

Leaf characteristics and surfactants affect primisulfuron droplet spread in three broadleaf weeds

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Laboratory studies were conducted to examine the leaf surface, epicuticular wax content, and spray droplet behavior on common lambsquarters, common purslane, and velvetleaf. Adaxial and abaxial leaf surfaces were examined using scanning electron microscopy, and leaf wax was extracted and quantified for all three weed species. The spread of 1- μ l droplets of distilled water, primisulfuron solution (without surfactant), primisulfuron solution with a nonionic low foam wetter/spreader adjuvant (0.25% v/v), and with an organosilicone wetting agent (0.1% v/v) was determined on the adaxial leaf surfaces of each of the weed species. Glands and trichomes were present on both the adaxial and abaxial leaf surfaces of velvetleaf. Common purslane had neither glands nor trichomes on either side of the leaf. Common lambsquarters did not have any glands or trichomes, but it had globular bladder hairs on both adaxial and abaxial leaf surfaces. Stomata were present on both adaxial and abaxial leaf surfaces in all three weed species. Common purslane had a much lower number of stomata per unit area of leaf as compared with velvetleaf or common lambsquarters. Common lambsquarters had the highest epicuticular wax content on the leaf surface (274.5 μ g cm⁻²), followed by common purslane (153.4 μ g cm⁻²) and velvetleaf (7.4 μ g cm⁻²). There were no significant variations in the spread of the 1- μ l droplet of distilled water and primisulfuron (without adjuvant) among the species. Spread of primisulfuron droplets with surfactant was highest on the leaf surface of velvetleaf that had the lowest wax content. Droplet spread was greatest with organosilicone surfactant followed by the nonionic surfactant.

Nomenclature: Primisulfuron; common lambsquarters, *Chenopodium album* L. CHEAL; common purslane, *Portulaca oleracea* L. POROL; velvetleaf, *Abutilon theophrasti* Medik, ABUTH.

Key words: Epicuticular wax, droplet spread, leaf surface morphology, scanning electron microscopy, surfactant.

Leaf surface characteristics affect the wetting and penetration behavior of foliarly applied herbicides (Hull et al. 1982; McWhorter 1985). Surface characteristics include the cuticle (epicuticular wax, cutin, and pectin), leaf angle and position, and the number of stomata, trichomes, and glands (Hess 1985; Wanamarta and Penner 1989). Herbicide absorption is facilitated by either cuticular or stomatal infiltration (Hess 1985; Wanamarta and Penner 1989). The epicuticular wax appears to be an effective barrier to herbicide absorption because the removal of epicuticular wax with chloroform greatly increased glyphosate absorption in coca (*Erythroxylum coca* var. *coca* Lam.) compared with plants with leaf epicuticular wax (Ferreira and Reddy 2000). Because the leaf surface influences the spreading and subsequent absorption of the herbicidal compounds into the leaf tissue, knowledge of the morphological and physicochemical characteristics of the leaf surface will help weed scientists better understand the behavior of a given herbicide on various weed species. This knowledge will also help in selection of surfactants to enhance the herbicidal activity in an integrated weed management program. Leaf surface micromorphology of various weed species was previously studied by Harr et al. (1991), and scanning electron micrographs were demonstrated. However, quantitative information on number of stomata, glands, and trichomes; comparative study of abaxial and adaxial surfaces of young and old leaves; and

quantification of leaf wax content are still lacking in literature for various weed species including the three weed species presented in this article.

Common lambsquarters (*Chenopodium album* L.) is one of the most widely distributed weed species in the world (Colquhoun et al. 2001). It is competitive with 40 crop species and is considered the principal weed in corn and soybean in the United States (Holm et al. 1977). Common purslane (*Portulaca oleracea* L.) is a frequent weed among vegetable crops, annual flowers and nursery trees, field and sweet corn, strawberries, tobacco, spring wheat, and newly planted orchards and is naturalized as a weed in 45 crops in 81 countries (Mitich 1997). Velvetleaf (*Abutilon theophrasti* Medik) causes significant crop yield losses in many parts of the world (Sattin et al. 1992; Spencer 1984; Warwick and Black 1988). Weed ecologists recommend that high priority be given to preventing velvetleaf establishment in areas where it is not yet present (Bauer and Mortensen 1992; Sattin et al. 1992; Warwick and Black 1988).

Primisulfuron is a selective POST herbicide for the control of certain broadleaf weeds and grasses (CPR 2002). Primisulfuron from 15 to 30 g ai ha⁻¹ controlled quackgrass [*Elytrigia repens* (L.) Nevski] more than 90% 6 wk after treatment (Bhowmik 1995). A single early POST application of primisulfuron at 40 g ha⁻¹ was as effective in controlling quackgrass as a split application of 20 g ha⁻¹ applied

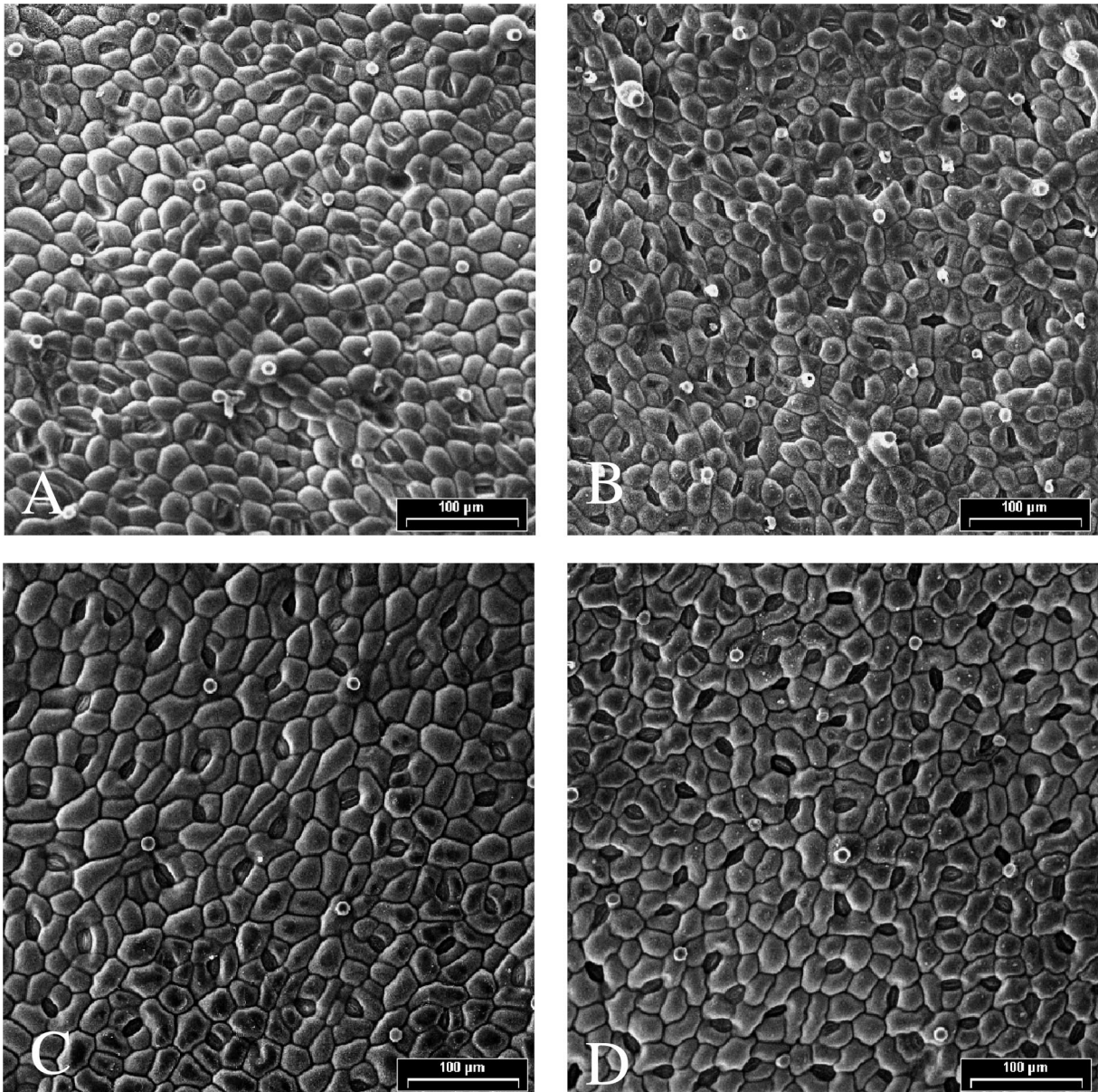


FIGURE 1. Scanning electron micrographs of common lambsquarters leaf surface: (A) adaxial surface of young leaf (first or second fully expanded leaf from the apical meristem); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (fifth or sixth fully expanded leaf from the apical meristem); and (D) abaxial surface of old leaf.

at the one- to three-leaf stage followed by a second application of 20 g ha^{-1} at the four- to six-leaf stage (Bhowmik 1999). According to the label of commercial formulation, primisulfuron partially controls common lambsquarters and requires crop oil concentrate to control velvetleaf (CPR 2002). There is no published information on the activity of primisulfuron on common purslane.

Surfactant activity is weed- and herbicide-specific (Johnson et al. 2002; Stock and Holloway 1993). Nandula et al. (1995) reported that primisulfuron provided greater wirestem muhly [*Muhlenbergia frondosa* (Poir.) Fern.] control with methylated vegetable oil concentrate as compared with nonionic surfactant whereas, in field experiments, primisulfuron (39 g ha^{-1}) provided greater control of itchgrass [*Rottboellia cochinchinensis* (Lour.) WD Clayton] when applied with nonionic surfactant than with methylated seed oil

blend or an organosilicone surfactant (Strahan et al. 2000). Green (2002) reported that increasing surfactant size generally increased rimsulfuron activity on velvetleaf, but activity was reduced on giant foxtail (*Setaria faberi* Herrm.) with surfactants having the longest alkyl chain and the highest number of ethylene oxides unit. Bellinder et al. (2003) reported that in general, adjuvant usage improved the efficacy of fomesafen more than it did with bentazone on velvetleaf, ragweed (*Ambrosia artemisiifolia* L.), eastern black nightshade (*Solanum ptycanthum* Dun.), and hairy nightshade (*Solanum sarrachoides* Sendtner). Though, in general, the use of nonionic surfactant or a good quality crop oil concentrate was recommended with primisulfuron (CPR 2002), it is apparent that for particular weed species the activity of primisulfuron can be increased by proper selection of surfactant.

The objectives of this study were to (1) examine the ab-

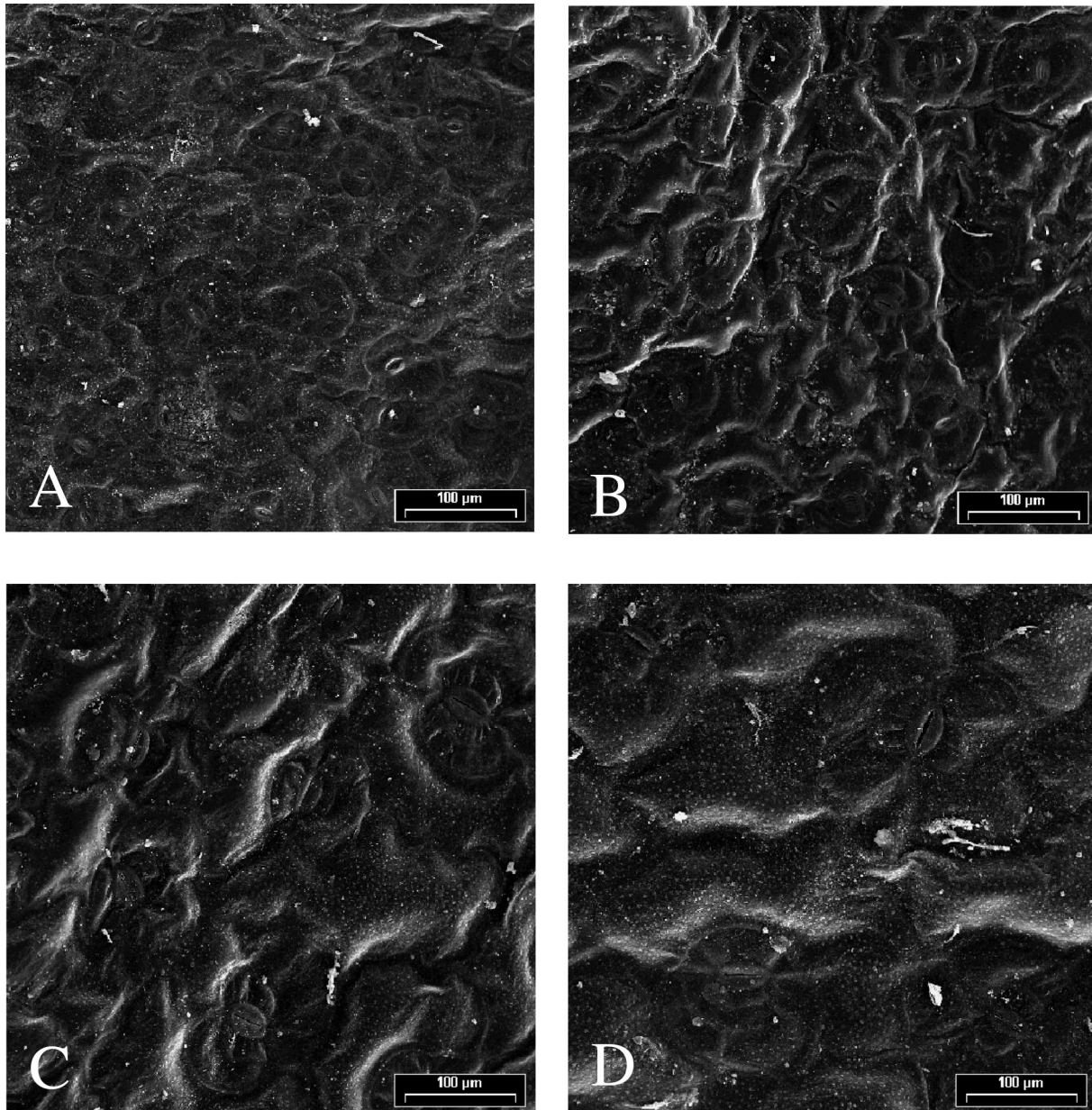


FIGURE 2. Scanning electron micrographs of common purslane leaf surface: (A) adaxial surface of young leaf (first or second fully expanded leaf from the apical meristem); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (fifth or sixth fully expanded leaf from the apical meristem); and (D) abaxial surface of old leaf.

axial and adaxial leaf surfaces of common lambsquarters, common purslane, and velvetleaf; (2) quantify wax content per unit of leaf area; and (3) determine the spread area of primisulfuron droplets with and without surfactants on leaf surface of these weed species.

Materials and Methods

Scanning Electron Microscopy of Leaf Surfaces

Leaves of common lambsquarters, common purslane, and velvetleaf were collected from plants at the eight- to ten-leaf stage, grown under natural field conditions at the U.S. Department of Agriculture (USDA) Southern Weed Science Research Unit farm, Stoneville, MS. Young leaves (first or second fully expanded leaf from the tip) and old leaves (fifth or

sixth fully expanded leaf from the tip) were collected from each plant. Plant specimens were prepared for scanning electron microscopy using similar procedures as those described by McWhorter et al. (1993). Leaf segments of approximately 20 mm² were fixed for 12 h in 4% glutaraldehyde and were rinsed three times with distilled water before dehydration in a graded ethanol series. Samples were dried in a critical point drier¹ and were mounted on aluminum stubs. Samples were gold-coated using a sputter coater² and examined under a scanning electron microscope.³ Leaf surfaces were photographed at $\times 200$ magnification for all species. The stomata, glands, and trichomes were studied and counted with four replications for each species, and the study was repeated. Data were subjected to ANOVA, and means were separated using Fisher's Protected LSD test at $P = 0.05$.

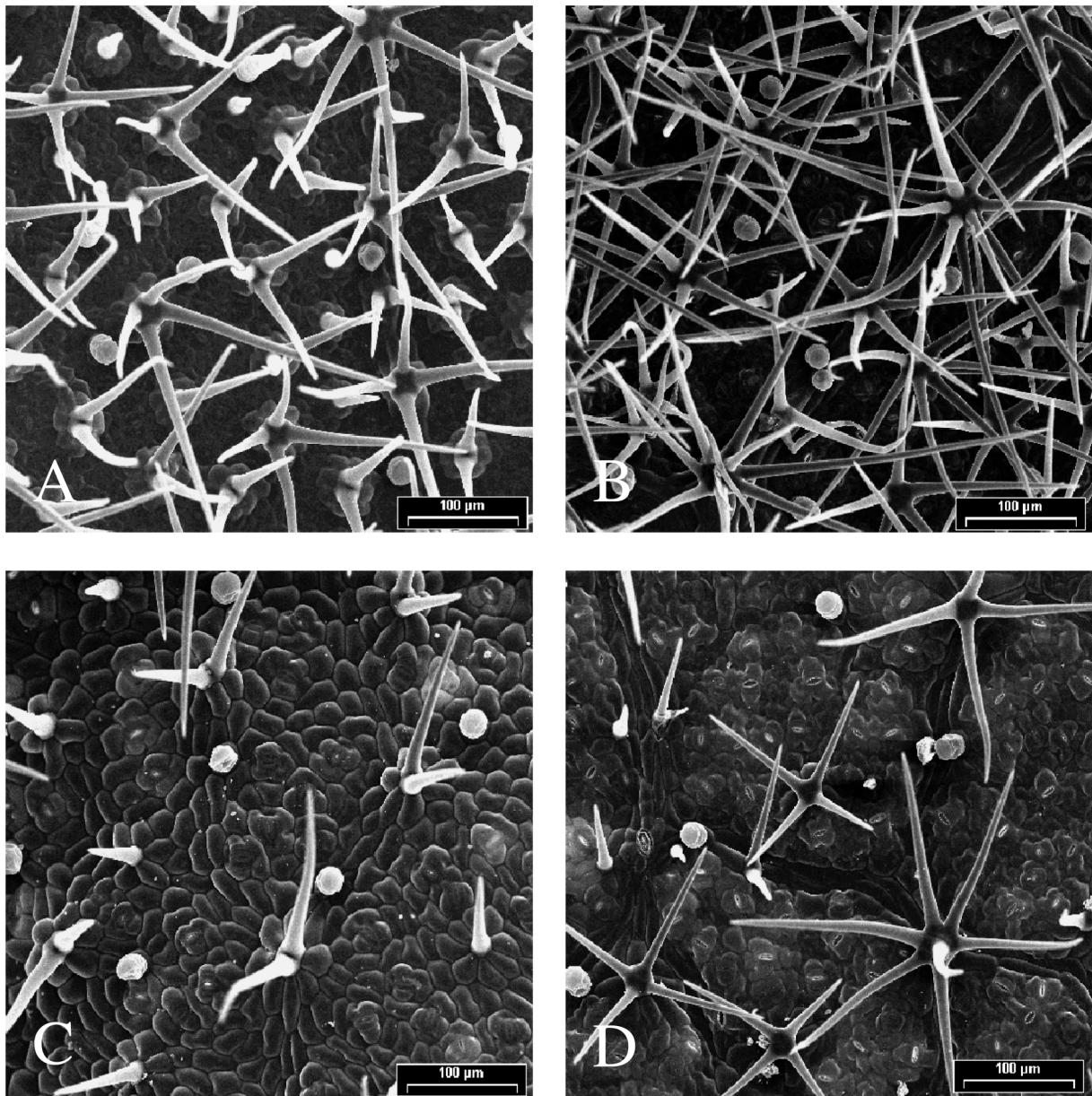


FIGURE 3. Scanning electron micrographs of velvetleaf leaf surface: (A) adaxial surface of young leaf (first or second fully expanded leaf from the apical meristem); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (fifth or sixth fully expanded leaf from the apical meristem); and (D) abaxial surface of old leaf.

Wax Content per Unit of Leaf Area

The leaves of common lambsquarters, common purslane, and velvetleaf were collected from field-grown plants at the eight- to ten-leaf stage at the USDA Southern Weed Science Research Unit farm in Stoneville, MS. Wax was extracted from the fourth to sixth (from apical meristem) fully expanded leaves in each species. The wax extraction procedure was followed as described by McWhorter (1993). The leaves were washed in running tap water and blotted dry with paper towels. Total leaf area of leaf samples was determined with a stationary leaf area meter.⁴ Wax extraction was done by immersing approximately 50 leaves for 30 s in 500 ml of high-performance liquid chromatography (HPLC)-grade chloroform in an ultrasonicator⁵ at room temperature. The chloroform-wax solution was filtered using a fritted glass funnel apparatus with Durapore⁶ membrane filters (0.22

µm, GV series), and the volume was reduced to approximately 20 ml in a rotary evaporator.⁷ The reduced chloroform-wax solution was transferred to a preweighed 25-ml glass scintillation vial. Chloroform was evaporated under a hood, and the vials were kept in a desiccator with silica gel blue for 7 d before recording the wax mass to ensure complete dryness of the wax sample. Each treatment had three replications, and the experiment was repeated. Data were subjected to ANOVA, and means were separated using Fisher's Protected LSD test at $P = 0.05$.

Spread Area of Primisulfuron Droplets

The leaves of individual weed species were collected at the eight- to ten-leaf stage from plants grown under natural field condition. The spread area of a 1-µl droplet of distilled

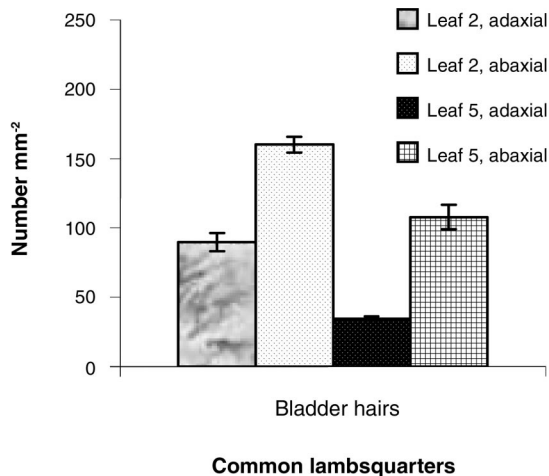


FIGURE 4. Number of bladder hairs on adaxial and abaxial leaf surfaces of young (first or second fully expanded leaf from the apical meristem) and old leaves (fifth or sixth fully expanded leaf from the apical meristem) of common lambsquarters. LSD (0.05) value was 13.51.

water, primisulfuron at 39.5 g ai ha⁻¹ (without surfactant), primisulfuron with a nonionic surfactant⁸ at 0.25% (v/v), and primisulfuron with an organosilicone wetting agent⁹ at 0.1% (v/v) was measured on the adaxial surface of the fourth to sixth (from apical meristem) fully expanded leaves of common lambsquarters, common purslane, and velvetleaf 3 min after the droplet application. Spread area was calculated using the formula πr^2 , where r was an estimate of the droplet radius based on the mean of the horizontal and vertical dimensions of the droplet. Spread area measurements were replicated five times, and the experiment was repeated. Data were subjected to ANOVA, and means were separated using Fisher's Protected LSD test at $P = 0.05$.

Results and Discussion

Scanning Electron Microscopy of Leaf Surfaces

There were distinct variations in leaf surface micromorphology of common lambsquarters, common purslane, and velvetleaf (Figures 1–3). In common purslane, both adaxial and abaxial leaf surfaces of young and old leaves were relatively smooth, and there were no trichomes or glands (Figure 2). In contrast, common lambsquarters had “bladder hairs” (Figure 1), and velvetleaf had glands and star-shaped trichomes on both adaxial and abaxial leaf surfaces (Figure 3). In common lambsquarters, the number of bladder hairs per unit area of the leaf was significantly higher in abaxial surfaces than adaxial, both in young and old leaves (Figure 4), and number of bladder hairs per unit area was higher in young leaves than old leaves. In velvetleaf, the number of trichomes and glands was significantly higher in younger leaves than in older leaves. In both leaf stages, the number of trichomes was higher in adaxial surfaces as compared with abaxial (Figure 5). Statistically, there was no variation in the number of glands between adaxial and abaxial surfaces in velvetleaf (Figure 5).

The presence of bladder hairs gives the “mealy” appearance to common lambsquarters leaves. Presence of trichomes on both adaxial and abaxial surface of velvetleaf would result in increased microroughness of the leaf surface. Trichomes

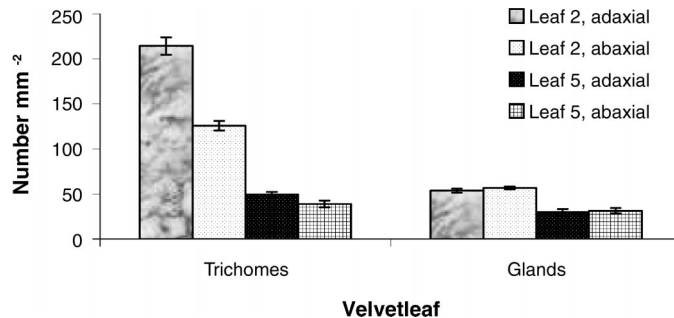


FIGURE 5. Number of glands and trichomes of adaxial and abaxial leaf surfaces of young (first or second fully expanded leaf from the apical meristem) and old (fifth or sixth fully expanded leaf from the apical meristem) leaves of velvetleaf. LSD (0.05) value for trichomes was 13.1; for glands, 5.9.

act in a complex way in relation to the spread of herbicide solution and adsorption of herbicide. Trichomes may cause reduced wetting and spreading of droplets (Hull et al. 1982). According to Hess et al. (1974), closely spaced trichomes might create air pockets beneath the droplets that would prevent leaf surface contact, and droplets may bounce upon or shatter due to the impact with trichomes. Benzing and Burt (1970) showed by using fluorescent dyes that trichomes might provide a site of entry to the foliar-applied herbicides.

In all three weed species, stomata were present in both adaxial and abaxial leaf surfaces (Figures 1–3). In common lambsquarters and velvetleaf, the number of stomata in abaxial surfaces was significantly higher than adaxial leaf surfaces, whereas common purslane had a higher number of stomata in adaxial surfaces than abaxial (Figure 6). In all three weed species, the number of stomata per mm² of leaf area was higher in the younger leaves than the older leaves (Figure 6). Stomata were larger in size in common purslane (Figure 2) than in common lambsquarters (Figure 1) or velvetleaf (Figure 3), which may influence the stomatal infiltration of herbicides. The number of stomata per mm² in common purslane was significantly less than that in common lambsquarters or velvetleaf (Figure 6), and it was also lower than that in some other weed species reported previously (Chachalis et al. 2001a; Ormrod and Renney 1968). Scanning electron micrographs of leaf surfaces of these weed

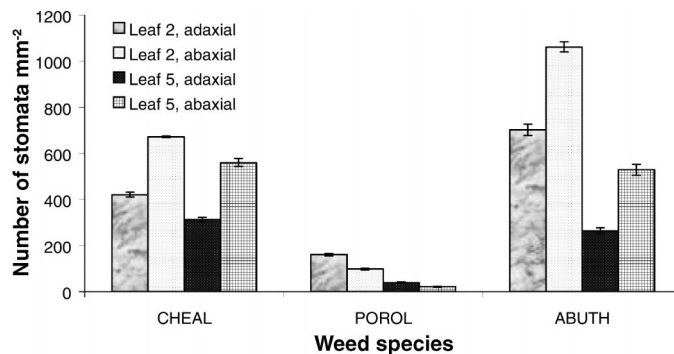


FIGURE 6. Number of stomata per unit area of adaxial and abaxial leaf surfaces of young (first or second fully expanded leaf from the apical meristem) and old (fifth or sixth fully expanded leaf from the apical meristem) leaves of common lambsquarters, common purslane, and velvetleaf. LSD (0.05) for species was 20.1, and for leaf age (young and old) and leaf surface (adaxial and abaxial) was 16.4.

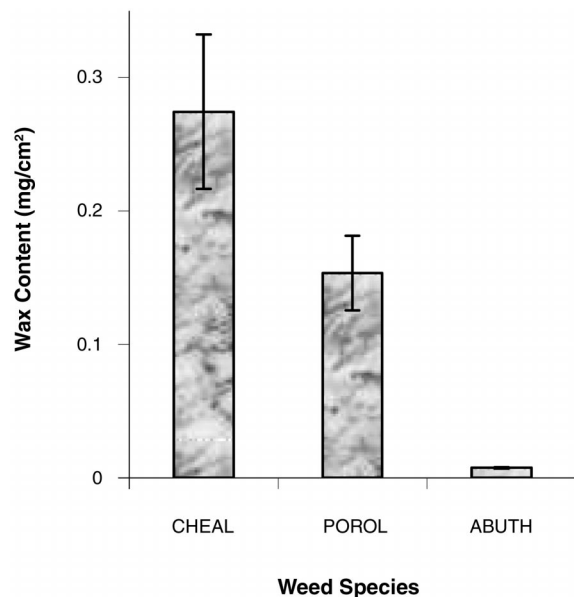


FIGURE 7. Wax content per unit of leaf area in common lambsquarters, common purslane, and velvetleaf. LSD (0.05) value was 0.25.

species were previously demonstrated by Harr et al. (1991), although quantitative information on number of stomata, glands, and trichomes and comparative study of abaxial and adaxial surfaces of young and old leaves of the above three weeds were lacking in literature.

Wax Content per Unit of Leaf Area

Among the three weed species, common lambsquarters had the highest wax content per unit of leaf area (274.5 $\mu\text{g cm}^{-2}$), and velvetleaf had the lowest (7.4 $\mu\text{g cm}^{-2}$) (Figure 7). Wax content of common purslane was 153.4 $\mu\text{g cm}^{-2}$. McWhorter (1993) reported that wax content varies from 10 to 200 $\mu\text{g cm}^{-2}$ for most species. Wax mass above 300 $\mu\text{g cm}^{-2}$ was reported by Baker (1982). McWhorter (1993) showed that the leaf wax content per unit area was inversely related to the total leaf surface area. The epicuticular wax appears to be the primary barrier to pesticide penetration. Removal of epicuticular wax with chloroform increased absorption of MCPB by broad bean (*Vicia faba* L.) leaves (Kirkwood et al. 1982). Thickness, chemical composition, and ultrastructure of the epicuticular wax differ among plant species, variety, age, and environment in which the plants are grown (Holloway 1970). Hull (1970) reported that the amount of wax produced by the plant is influenced by light, temperature, and relative humidity. Leaf wax content plays an important role in herbicide spread on the leaf surface. In general, leaf wax content and the spread area of herbicide droplet are inversely related (Chachalis et al. 2001b).

Spread Area of Primisulfuron Droplets

In all three weed species, primisulfuron with a nonionic surfactant had more spread area than that without a surfactant, and the spread was even greater with an organosilicone wetting agent. The spread of primisulfuron droplets was higher on the leaf surface of velvetleaf than on leaves of common lambsquarters or common purslane (Figure 8). There was no variation in spread of the 1- μl droplet of pure

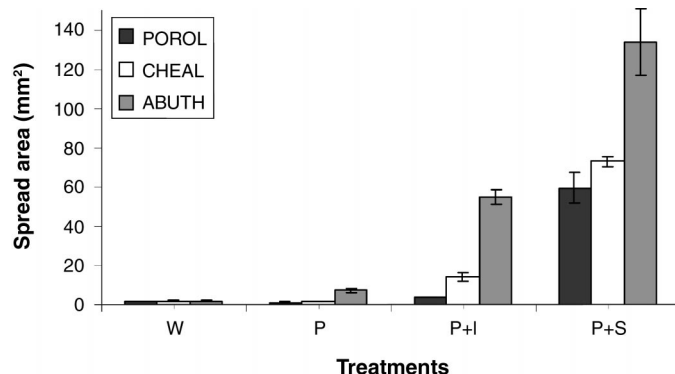


FIGURE 8. Spread area of 1 μl droplets of distilled water (W), primisulfuron (P), primisulfuron with a nonionic surfactant (P+I), and primisulfuron with an organosilicone wetting agent (P+S). Primisulfuron was used at 39.5 g ai ha⁻¹, nonionic surfactant at 0.25% (v/v), and the organosilicone wetting agent at 0.1% (v/v). LSD (0.05) for species was 7.9; and for treatments, 9.2.

distilled water among three weed species (Figure 8). These results showed an inverse relationship between leaf wax content and the spread area of the spray droplet in common lambsquarters and velvetleaf, which agrees with the finding of Chachalis et al. (2001b), whereas common purslane had lower wax content than common lambsquarters, but the droplet spread was not higher. This result indicates that herbicide spread is not dependent solely on the wax content per unit area of leaf surface. Composition, physical structure, and orientation of leaf wax play important roles in this regard (Juniper 1960; Whitehouse et al. 1982). In general, waxes with significant quantities of long-chain ketones and alkanes were the most difficult to wet regardless of the cuticle thickness (Holloway 1970; Juniper 1960; Juniper and Bradley 1958). The relatively nonrepellent waxes consist largely of diols, sterols, and triterpenoids (Holloway 1970). Holloway (1970) also reported that the amount of wax had a positive correlation with herbicide absorption.

The number of stomata in abaxial leaf surface was higher than on the adaxial surface in common lambsquarters and velvetleaf, whereas common purslane had higher numbers of stomata in adaxial surfaces than abaxial (Figure 6). Higher numbers of stomata cause greater infiltration of herbicides into the leaf tissue (Wanamarta and Penner 1989). In all three weed species, the number of stomata per unit area of leaf surface was higher in the younger leaves than the older leaves. Bladder hairs were present in common lambsquarters, and the number was higher on the abaxial surface. Velvetleaf had glands and star-shaped trichomes, and the number of the trichomes was higher in adaxial surfaces, although the trichomes in the abaxial surface were more branched and bigger in size. Trichomes and leaf hairs may cause reduced contact of herbicide droplets with the leaf surface by creating air pockets if they are densely spaced (Hess et al. 1974; Hull et al. 1982). In general, the leaves with higher wax content per unit of leaf area had lower herbicide spread on leaf surface. Organosilicone wetting agent spread the primisulfuron droplets significantly more than the nonionic surfactant, and the spread was more in case of velvetleaf as compared with common lambsquarters or common purslane.

Overall, these results give basic support to the concept that the morphological and physicochemical characteristics

of leaves of various weed species influence the behavior of herbicide on leaf surface, which may lead to differential activity of a given herbicide from weed species to species and can be optimized by using specific surfactant. Addition of an organosilicone wetting agent with primisulfuron spreads the herbicide better and covers more surface area on leaves, which may lead to higher absorption of herbicidal compound into the leaf tissue, resulting in greater weed control.

Sources of Materials

¹ Balzers CPD 020, Balzers, 8 Sagamore Park Road, Hudson, NH 03051.

² Hummer X, Anatech, Ltd., 5510 Vine Street, Alexandria, VA 22310.

³ JEOL-JSM 840 (USA), 11 Dearborn Road, Peabody, MA 10960.

⁴ Leaf Area Meter, model LI-3100, LI-COR, Inc., Lincoln, NE 68501.

⁵ Branson 2210 Sonicator, Branson Ultrasonic Corporation, 41 Eagle Road, Dunbury, CT 06813-1961.

⁶ Durapore Membrane Filters, Millipore Corporation, 80 Ashby Road, Bedford, MA 01730.

⁷ Buchi R-124 Rotavapor, Buchi Analytical Inc. 19 Lukens Dr., New Castle, DE 19720.

⁸ A nonionic surfactant, blend of alkyl aryl polyoxyalkane ethers, free fatty acids, and dimethyl polysiloxane, Helena Chemical Company, 225 Schilling Boulevard, Collierville, TN 38017.

⁹ An organosilicone wetting agent, polyalkyleneoxide-modified heptamethyltrisiloxane 7.5 EO, 100%, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591.

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