

# Influence of leaf surface micromorphology, wax content, and surfactant on primisulfuron droplet spread on barnyardgrass (*Echinochloa crus-galli*) and green foxtail (*Setaria viridis*)

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Laboratory studies were conducted to examine the leaf surface, epicuticular wax content, and spread area of primisulfuron spray droplet with and without surfactant on leaf surface of barnyardgrass and green foxtail. Adaxial and abaxial leaf surfaces were examined using scanning electron microscopy and leaf wax was extracted and quantified. The spread of 1- $\mu$ 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. Stomata and trichomes were present on adaxial and abaxial leaf surfaces in both species. Green foxtail had more stomata per unit area on the adaxial as compared to the abaxial leaf surface. Barnyardgrass had more stomata on the abaxial than on the adaxial leaf surface. There was no significant variation in the number of trichomes per unit leaf area of green foxtail, and the number of prickles per unit area of leaf was significantly higher in adaxial than the abaxial leaf surface, in both young and old leaves. In barnyardgrass, there were more trichomes on abaxial than adaxial leaf surface. The mean value of the wax content per unit of leaf area in barnyardgrass and green foxtail was 35.9  $\mu$ g cm<sup>-2</sup> and 19.1  $\mu$ g cm<sup>-2</sup>, respectively. On both species primisulfuron with a nonionic surfactant had more spread area than that without a surfactant, and the spread was even greater with organosilicone wetting agent. The spread area of primisulfuron droplet was higher on the leaf surface of barnyardgrass than on green foxtail when surfactant was added.

**Nomenclature:** Primisulfuron; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv. ECHCG; green foxtail, *Setaria viridis* (L.) Beauv.

**Key words:** Epicuticular wax, herbicide spread, leaf surface micromorphology, scanning electron microscopy, surfactant.

Barnyardgrass is one of the most important annual weeds in world agriculture and has been reported to be a problem in 36 different crops in at least 61 countries (Holm et al. 1977). The weed is a very aggressive invader, difficult to control, and causes major losses in rice (*Oryza sativa* L.) production (Lopez-Martinez et al. 1999). VanDevender et al. (1997) reported that 20 barnyardgrass plants m<sup>-2</sup> can reduce rice yield by 80%. Barnyardgrass, depending on its density, can cause 26 to 84% reductions in marketable fruit yields of transplanted tomato (*Lycopersicon esculentum* Mill.) in season-long competition (Bhowmik and Reddy 1988).

Green foxtail is primarily a weed of cultivated cereals, vegetables, and pulse crops and is also common in pastures, turf, orchards, gardens, and other frequently disturbed sites (Douglas et al. 1985; Holm et al. 1991). Green foxtail competition has reduced wheat (*Triticum aestivum* L.) yields by up to 44% (Blackshaw et al. 1981) and soybean [*Glycine max* (L.) Merr.] yields by up to 29% (Staniforth 1965). Significant yield reductions have also been reported in alfalfa (*Medicago sativa* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], rice, sugar beet (*Beta vulgaris* L.), vineyards, and many other crops (Douglas et al. 1985; Gates 1941; Holm et al. 1991). The roots of green foxtail are reported to produce a chemical that is toxic to cabbage (*Brassica oleracea*

L.) and tomato (Holm et al. 1991), and are also an alternate host for a number of insects, viruses, and nematodes that affect crops (Douglas et al. 1985; Gates 1941; Holm et al. 1991). Understanding the biology of these weeds can aid in developing the most effective and economical weed management programs.

Leaf surface micromorphology is an important feature in determining deposition and spread of spray droplets (Boize et al. 1976; Gudin et al. 1976). It was previously demonstrated that the wetting and penetration behavior of foliarly applied herbicides was affected by leaf characteristics (Hull et al. 1982; McWhorter 1985). Surface characteristics include the cuticle (epicuticular wax, cutin, and pectin), leaf angle and position, 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). Previous research has shown that spray droplets impacting on large trichomes may shatter and bounce off the leaf (Boize et al. 1976). The epicuticular wax appears to be an effective barrier to herbicide absorption as the removal of epicuticular wax with chloroform increased absorption of MCPB by broad bean (*Vicia faba* L.) leaves (Kirkwood et al. 1982). As the leaf surface influences the spread and subsequent ab-

sorption of herbicides into the leaf tissue, knowledge of the morphological and physicochemical characteristics of the leaf surface will help weed scientists to better understand the behavior of herbicides on different weed species. This knowledge will also help in selection of surfactant to enhance the herbicidal activity. Leaf surface micromorphology of various weed species was previously studied by Harr et al. (1991). 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 lacking in the literature for the two species studied in this paper.

The importance of postemergence herbicides has risen dramatically in the past decade. Underwood et al. (2001) reported that 70% of U.S. agrochemical market was occupied by herbicides and 50% of those were postemergence herbicides. Primisulfuron is a selective postemergence herbicide for the control of certain broadleaf weeds and grasses (CPR 2005). Bhowmik (1995) reported that rates from 15 to 30 g ai ha<sup>-1</sup> of primisulfuron controlled quackgrass [*Elytrigia repens* (L.) Nevski] over 90% 6 wk after treatment. A single early postemergence application of primisulfuron at 40 g ai ha<sup>-1</sup> was as effective in controlling quackgrass as a split application of 20 g ai ha<sup>-1</sup> applied at the one- to three-leaf stage followed by a second application of 20 g ai ha<sup>-1</sup> at four- to six-leaf stage (Bhowmik 1999). According to Tweedy and Kapusta (1995), primisulfuron at 40 g ha<sup>-1</sup> controlled johnsongrass (*Sorghum halepense* L.) 85 to 100% from 1992 to 1994 and corn (*Zea mays* L.) yield was more than double as compared to the control plots. According to the label of commercial formulation primisulfuron partially controls foxtails (*Setaria* spp.) (CPR 2005).

Surfactant activity is weed and herbicide specific (Johnson et al. 2002; Stock and Holloway 1993). Nandula et al. (1995) reported that primisulfuron provided greater wire-stem muhly [*Muhlenbergia frondosa* (Poir.) Fern.] control with methylated vegetable oil concentrate as compared to nonionic surfactant, whereas, in field experiments, primisulfuron (40 g ai ha<sup>-1</sup>) provided greater control of itchgrass [*Rottboellia cochinchinensis* (Lour.) W.D. 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 molecular size of surfactant generally increased rimsulfuron activity on velvetleaf (*Abutilon theophrasti* Medicus) 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 bentazon on velvetleaf, ragweed (*Ambrosia artemisiifolia* L.), eastern black nightshade (*Solanum ptycanthum* Dun.), and hairy nightshade (*Solanum sar-rachoides* Sendtner). The use of nonionic surfactant or a good quality crop oil concentrate was recommended with primisulfuron (CPR 2005), it is apparent that for a particular weed species the activity of primisulfuron can be increased by proper selection of surfactant.

The objective of this study was to (1) examine the abaxial and adaxial leaf surfaces of barnyardgrass and green foxtail; (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 barnyardgrass and green foxtail were collected from plants at the five- to six-leaf stage, grown under natural field condition at the USDA Southern Weed Science Research Unit farm, Stoneville, MS. Young leaves (first fully expanded leaf from the tip) and old leaves (third or fourth fully expanded leaf from the tip) were collected from each plant. Plant specimens were prepared for scanning electron microscopy using similar procedures as described by McWhorter et al. (1993). Leaf segments of approximately 20 mm<sup>2</sup> 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 drier<sup>1</sup> and were mounted on aluminum stubs. Samples were gold-coated using a sputter coater<sup>2</sup> and examined under a scanning electron microscope.<sup>3</sup> Leaf surfaces were photographed at 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.

### Wax Content per Unit of Leaf Area

The leaves of barnyardgrass and green foxtail were collected from five- to six-leaf stage field-grown plants at the USDA Southern Weed Science Research Unit farm, Stoneville, MS. Wax was extracted from the third to fourth (from tip) 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.<sup>4</sup> Wax extraction was done by immersing approximately 50 leaves for 30 s in 500-ml HPLC-grade chloroform in an ultrasonicator<sup>5</sup> at room temperature. The chloroform-wax solution was filtered using a fritted glass funnel apparatus with Durapore<sup>6</sup> membrane filters (0.22 μm, GV series), and the volume was reduced to approximately 20 ml in a rotary evaporator.<sup>7</sup> The reduced chloroform-wax solution was transferred to a pre-weighed 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 to ensure complete dryness of the wax sample before recording the wax mass. 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 from five- to six-leaf stage plants grown under natural field condition. The spread area of a 1 μl droplet of distilled water, primisulfuron at 40 g ai ha<sup>-1</sup> (without surfactant), primisulfuron with a nonionic surfactant<sup>8</sup> at 0.25% (v/v), and primisulfuron with an organosilicone wetting agent<sup>9</sup> at 0.1% (v/v) was measured on the adaxial surface of third to fourth (from tip) fully expanded leaves of barnyardgrass and green foxtail, 3 min after the droplet application. Spread area was calculated using the formula  $\pi r^2$ , where r was an estimate of the droplet radius based on the mean of hori-

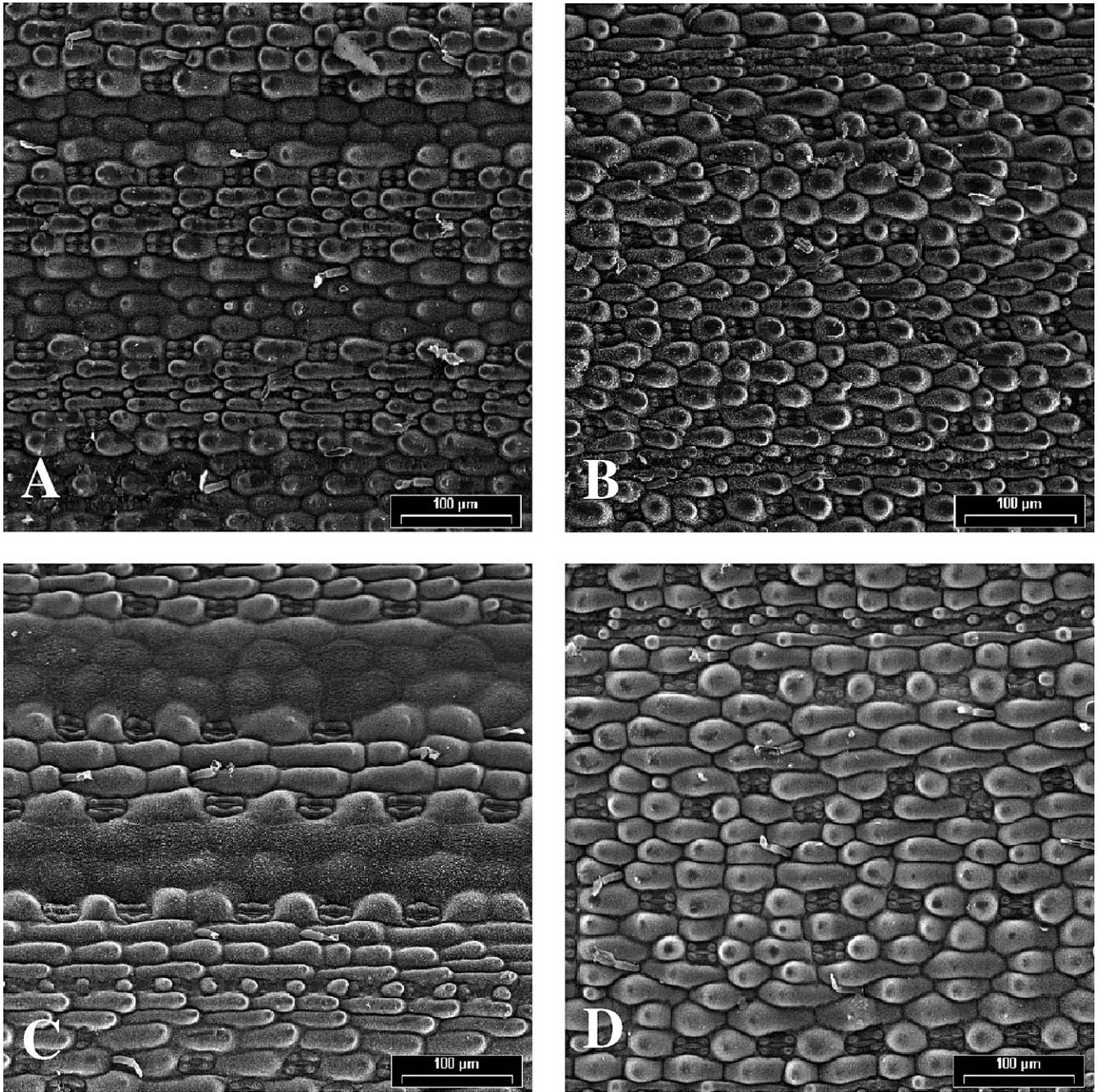


FIGURE 1. Scanning electron micrographs of barnyardgrass leaf surface: (A) adaxial surface of young leaf (first fully expanded leaf from the tip); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (third or fourth fully expanded leaf from the tip); (D) abaxial surface of old leaf.

zontal 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

The epidermis of barnyardgrass and green foxtail are made up of cells of various shapes (Figures 1 and 2). In

barnyardgrass, on adaxial leaf surface the epidermal cells are mostly elongated, whereas, the cells are mostly dome shaped on the abaxial surface (Figure 1). Epidermal cells of green foxtail are polygonal and on the abaxial surface cells are longer than cells on the adaxial surface (Figure 2). Both species have stomata and trichomes on both adaxial and abaxial surfaces. The adaxial and abaxial leaf surfaces of young and old leaves of barnyardgrass were relatively smooth as there were no prickles (Figure 1). In contrast, green foxtail had short, one-celled, hook-shaped prickles on both adaxial and abaxial surfaces (Figure 2).

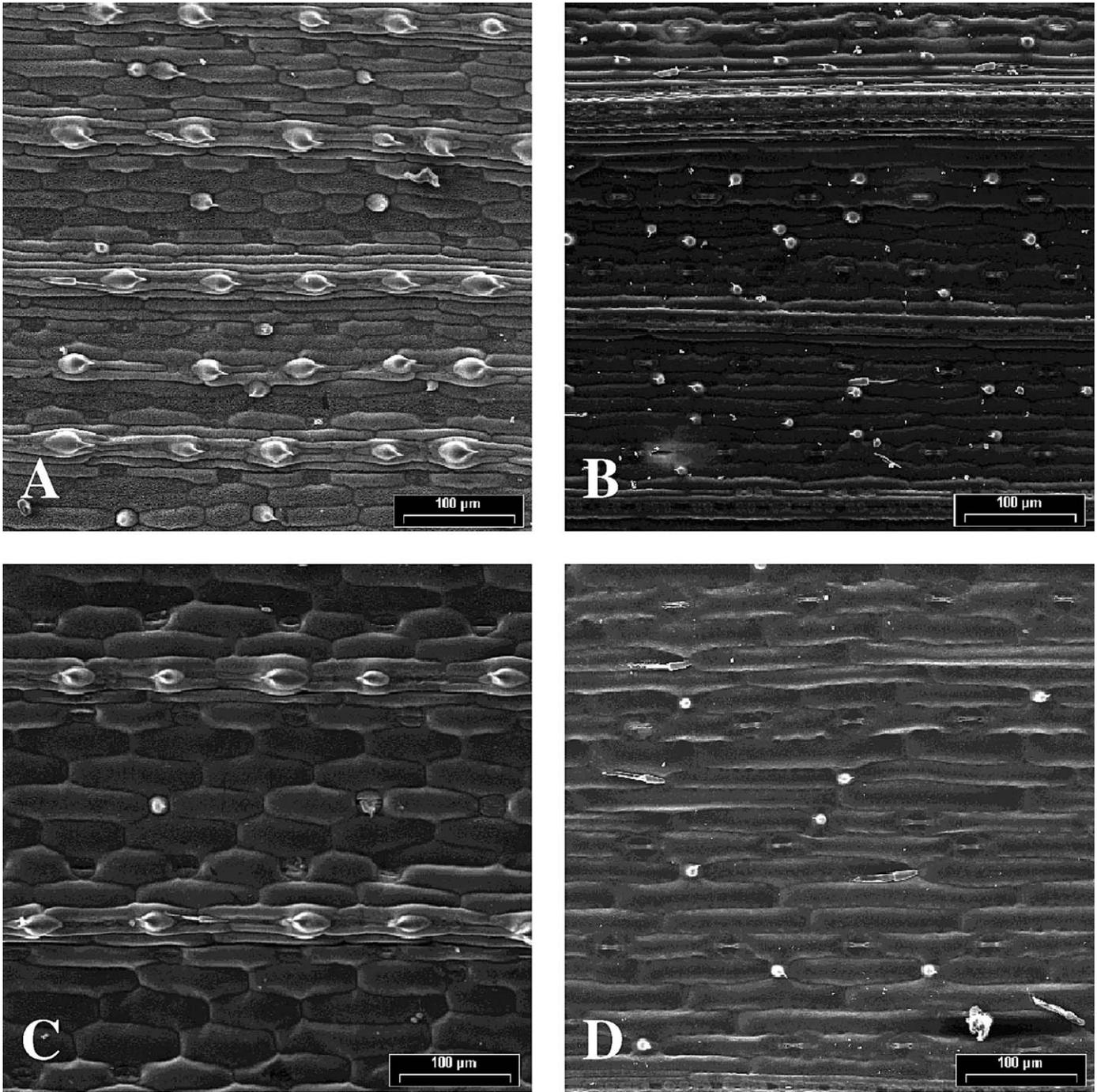


FIGURE 2. Scanning electron micrographs of green foxtail leaf surface: (A) adaxial surface of young leaf (first fully expanded leaf from the tip); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (third or fourth fully expanded leaf from the tip); (D) abaxial surface of old leaf.

In green foxtail the number of stomata per unit area was more on adaxial surface as compared to abaxial surface, whereas, the number of stomata was more on abaxial surface than adaxial surface in barnyardgrass (Figure 3). In both weed species the number of stomata per unit area of leaf area was higher in the younger leaves as compared to the older leaves, which was also true in case of johnsongrass as reported by McWhorter et al. (1993). Several reports have indicated herbicide infiltration of stomata (Wanamarta and Penner 1989). In a recent study it has been shown that uptake of anions (fluorescein) and cations ( $\text{Fe}^{3+}$ ) in garden

leek (*Allium porrum* L.), Asiatic dayflower (*Commelina communis* L.), and CAM plant (*Sedum telephium* L.) leaves was increased with stomatal aperture and stomatal density (Eichert and Burkhardt 2001). This complements previously published results with *A. porrum* (Eichert et al. 1998), where stomatal uptake of uranine was demonstrated for both intact leaves and epidermal peels. Fewer numbers of stomata on the adaxial leaf surface reduces stomatal infiltration of herbicides as the herbicide spray droplet is primarily intercepted by the adaxial leaf surface.

Both barnyardgrass and green foxtail had bicellular tri-

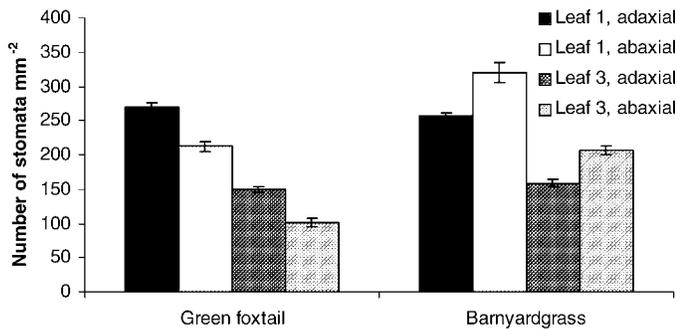


FIGURE 3. Number of stomata per unit area of adaxial and abaxial leaf surfaces of young (first fully expanded leaf from the tip) and old (third or fourth fully expanded leaf from the tip) leaves of barnyardgrass and green foxtail. LSD (0.05) value was 10.63.

chomes (Figures 1 and 2). There was no significant variation in the number of trichomes per unit leaf area of green foxtail (Figure 4). In barnyardgrass the number of trichomes was higher on the abaxial surface than the adaxial surface (Figure 4) similar to that observed in johnsongrass leaves by McWhorter et al. (1993). Number of trichomes per unit area of leaf area was higher in the younger leaves as compared to the older leaves in both species. Trichomes act in a complex way in relation to spread of herbicide solution and adsorption of herbicide. Trichomes may cause reduced wetting and spreading of droplets (Hull et al. 1982). Bicellular trichomes discharge a mucilage-type secretion, which contains callose, a carbohydrate component ( $\beta$ 1, 3-glucan) usually associated with “walling off responses” same as those associated with injured plant tissues (Paul et al. 1992). 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 fluorescent dyes that trichomes might provide a site of entry to the foliar applied herbicides.

Green foxtail leaves had prickles on both surfaces, which are tough pointed structures that appear to be spines or barbs (Figure 2). The number of prickles per unit area of leaf was significantly higher in adaxial surfaces than the abaxial, both in young and old leaves (Figure 5). The size of individual prickles was also larger on adaxial leaf surfaces as compared to abaxial surfaces. Number of prickles per unit area was higher in young leaves as compared to old leaves. Prickles are heavily silicified structures that often have wax

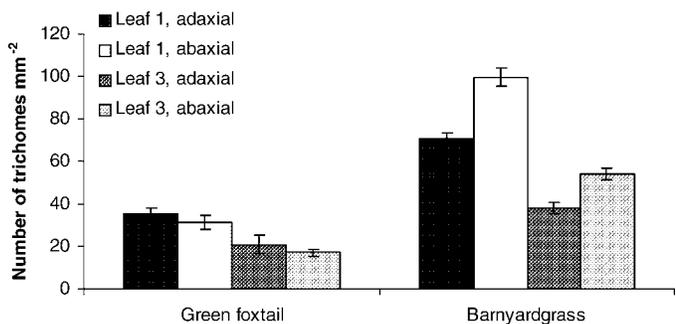


FIGURE 4. Number of trichomes on adaxial and abaxial leaf surfaces of young (first fully expanded leaf from the tip) and old (third or fourth fully expanded leaf from the tip) leaves of barnyardgrass and green foxtail. LSD (0.05) value was 4.47.

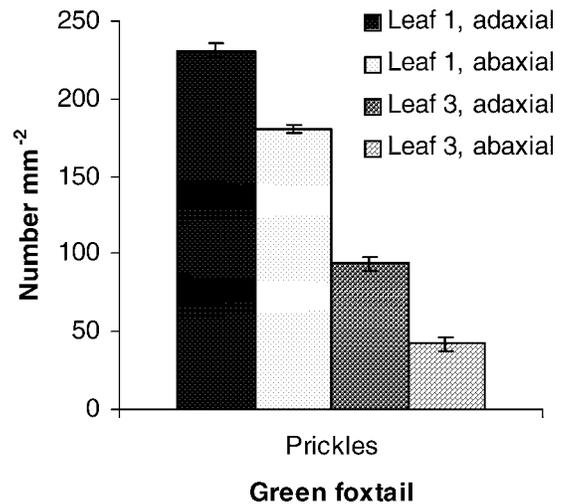


FIGURE 5. Number of prickles on adaxial and abaxial leaf surfaces of young (first fully expanded leaf from the tip) and old leaves (third or fourth fully expanded leaf from the tip) of green foxtail. LSD (0.05) value was 8.54.

crystals around the base that would likely increase the rate of spread of oil but inhibit spread of water droplets (McWhorter et al. 1993). Presence of prickles would result in increased microroughness of the leaf surface. Presence of short and macroprickles was reported in other grass species by McWhorter et al. (1993).

#### Wax Content per Unit of Leaf Area

The mean value of the wax content per unit area of leaf in barnyardgrass and green foxtail was 35.9 and 19.1  $\mu\text{g cm}^{-2}$ , respectively and the difference was not statistically significant (Figure 6). In our previous work with three broadleaf weeds, common lambsquarters (*Chenopodium album* L.) had the highest wax content (275  $\mu\text{g cm}^{-2}$ ) compared to velvetleaf (7  $\mu\text{g cm}^{-2}$ ) and common purslane (*Portulaca oleracea* L.; 153  $\mu\text{g cm}^{-2}$ ) (Sanyal et al. 2006). The epicuticular wax content 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). Hull (1970) reported that the amount of wax produced by the plant is influenced by light, temperature, and relative humidity. Leaf

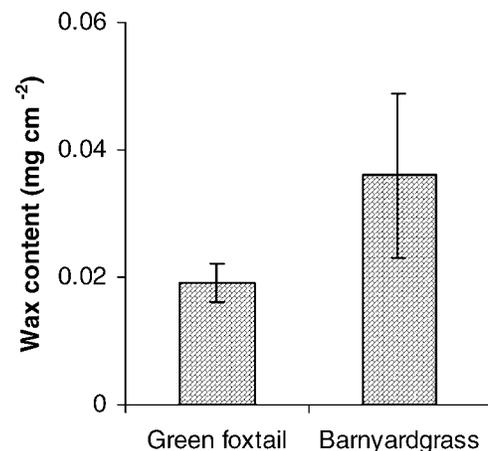


FIGURE 6. Wax content per unit of leaf area in barnyardgrass and green foxtail. LSD (0.05) value was 0.03.

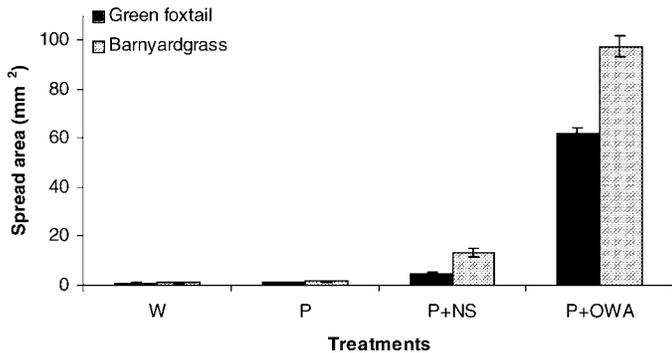


FIGURE 7. Spread area of 1- $\mu$ l droplets of distilled water (W), primisulfuron (P), primisulfuron with a nonionic surfactant (P+NS), and primisulfuron with an organosilicone wetting agent (P+OWA). Primisulfuron at 40 g ai ha<sup>-1</sup>, nonionic surfactant at 0.25% (v/v), and the organosilicone wetting agent at 0.1% (v/v) were used. LSD (0.05) for species 2.61 and for treatments 3.69.

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. 2001). Norsworthy et al. (2001) reported that among four species, pitted morningglory (*Ipomoea lacunosa* L.) and prickly sida (*Sida spinosa* L.) contained the least leaf wax and had smaller contact angles or higher leaf wettability than the species with more waxy leaves. The epicuticular wax appears to be the primary barrier to pesticide penetration. Removal of epicuticular wax with chloroform greatly increased glyphosate absorption in coca [*Erythroxylum coca* var. *coca* (Lam.)] compared to plants with leaf epicuticular wax (Ferreira and Reddy 2000). 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).

### Spread Area of Primisulfuron Droplets

There was no significant variation in spread of 1  $\mu$ l droplet of primisulfuron (without surfactant) or pure distilled water on both the species (Figure 7). On both barnyardgrass and green foxtail primisulfuron with a nonionic surfactant had more spread area than that without a surfactant, and the spread was even greater with organosilicone wetting agent (Figure 7). There was no variation in spread of 1  $\mu$ l droplet of pure distilled water between two species. The spread of primisulfuron droplets was higher on the leaf surface of barnyardgrass than the spread on green foxtail when surfactant was added (Figure 7). These results did not show the inverse relationship between leaf wax content and the spread area of the spray droplet as described by Chachalis et al. (2001). 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 a significant quantity 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.

Our results show that the addition of organosilicone wetting agent with primisulfuron spreads the herbicide better and covers more surface area on leaves, which may lead to higher absorption of herbicide into the leaf tissue resulting in a greater weed control. These results also 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.

### Sources of Materials

- 1 Critical point drier, Balzers CPD 020, Balzers, 8 Sagamore Park Road, Hudson, NH 03051.
- 2 Sputter coater, Hummer X, Anatech, Ltd., 5510 Vine Street, Alexandria, VA 22310.
- 3 Scanning electron microscope, 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 Rotary evaporator, Buchi R-124 Rotavapor, Buchi Analytical Inc., 19 Lukens Drive, New Castle, DE 19720.
- 8 Induce®, a nonionic surfactant, blend of alkyl aryl polyoxyalkane ethers, free fatty acids, and dimethyl polysiloxane, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.
- 9 Silwet L-77®, an organosilicone wetting agent, polyalkyleneoxide-modified heptamethyltrisiloxane 7.5 EO, 100%. Formerly, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591.

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