inc., P.O. Box 1289, Greeley, CO 80632.

<sup>10</sup> Optima, nonionic surfactant, Helena Chemicals Co., 5100 Poplar Avenue, Memphis, TN 38137.

<sup>11</sup> Silwet L-77, polyalkyleneoxide-modified heptamethyltrisiloxane 7.5 EO, 100%, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591.

<sup>12</sup> Methylated seed oil, Helena Chemicals Co., 5100 Poplar Avenue, Memphis, TN 38137.

<sup>13</sup> Agri-Dex, paraffin-based petroleum oil (83%) and surfactant blend (17%), Helena Chemicals Co., 5100 Poplar Avenue, Memphis, TN 38137.

<sup>14</sup> Dyne-Amic, 80% methylated seed oil and 20% alkyne oxide, silicone and carbon-based nonionic surfactants (emulsifiers), Helena Chemicals Co., 5100 Poplar Avenue, Memphis, TN 38137.

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