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ACTIVITY OF PRIMISULFURON AND *Alternaria helianthi* AS AFFECTED BY LEAF SURFACE MICRO-MORPHOLOGY AND SURFACTANTS

A Dissertation Presented

by

DEBANJAN SANYAL

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University of Massachusetts Amherst in partial fulfillment
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DEDICATION

To

Nibedita, my beloved wife.

It would not have been possible for me to achieve my goal without her.

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ABSTRACT

ACTIVITY OF PRIMISULFURON AND *Alternaria helianthi* AS AFFECTED BY LEAF SURFACE MICRO-MORPHOLOGY AND SURFACTANTS

MAY 2006

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Laboratory and greenhouse studies were conducted to examine the leaf surface, epicuticular wax content, spray droplet behavior, and primisulfuron activity (with and without surfactants) on common lambsquarters, common purslane, velvetleaf, barnyardgrass, and green foxtail. Adaxial and abaxial leaf surfaces were examined using scanning electron microscopy. Leaf wax was extracted and quantified. The spread of 1 µl droplets of distilled water, primisulfuron solution (without surfactant), primisulfuron solution with a nonionic surfactant and with an organosilicone surfactant was determined on the adaxial leaf surfaces of each of the weed species. The activity of primisulfuron without or with surfactants was assessed 3 weeks after treatment in terms of percent injury and plant fresh weight. Greenhouse studies were also conducted to investigate the bioherbicidal activity of *Alternaria helianthi* (Hansf.) Tubaki & Nishih. on multiple-seeded cocklebur as affected by various surfactants.

The number of stomata per unit area on abaxial surface was more than on adaxial leaf surface of barnyardgrass, common lambsquarters and velvetleaf, whereas, common purslane and green foxtail had more stomata on adaxial surface than abaxial. Common lambsquarters had the highest wax content per unit of leaf area (274.5 μg cm^{-2}) and velvetleaf had the lowest (7.4 μg cm^{-2}). Wax content of common purslane was 153.4 μg cm^{-2}. The mean values of the wax content per unit of leaf area in barnyardgrass and green foxtail were 35.91 μg cm^{-2} and 19.14 μg cm^{-2}, respectively. Surfactants increased primisulfuron activity on common lambsquarters, common purslane, velvetleaf, and green foxtail. In general, organosilicone surfactant reduced the contact angle with increased spread area of the primisulfuron droplets more than the nonionic surfactant treatments and resulted in enhanced activity of primisulfuron. *Alternaria helianthi* resulted in significant reduction of fresh weight of multiple-seeded cockleburs when followed by a 12 h dew period as compared to a 6 h dew. Under short dew period (6 h), greater control of multiple-seeded cocklebur was achieved using higher rates of Activator 90 and Silwet L-77 and may have great potentials for achieving effective biological control.

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CHAPTER I

INTRODUCTION

Weed management: an important component of agriculture

Weeds have long been recognized as a source of considerable economic loss and there are lots of information on the nature and extent of weed interference in many crops (Knake and Slife 1962; Nieto et al. 1968; Shadbolt and Holm 1956; Sharma et al. 1977). Weeds resulted in yield reduction by competing with crops for moisture, nutrients, and light (Pavlychenko 1949; Rejmanek et al. 1989; Pantone and Baker 1991; Singh et al. 1991; Vitta and Satorre 1999; Ponce and Santin 2004; Tepe et al. 2005). Interference from weeds not only causes yield loss, it also reduces quality, and harvesting efficiency (Chisaka 1977; Bhowmik and Reddy 1988a; Bhowmik and Reddy 1988b). The degree of interference depends on weed and crop species, density and distribution of weeds, and duration of interference (Bleasdale 1960; Bhowmik and Reddy 1988a; Bhowmik and Reddy 1988b). From historical perspective, weed control was mostly incidental to tillage and to growing of thickly planted crops, for many centuries (Timmons 2005). Occasional references in literature previous to 1900 mentioned use of mechanical devices and a few inorganic herbicides specifically for weed control (Timmons 2005).

The arsenicals were the first chemicals to be tested widely for weed control and to gain rather general use as herbicides (Hanson 1962). Sodium arsenite was used extensively in Hawaii for controlling annual weeds, particularly forbs, during 1913 to

1945 (Hanson 1962). However, the discovery of the weed killing properties of the phenoxyacetic herbicides in Britain and in the United States during 1942 to 1944 (Blackman 1948; Hamner and Tukey 1944; Mitchell and Hamner 1944) marked the real beginning of the herbicide phase of the "Chemical Era of Agriculture". Soil-applied herbicides have been used to control weeds for several decades. However, the efficacy of soil-applied herbicides is highly dependent on rainfall, with reduced weed control under extremely low or high rainfall conditions. The importance of postemergence herbicides has risen dramatically in the past decade. Several factors, such as maintaining ground- and surface-water quality, reducing herbicide carryover, and more economical weed control, have promoted a trend toward the use of postemergence herbicides (Stoller et al. 1993). In a postemergence herbicide program, herbicides are applied within the limits of weed height and crop stage to ensure optimal herbicide performance and yield potential (Carey and Kells 1995; DeFelice et al. 1989; Devlin et al. 1991). In 2001, Underwood reported that 70% of U.S.-agrochemical market was occupied by herbicides and 50% of those were post-emergence herbicides.

Sulfonylurea herbicides

In 1980, chlorsulfuron, the first sulfonylurea herbicide was introduced (Appleby 2005). The mode of action of sulfonylurea herbicide is via inhibition of acetolactate synthase (ALS) in plants (Ray 1984; Ray 1985; Matsunaka et al. 1985; Scheel and Casida 1985; Rost and Reynolds 1985; Falco et al. 1987). Acetolactate synthase is the first and the key enzyme in the biosynthesis of valine, leucine, and isoleucine, which are the essential branched chain amino acids for normal plant growth. The sulfonylureas are effective in very low rates like 2 g ha^{-1} (Devine and Vanden Born, 1985), and they have quite low mammalian toxicity (LD_{50}>5,000 mg kg^{-1} in rats). Many more sulfonylureas were introduced gradually by several manufacturers.

In any sulfonylurea herbicide the molecule generally consists of three parts; an aryl group, the sulfonylurea bridge, and a nitrogen-including heterocycle and when the heterocyclic portion of this structure is a symmetrical-pyrimidine or symmetrical-triazine containing lower alkyl or lower alkoxy substitutes, the substance has a high activity (Beyer et al. 1988). Minor adjustments in chemical structure drastically changed selectivity and soil persistence (Appleby 2005). Sulfonylureas can control grasses as well as broadleaf weeds and has excellent crop selectivity. Hageman and Behrens (1984) reported that susceptible velvetleaf (*Abutilon theophrasti* Medicus) was 20,000 times more sensitive to chlorosulfuron than was eastern black nightshade (*Solanum ptycanthum* Dun.). Most of the herbicides of this class have an acidic pKa, which is attributed to acidic sulfonamide nitrogen (Beyer et al. 1988).

Sulfonylurea herbicides can be easily absorbed by both roots and foliage of plants and it can be translocated through both xylem and phloem (Beyer et al. 1988). The most noticeable plant response caused by sulfonylureas, is the strong and rapid inhibition of plant growth. Also, there may be some secondary plant responses like enhanced anthcyanin formation, abscission, terminal bud death, loss of leaf nyctinasty, vein discoloration, chlorosis or necrosis.

There are many sulfonylurea herbicides. Primisulfuron {methyl 2-[[[[[4,6-bis(difluromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate} is a selective herbicide applied post-emergence for the control of grasses, especially some difficult to control perennial grasses, and certain broadleaf weeds in field corn for silage or grain and in popcorn. (CPR 2005). Bhowmik (1995) reported that rates from 15 to 30 g ai ha^{-1} of primisulfuron controlled quackgrass over 90% 6 weeks after treatment. With a single early postemergence application of primisulfuron at 40 g ai ha^{-1} was as effective in controlling quackgrass as a split application of 20 g ai ha^{-1} applied at the one- to three-leaf stage followed by a second application of 20 g ai ha^{-1} at four- to six-leaf stage (Bhowmik 1999). According to Tweedy and Kapusta (1995), primisulfuron at 40 g ha^{-1} controlled johnsongrass (*Sorghum halepense* L.) 85 to 100% from 1992 to 1994 and corn yield was more than double as compared to the control plots. Sprague et al. (1999) reported that primisulfuron at 40 g ha^{-1} rate with 2% (v/v) crop oil concentrate had 73% control of Canada thistle (*Cirsium arvense* (L.) Scop.). The same rate of primisulfuron with a nonionic surfactant and 28% N resulted in 69% control of wild carrot (*Daucus carota* L.) seedling in greenhouse condition (Stachler and Kells 1997). Prostko et al. (1994) reported that primisulfuron reduced horsenettle (*Solanum carolinense* L.) biomass by 68%. Efficacy of primisulfuron (40 g ai ha^{-1}) on velvetleaf control was reduced by 22% when applied in combination with atrazine at 1.7 kg ai ha^{-1} (Hart and Penner 1993).

Wetting of leaf surface: a complex phenomenon

The exact manner in which a herbicide spray adheres to and penetrates a leaf surface is a function of the leaf surface and of the chemical and physical nature of the spray itself. The mechanism of foliar wetting by agricultural sprays has been discussed in detail by Johnstone (1973). The physical and chemical properties of the spray formulation include factors such as surface tension, density, viscosity, volatility, solubility characteristics etc. These properties, as they relate to the spreading, retention and penetration of agricultural sprays in general, have been discussed by several authors (Crafts and Foy 1962; Dimond 1962; Franke 1967; Hull 1970). Physiochemical properties of the spray solution are variously influenced by adjuvants, particularly surfactants. Reduction of surface tension can influence droplet size, which in turn affects impact velocity and contact angle (θ) of the impinging droplet. These phenomena are interrelated with energy transitions involved in droplet impaction, which consists of initial spreading, retraction of the droplet, and a final secondary spreading. Technical aspects of these relationships were considered by Johnston (1973). There is likewise an "energy barrier" involved in the transfer of a herbicide from the external phase (the droplet on the leaf surface) to the internal phase within the leaf. The baseline values for these energy barriers are quite different for different herbicides, and are generally reduced upon the addition of surfactants. The kinetics of this relationship have been discussed by Freed and Witt (1969). Zisman (1964) demonstrated that in order to wet a substrate the surface tension of the wetting liquid must not exceed a certain critical value that is characteristic of the particular substrate. According to Young's equation, when a liquid droplet is at equilibrium on a solid surface, $\gamma_{LA} \cos\theta = \gamma_{SA} - \gamma_{SL}$, where, γ_{SL}, γ_{LA}, and γ_{SA} represent the interfacial free energy per unit area at the solid-liquid, liquid-air, and solid-air interface, respectively (Grayson et al. 1991). Low-melting solids, such as organic polymers, waxes, and covalent compounds, in general have surface free energies ranging from 100 to 25 mJ/m^2 and in order to spread on these surfaces the free surface energy of the liquid has to be lower than that (Rosen 2004).

Leaf-surface characteristics: a key factor in foliar wetting and penetration of herbicides

Leaf surface affects the wetting and penetration behavior of foliar applied herbicides (Hess 1985; Hull et al. 1982; McWhorter 1985; Wanamarta and Penner 1989). Surface characteristic includes the cuticle (epicuticula wax, cutin, and pectin) and numbers of stomata, glands, and trichomes (Hess 1985; Hull et al. 1982; McWhorter 1985; Wanamarta and Penner 1989). Herbicide absorbtion is facilitated with either cuticular or stomatal infiltration, but there are no conclusive evidence indicating the comparative level of each rout of penetration (Hess 1985; Wanamarta and Penner 1989).

Cuticle composition and structure of modern higher plants vary greatly from species to species but are generally 0.1–10 μm thick, being composed of two major classes of lipids: (i) solid waxes, usually mixes of long chain aliphatic compounds and (ii) insoluble, high molecular weight polyester cutins (Holloway 1993; Riederer and Schreiber 2001; Hess and Foy 2000). Generally, the plant cuticle is composed of two regions. The lower region consists of cutin, long chain alkyl ketones, alcohols, and closest to the cells, polysaccharides; the upper region consists of epicuticular wax, mostly very long chain alkanes (Holloway 1993; Hess and Foy 2000). Plant cuticles also contain terpenoids and phenolic compounds, some of which serve as anti-feedants to discourage grazing by herbivores. Cuticle structure and composition varies from species to species, although there appear to be five basic types: smooth, ridged, papillose, glaucous (having an additional covering of microcrystalline wax), and glandular where trichomes are present in high number and comprise the main 'surface' of the leaf (Holloway 1993).

Epicuticular leaf wax commonly occurs as a wax crystal deposition (Baker 1982; Hess 1985) and appears to be an effective barrier to herbicide 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). Absorption of MCPB by broad bean (*Vicia faba* L.) leaves was also increased by removal of epicuticular wax with chloroform (Kirkwood et al. 1982). Thickness, chemical composition, and ultrastructure of the epicuticular wax differ among plant species, 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 also 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. 2001a; Chachalis et al. 2001b). Composition, physical structure, and orientation of leaf wax also play important roles in this regard (Juniper 1960; Whitehouse et al. 1982). Leaf wax contains primarily a variety of short and long chain hydrocarbons, alcohols, acids, esters, aldehydes, and triterpenes (Baker 1982). Wax is considered to be largely non-polar and therefore hydrophobic, but variation exists among species. 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. Recent studies indicate that the largest fraction of the cuticle surface is covered by the lipophilic domains of cutin and wax, but to a certain extent polar domains are also present in the cuticle, which form preferential sites of penetration for polar compounds (Schreiber 2005). Limited information is available on the characterization of epicuticular wax in many weed species.

Stomata appear to play role in foliar penetration in two ways. The guard and accessory cells may act as preferred sites of entry (Franke 1964). Stomatal infiltration of spray solution was also reported by several authors (Dybing and Currier 1961; Schönherr and Bukovac 1972; Greene and Bukovac 1974). Christensen (1972) reported a positive relationship between the rate of water uptake and stomatal pore area, which may vary in the course of a day if stomata are functional. In earlier studies, increased water uptake was observed as stomatal density increased (Beyer and Knoche 2002). Theoretically, flow of water through an open stomatal pore would occur if water droplets wetted the surface completely, resulting in a 0° contact angle (θ) (Schönherr and Bukovac 1972). Aqueous solutions with a surface tension approaching that of pure water do not pass through the stomatal pore (Schönherr and Bukovac 1972). However, penetration has been observed when the surface tension was sufficiently decreased by addition of a surfactant (Dybing and Currier 1961). Greene and Bukovac (1974) observed that mass stomatal penetration of NAA and silver nitrate into pear leaves was promoted by surfactants when stomata were open.

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). Previous research has shown that 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 (Boize et al. 1976; Hess et al. 1974). Benzing and Burt (1970) showed by fluorescent dyes that trichomes might provide a site of entry to the foliar applied herbicides.

Surfactants: role in wetting, penetration, and subsequent activity of herbicides

To achieve weed control by postemergence herbicides, the herbicides have to come in contact and retain on the leaf surface prior to absorption into plant, able to reach the site of action, and finally induce phytotoxic responses. The ability of spray droplet to wet the leaf surface is generally determined as the contact angle that the droplet makes with the leaf surface. Addition of surfactant into the spray solution reduces the surface tension and enhances the solution's ability to wet leaf surface. However, there are lots of variations of wetting properties among surfactants (Wells 1989; Knight and Kirkwood 1991).

There are many reports showing surfactants improving herbicide uptake into whole plants and isolated leaf tissue (Stevens and Bukovac 1987a; Stevens and Bukovac 1987b; Riederer and Schönherr 1990; Field et al. 1992; Kirkwood et al. 1992; De Ruiter et al. 1992; Gaskin and Holloway 1992; Stock and Holloway 1993; Coret et al. 1993; Coret and Chamel 1993; Coret and Chamel 1994; Coret and Chamel 1995; Holloway and Edgerton 1992). Jansen et al. (1961) noted that inclusion of an appropriate surfactant in herbicidal formulation enhanced retention and penetration only in certain plant species, and thus measurably altered selectivity of the herbicide as compared to its activity without a surfactant. Within a single species, the extent to which a specific surfactant may enhance the activity of a herbicide depends to a considerable extent upon the various components of the formulation and upon the physical properties of the active ingredient. In general, the addition of a surfactant or surfactant tends to equalize the foliar absorption of herbicides (Klingman and Ashton 1975).

Enhanced uptake into the plant system leads to higher activity of herbicides when applied with surfactants. In general, most postemergence herbicides have been used with one of the surfactant types such as nonionic surfactants (NIS), crop oil concentrates (COC), nitrogen-surfactant blends, esterified seed oils, or organosilicones. Mitra et al. (1998) reported that the addition of an oil to rimsulfuron increased quackgrass (*Elytrigia repens*(L.) Beauv.) control by 40% 5 weeks after treatment, common lambsquarter (*Chenopodium album*(L.)) and yellow foxtail (*Setaria lutescens* (Weigel.) Hubb.) control by 30% 3 weeks after treatment, compared to control by rimsulfuron alone. Mitra et al. (1998) reported 70% quackgrass control by rimsulfuron (1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonyl)urea) and the same treatment increased quackgrass control over 90% with Silwet, and 80% with Atplus S-12, Induce, or Renex. The effect of surfactant on herbicidal activity varies with herbicides and with weed species. Nandula et al. (1995) reported that primisulfuron provided greater wirestem muhly (*Muhlenbergia frondosa* (Poir.) Fern.) control with methylated vegetable oil concentrate as compared to nonionic surfactant. In field experiments, at 39 g ai ha^{-1} rate primisulfuron provided greater control to itchgrass (*Rottboellia cochinchinensis* (Lour.) W.D. Clayton) when applied with a nonionic surfactant than with an organosilicone or methylated seed oil blend (Strahan et al. 2000). Even within a class, various surfactants exhibit selective effect. Thus S309 enhanced the herbicidal efficacy of a picloram (4-amino-3,5,6-trichloropicolinic acid) + 2,4-D (2,4-Dichlorophenoxyacetic acid) mixture on both black spruce and balsam fir (Haagsma and Mihajlovich 1992). In contrast, L-77 did not, but was beneficial with triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid) + dicamba (3,6-dichloro-2-methoxybenzoic acid) on black spruce, but not balsam fir. In general, increasing amount of surfactants increases agrochemical uptake, although in some cases, such as with glyphosate, if this markedly increases the contact area of the droplet with the leaf surface, it may reduce the concentration per unit area and reduce the uptake of the herbicide (Liu and Zabkiewicz 1998).

Research results till to date indicate that surfactants do not have a direct effect on herbicide translocation (Coupland 1988; Field and Dastgheib 1996). However, because the addition of surfactants will enhance uptake, the percentage of absorbed herbicide translocated falls with increasing uptake, regardless of whether it is cuticular (Stevens and Bukovac 1987a; Stevens and Bukovac 1987b; Field et al. 1995; Holloway 1998) or stomatal uptake (Stevens and Zabkiewicz 1988; Zabkiewicz and Gaskin 1989).

Fungal bio-control agents for weed control

A number of phytopathogenic and non-phytopathogenic bacteria and fungi or their secondary metabolites exhibit potential as biological weed control agents (Lax et al. 1988). Collectively these organisms and their natural products are called bio-herbicides. Fungi with potential bio-herbicidal activity are termed as mycoherbicides. Numerous fungi have been screened for phytotoxic potential and several have been more closely examined as mycoherbicides (Hoagland 1990; TeBeest 1991).

Mycoherbicides may be nonspecific or it may be host-specific. Examples of some host-specific toxins produced by phytopathogenic fungi are, HV toxins by *Cochliobolus victoriae* against *Avena* spp.(induce tissue leakage), HC toxins by *Cochliobolus carbonum* against specific maize (*Zea mays*) (plasma membrane disruptor), AAL toxin by *Alternaria alternata* f.sp. *lycopersici* against tomato (*Lycopersicon esculentam*) (disrupts pymiridine synthesis by inhibiting aspertate carbamoyl transferase) etc. (Yoder and Tergeon 1985; Hoagland 1990). Host-specific phytotoxins cause visible and physiological changes that are characteristic of infected plants. Most of the changes appear to be secondary, relative to the primary or initial biochemical lesions, as indicated by the single-gene control of sensitivity and by experiments with isolated organelles (Scheffer and Livingston 1984).

Nonspecific phytotoxins are produced by both specialist and generalist plant pathogens. For example, *Alternaria alternata* f.sp. *citri*, causal agent of brown spot disease of tangerines and mandarins, simultaneously produces host-specific toxins and nonspecific phytotoxins (e.g. tentoxin and tenuazonic acid) in culture broth (Kono et al. 1986). The phytopathogenic species of *Fusarium* produce a wide variety of chemical contaminants of plant tissue including trichothecenes (T2-toxin and others), fumonisins, napthazarins, fusaric acid, and related pyridine derivatives (Desjardins 1992). The phytotoxicity of *Fusarium* spp. and some of their natural products has been reviewed by Hoagland and Abbas (1995). In turfgrass, a reduction in seed-head formation of annual bluegrass was observed a month after the *Xanthomonas campestris* pv. *poannua* application when combined with different plant growth regulators (Mitra et al. 2001).

Because of environmental concerns about some herbicide uses, and the increasing number of weeds that have developed resistance to one or more herbicides, there is a need to develop new efficacious herbicides with relatively short half-lives, low toxicity to non-target organisms, and different mode of action from the existing commercial herbicides in the market. Interest in phytotoxic microorganisms (either directly or as sources of naturally occurring phytotoxins) has increased recently, due to the search for less persistent, more selective and more environmentally benign herbicides, and as a result, many fungi have been discovered that have potential as mycoherbicides for the control of many terrestrial and aquatic weeds (Charudattan 1990). Although there are numerous pathogens that attack weeds, thus far only few pathogens have been registered and marketed as bioherbicides in United States and Canada for control of weeds in agricultural systems (Hoagland 2000). *Phytophthora palmivora* has been registered as DeVine® for the control of strangler vine in citrus groves, *Colletotrichum gleosporioides* f.sp. *aeschynomene* as Collego® for control of northern joint vetch in rice (*Oryza satvia*) and soybean fields (Templeton and Heiny 1989), *Colletotrichum gleosporioides* f.sp. *malvae* as BioMal®, for round leaved mallow (*Malva pusilla*) control, and *Alternaria cassiae* for control of sicklepod (*Cassia obtusifolia*) (Auld and Morin 1995; TeBeest 1991; Hoagland 1990; Hoagland 2000).

Influence of surfactants on the activity of bio-control agents

Formulation has often been recognized as a critical component in helping a biological control pathogen to germinate and infect a weed host resulting in bioherbicidal activity (Boyette et al. 1996; Connick et al. 1990; Diagle and Connick 1990; Watson and Wymore 1990). Surfactants may be added to a bioherbicide to enhance activity by (1) prolonging water retention to overcome dew period requirement; (2) adding nutrients to maintain fungal viability and stimulate spore germination, penetration, and infection; (3) modifying leaf wettability to improve spore deposition and retention on sprayed leaves; or (4) mixing with proper fillers for extended shelf-life (Green et al. 1998). Surfactants may also help to trap conidia for longer time on the leaf surface. Iqbal (1995) reported that artificial foam traps conidia of rare species in rich communities of fresh-water hypomycetes as efficiently as it traps conidia in a community showing poor species composition with low conidial numbers. Growth chamber experiments by Gronwald et al. (2002) indicated that maximum population of *Pseudomonas syringae* pv. *tagetis* on Canada thistle (*Cirsium arvense* (L.) scop.) leaves, was obtained with Silwet L-77 at 0.3% concentration (v/v), and the shoot dry weight was reduced by 52% after 14 days of treatment. Boyette et al. (2002) reported that *Myrothecium verrucaria* when applied with 0.2% of Silwet L-77, killed kudzu plant (*Pueraria lobata*) by 100%, 7 days after treatment. Winder (1999) reported that *Colletotrichum* isolate PFC 13 caused up to 54% damage of marsh reed grass (*Calamagrostis canadensis*) when formulated in powdered alginate applied with a vegetable oil and surfactant combination.

Different types of surfactants may have a range of effects on fungal spore germination and mycelial growth. Prasad (1994) investigated the effects of surfactants and sunscreens on mycelial growth of *Chondrostereum purpureum* Fr. Pouzar and result showed that the surfactants Silwet L-77, Triton X-1000, nonylphenol ethoxylate, Ethokem, Sunblock, Coppertone-15, and Sunscreen-8 were fungitoxic at a concentration of 0.1%. Only Bond and Suntangel-2 were nontoxic to fungi. Common laboratory surfactants such as Tween, Tergitol NP, Sorbitol, and Gelatin are often used to formulate spore suspensions at initial screening stages of bioherbicides (Greaves et al. 1998). Zhang et al. (2003) has shown that gelatin, Tween 40 and Tween 80 were useful components for bioherbicide formulations to increase conidial germination and mycelial growth of *Phoma*, whereas Tween 40 and Tween 80 were useful for *Colletotrichum*. Elston and Bhowmik (2002) studied bio-control of annual bluegrass (*Poa annua* L.) by *Xanthomonas campestris* pv. *poannua* with different surfactants, in putting green, and found that among Agri-dex, Break-thru, Rely, Silwet L-77, and X-77 only Agri-dex was safe to the bacterium.

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CHAPTER II

LEAF CHARACTERISTICS AND SURFACTANTS AFFECT PRIMISULFURON DROPLET SPREAD IN THREE BROADLEAF WEEDS[1]

Abstract

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-μl 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 surfactant (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

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to velvetleaf or common lambsquarters. Common lambsquarters had the highest epicuticular wax content on the leaf surface (274.5 μg cm^{-2}), followed by common purslane (153.4 μg cm^{-2}), and velvetleaf (7.4 μg cm^{-2}). There were no significant variations in the spread of the 1 μl 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.

Introduction

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 behaviour 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 micro-morphology 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 present 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 weeks 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 at the one- to three-leaf stage followed by a second application of 20 g ha^{-1} at 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.) 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 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 a 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 abaxial 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 mm^2 were fixed for 12 hours 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 (Balzers CPD 020, Balzers, 8 Sagamore Park Road, Hudson, NH 03051) and were mounted on aluminum stubs. Samples were gold-coated using a sputter coater (Hummer X, Anatech, Ltd., 5510 Vine Street, Alexandria, VA 22310) and examined under a scanning electron microscope [JEOL-JSM 840 (USA), 11 Dearborn Road, Peabody, MA 10960]. Leaf surfaces were photographed at 200X 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 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, Stoneville, Mississippi. 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 (Leaf Area Meter, model LI-3100, LI-COR, Inc., Lincoln, NE 68501). Wax extraction was done by immersing approximately 50 leaves for 30 seconds in 500ml of high-performance liquid chromatography (HPLC) grade chloroform in an ultra-sonicator (Branson 2210 Sonicator, Branson Ultrasonic Corporation, 41 Eagle Road, Dunbury, CT 06813-1961) at room temperature. The chloroform-wax solution was filtered using a fritted glass funnel apparatus with Durapore membrane filters (0.22 µm, GV series) (Durapore Membrane Filters, Millipore Corporation, 80 Ashby Road, Bedford, MA 01730), and the volume was reduced to approximately 20 ml in a rotary evaporator (Buchi R-124 Rotavapor, Buchi Analytical Inc. 19 Lukens Drive, New Castle, DE 19720). The reduced chloroform-wax solution was transferred to a pre-weighed 25ml 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 plants grown under natural field condition. The spread area of a 1-µl droplet of distilled water, primisulfuron at 39.5 g ai ha^{-1} (without surfactant), primisulfuron with a nonionic surfactant (blend of alkyl aryl polyoxylkane ethers, free fatty acids, and dimothyl polysiloxane, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017) at 0.25% (v/v), and primisulfuron with an organosilicone surfactant (polyalkyleneoxide-modified heptamethyltrisiloxane 7.5 EO, 100%, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591) 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 minutes 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 micro-morphology of common lambsquarters, common purslane, and velvetleaf (Figures 2.1, 2.2, and 2.3). 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 2.2). In contrast, common lambsquarters had "bladder hairs" (Figure 2.1), and velvetleaf had glands and star-shaped trichomes on both adaxial and abaxial leaf surfaces (Figure 2.3). In common lambsquarters, the number of bladder hairs per unit area of the leaf was significantly higher in abaxial surfaces than the adaxial, both in young and old leaves (Figure 2.4) and number of bladder hairs per unit area was higher in young leaves than the old leaves. In velvetleaf, the number of trichomes and glands was significantly higher in younger leaves than in the older leaves. In both leaf stages, the number of trichomes was higher in adaxial surfaces as compared with abaxial (Figure 2.5). Statistically, there was no variation in the number of glands between adaxial and abaxial surfaces in velvetleaf (Figure 2.5).

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 micro-roughness of the leaf surface. Trichomes 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

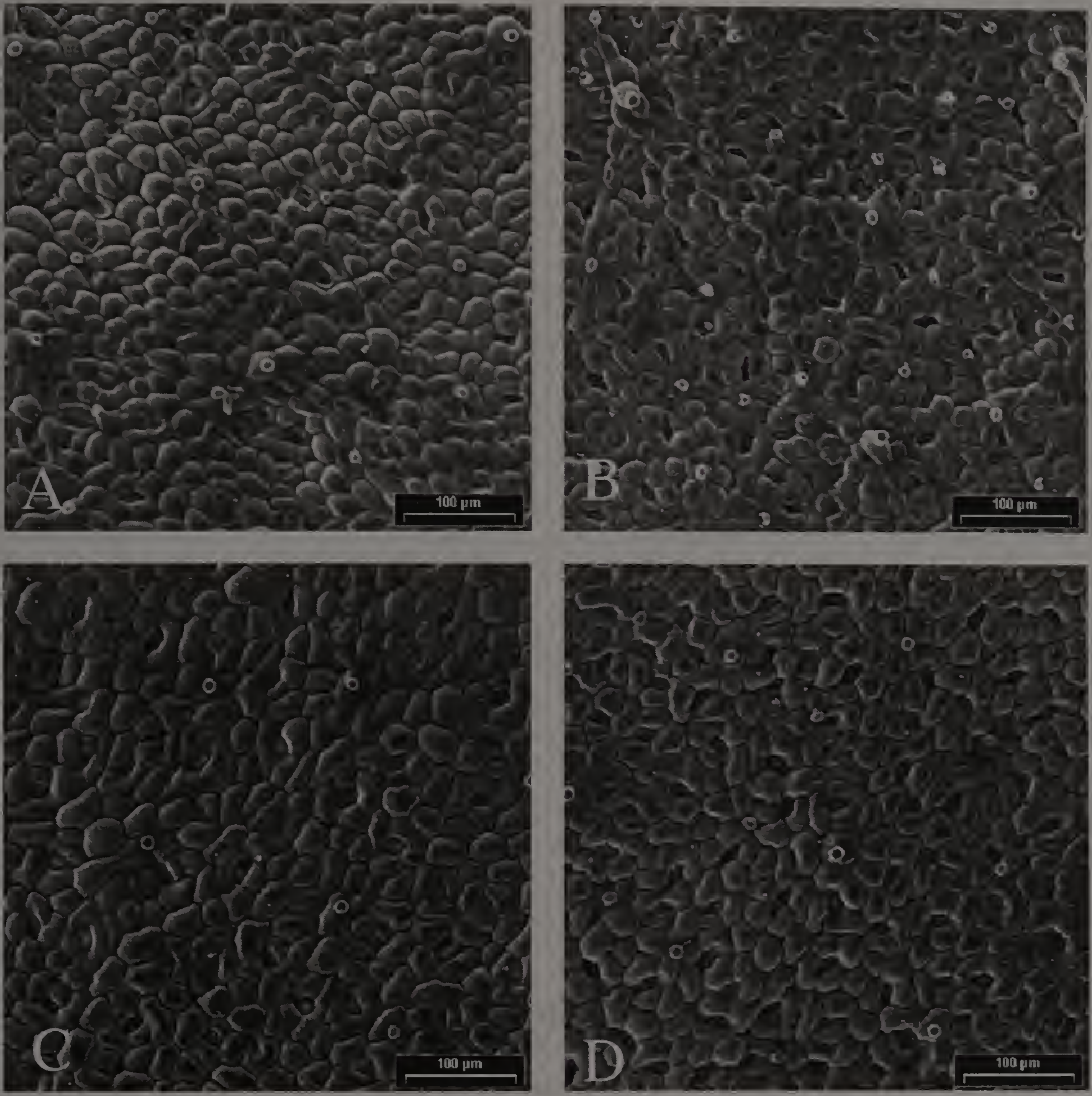

Figure 2.1 Scanning electron micrographs of common lambsquarters leaf surface: (A) adaxial surface of young leaf (first or second fully expanded leaf from the apical meristem); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (fifth or sixth fully expanded leaf from the apical meristem); (D) abaxial surface of old leaf.

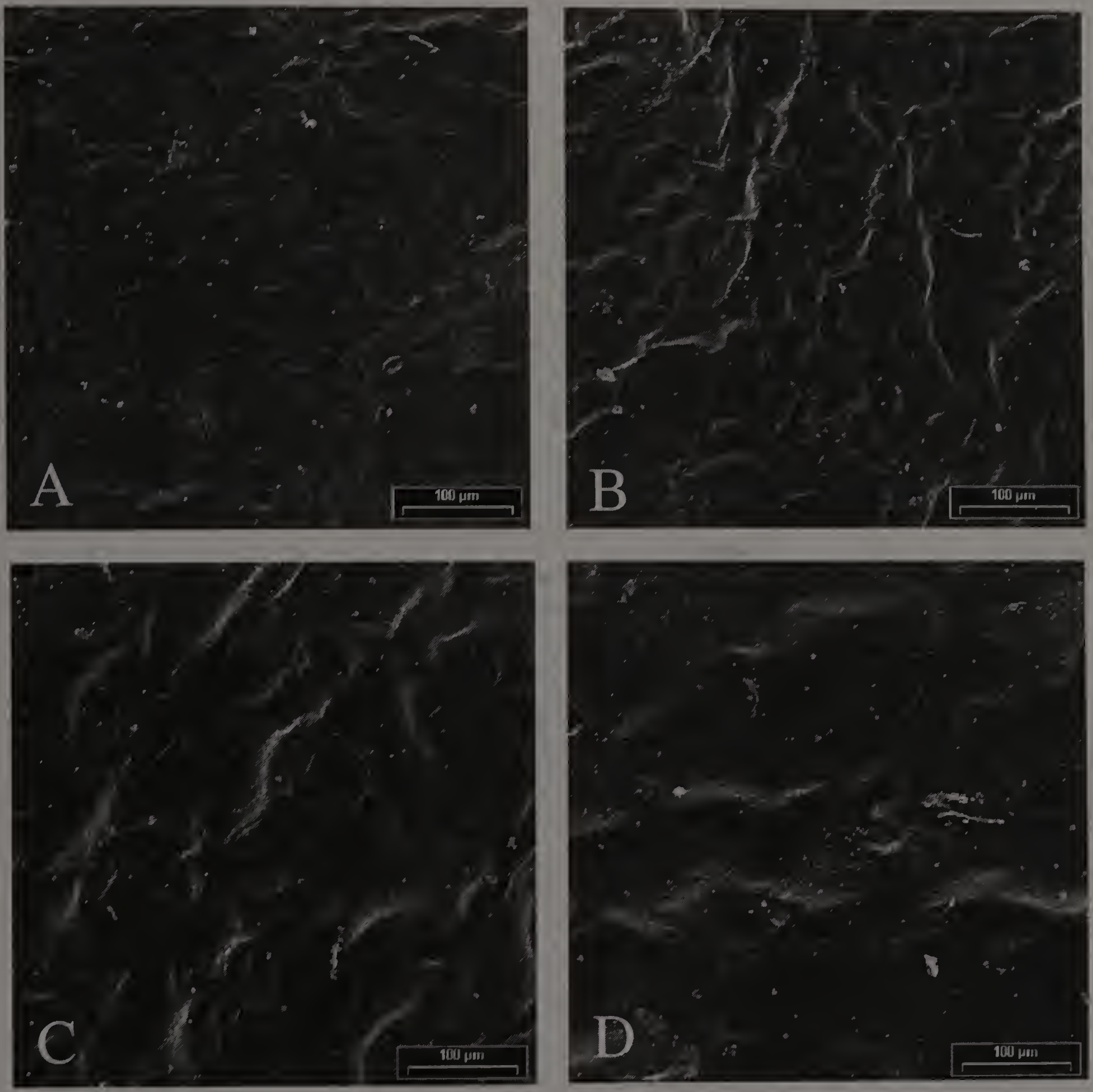

Figure 2.2 Scanning electron micrographs of common purslane leaf surface: (A) adaxial surface of young leaf (first or second fully expanded leaf from the apical meristem); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (fifth or sixth fully expanded leaf from the apical meristem); (D) abaxial surface of old leaf.

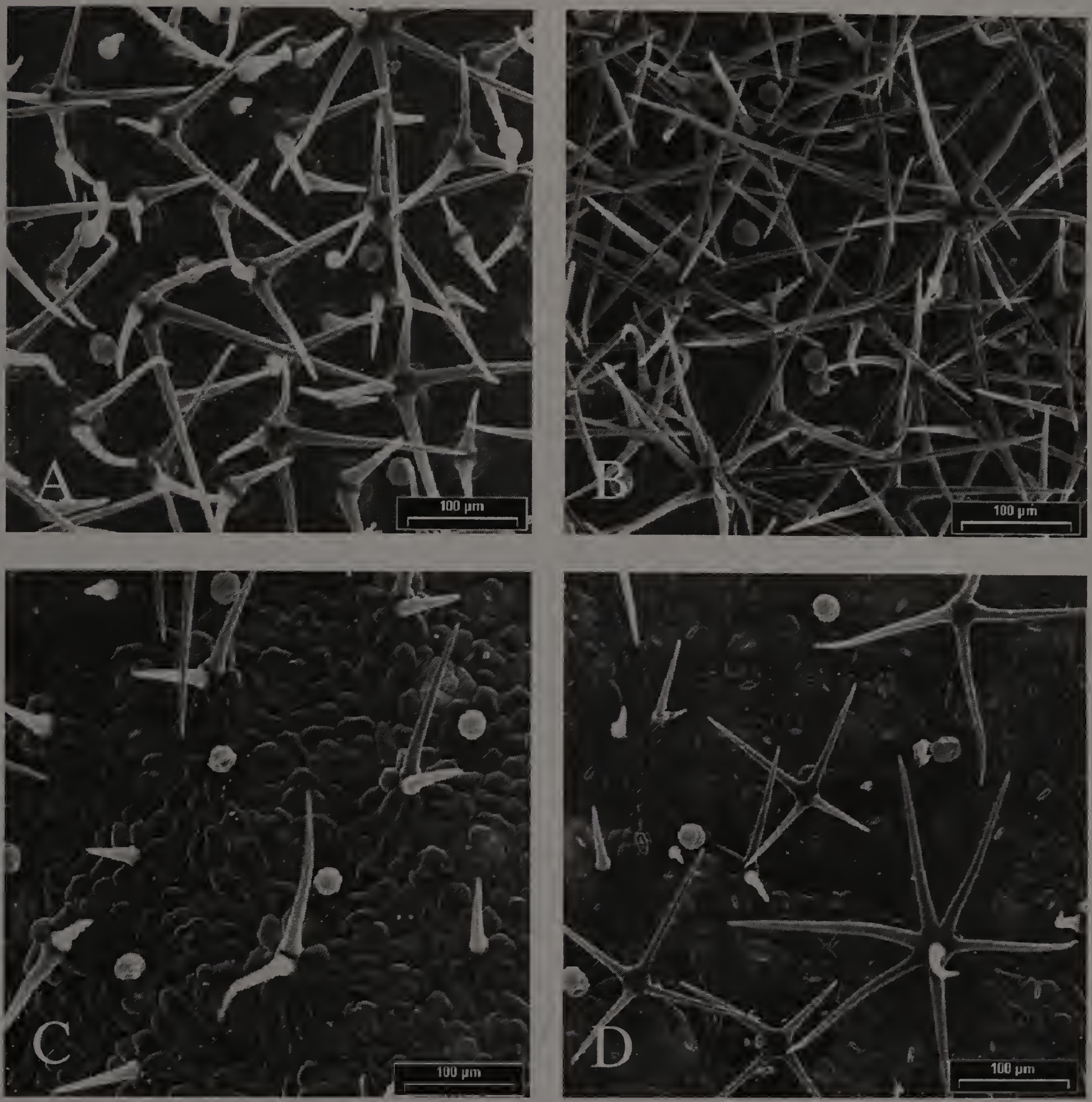

Figure 2.3 Scanning electron micrographs of velvetleaf leaf surface: (A) adaxial surface of young leaf (first or second fully expanded leaf from the apical meristem); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (fifth or sixth fully expanded leaf from the apical meristem); (D) abaxial surface of old leaf.

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 2.1, 2.2, and 2.3). In common lambsquarters and velvetleaf, the number of stomata in abaxial surfaces was significantly higher than adaxial leaf surfaces, whereas, common purslane had higher number of stomata in adaxial surfaces than abaxial (Figure 2.6). In all three weed species, the number of stomata per mm^2 of leaf area was higher in the younger leaves than the older leaves (Figure 2.6). Stomata were larger in size in common purslane (Figure 2.2) than in common lambsquarters (Figure 2.1) or velvetleaf (Figure 2.3), which may influence the stomatal infiltration of herbicides. The number of stomata per mm^2 in common purslane was significantly less than that in common lambsquarters or velvetleaf (Figure 2.6), 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 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.

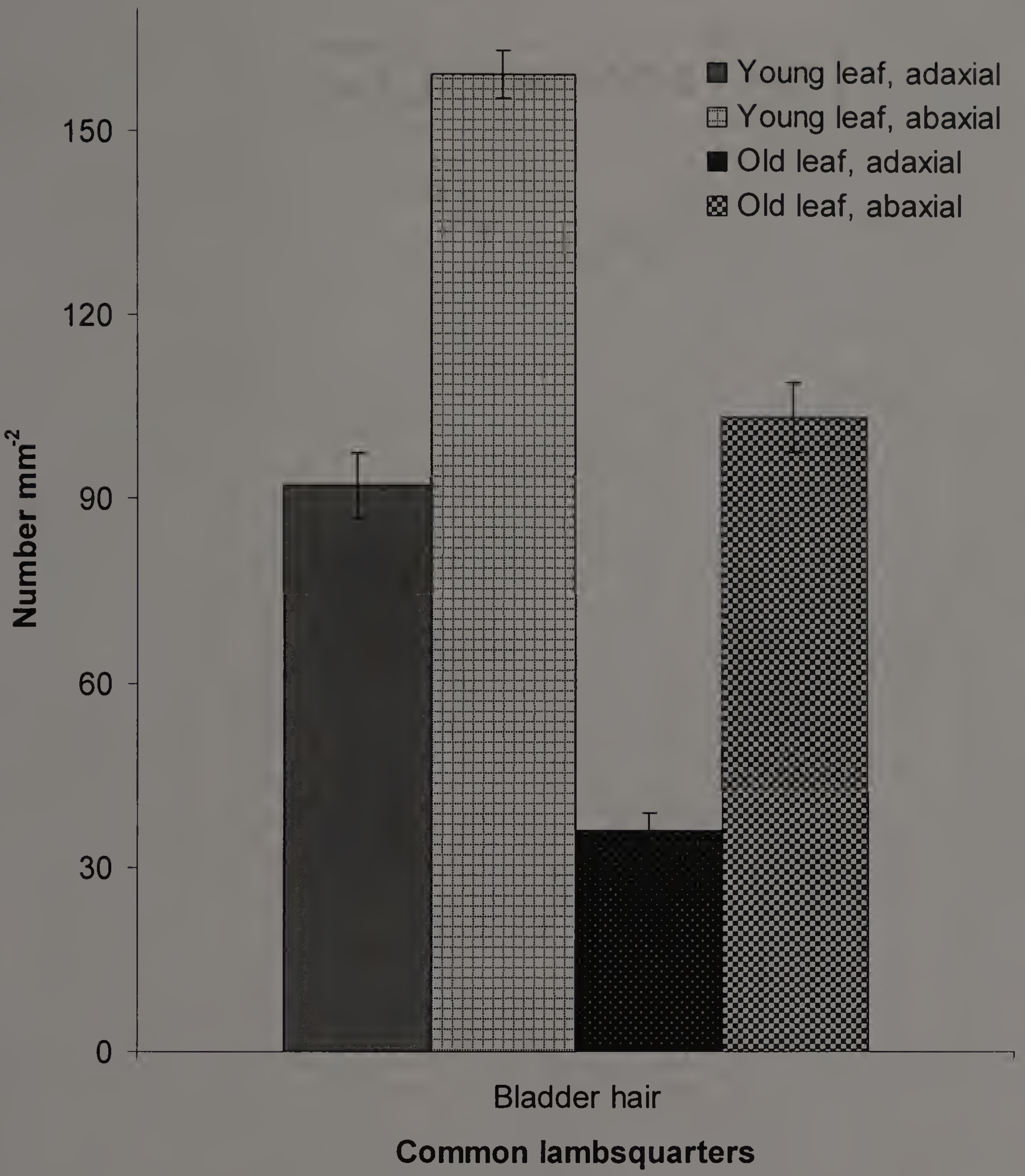

Figure 2.4 Number of bladder hairs on adaxial and abaxial leaf surfaces of young (first or second fully expanded leaf from the apical meristem) and old leaves (fifth or sixth fully expanded leaf from the apical meristem) of common lambsquarters. LSD (0.05) value was 13.51.

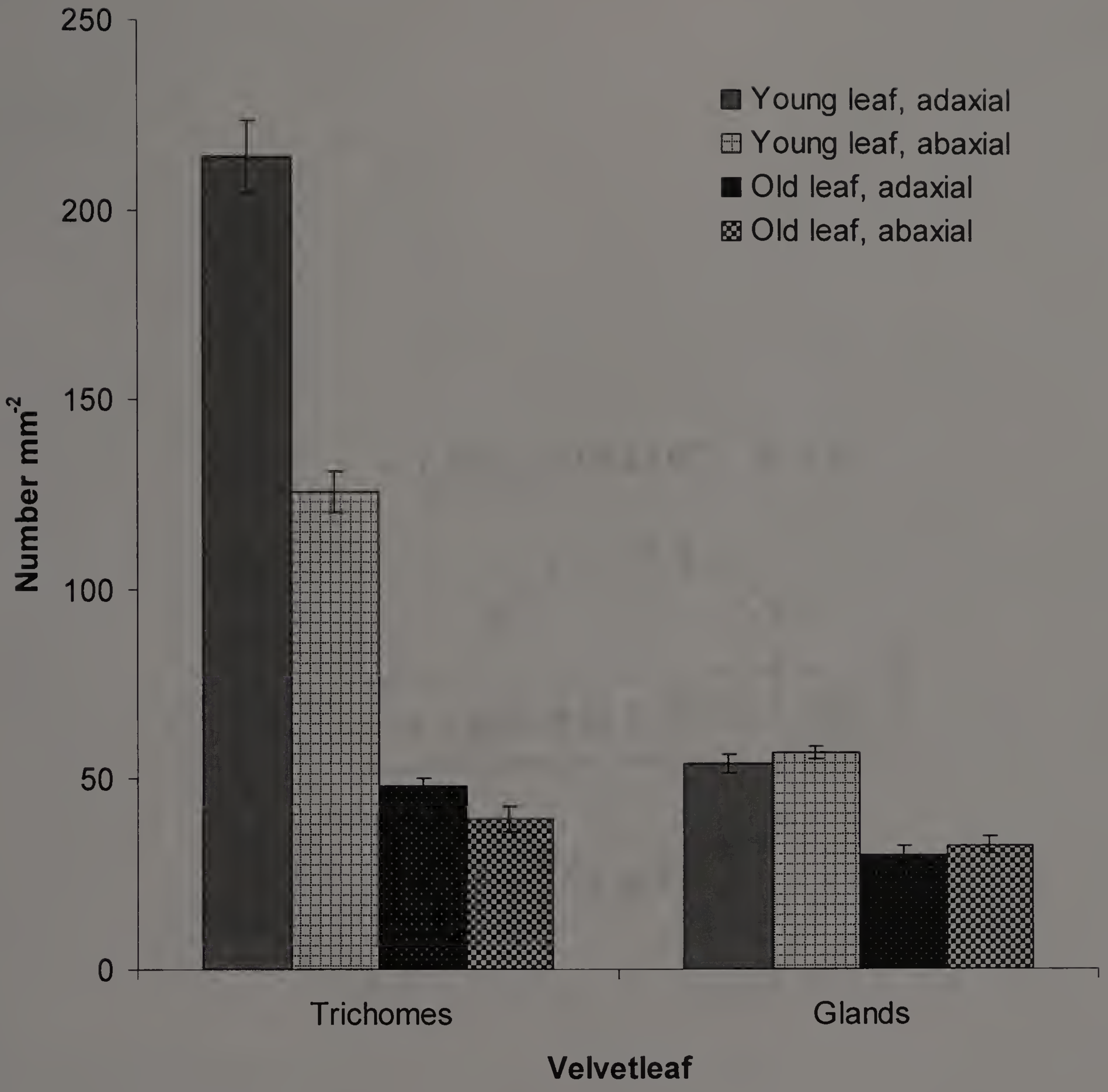

Figure 2.5 Number of glands and trichomes of adaxial and abaxial leaf surfaces of young (first or second fully expanded leaf from the apical meristem) and old (fifth or sixth fully expanded leaf from the apical meristem) leaves of velvetleaf. LSD (0.05) for trichomes 13.1, for glands 5.9.

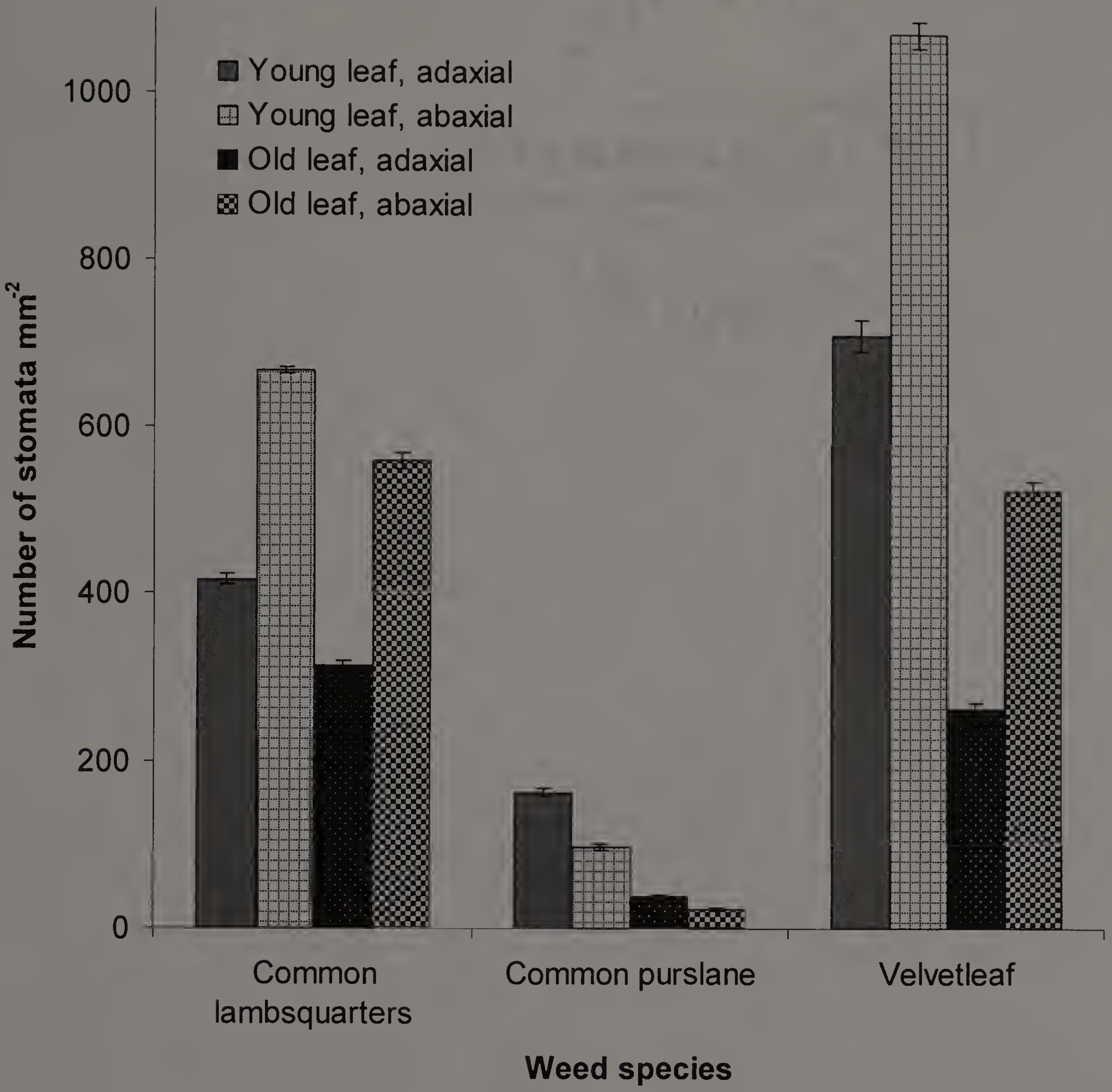

Figure 2.6 Number of stomata per unit area of adaxial and abaxial leaf surfaces of young (first or second fully expanded leaf from the apical meristem) and old (fifth or sixth fully expanded leaf from the apical meristem) leaves of common lambsquarters, common purslane, and velvetleaf. LSD (0.05) for species was 20.1, and for leaf age (young and old) and leaf surface (adaxial and abaxial) was 16.4.

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 μg cm^{-2}) and velvetleaf had the lowest (7.4 μg cm^{-2}) (Figure 2.7). Wax content of common purslane was 153.4 μg cm^{-2}. McWhorter (1993) reported that wax content varies from 10 to 200 μg cm^{-2} for most species. Wax mass above 300 μg 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

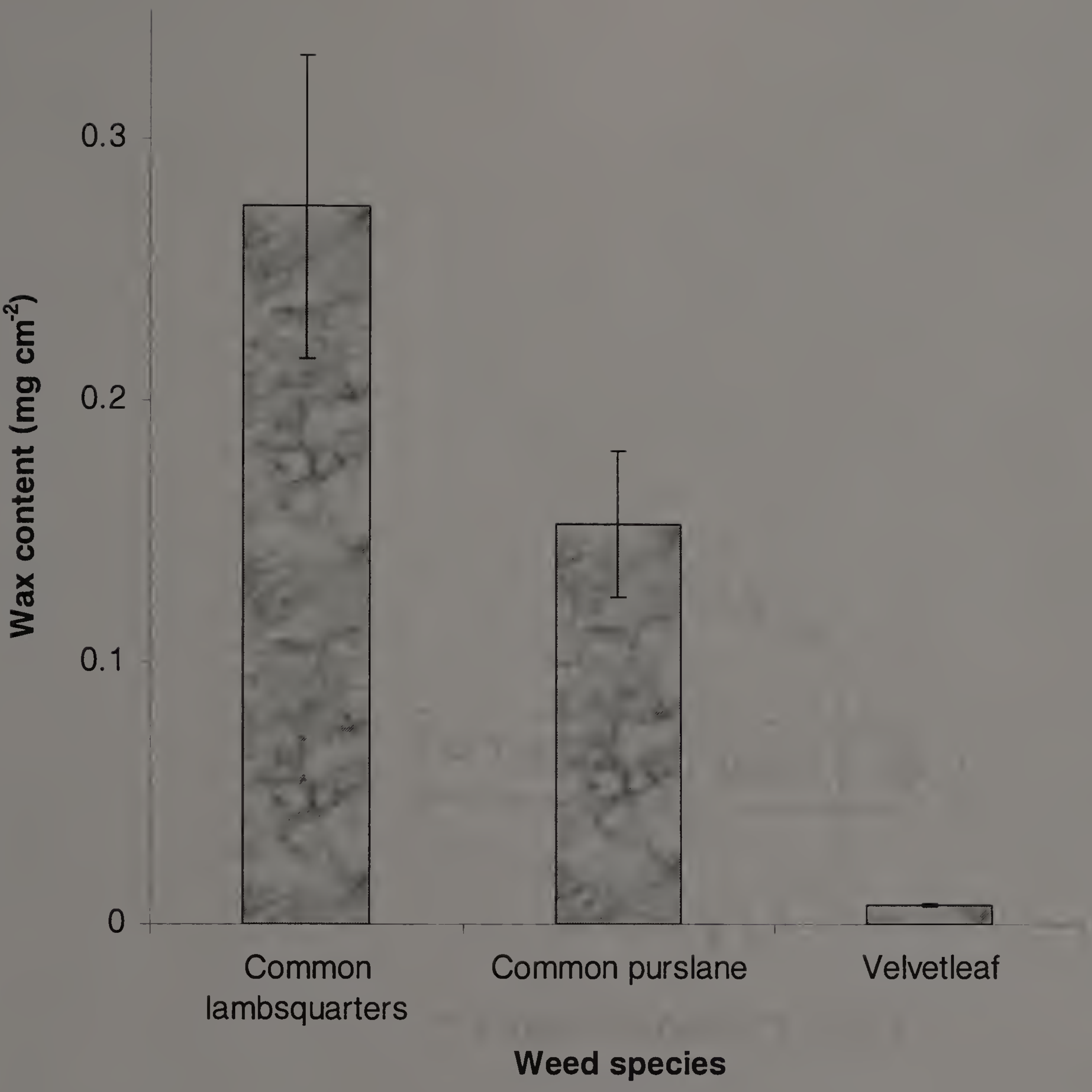

Figure 2.7 Wax content per unit of leaf area in common lambsquarters, common purslane, and velvetleaf. LSD (0.05) value was 0.25.

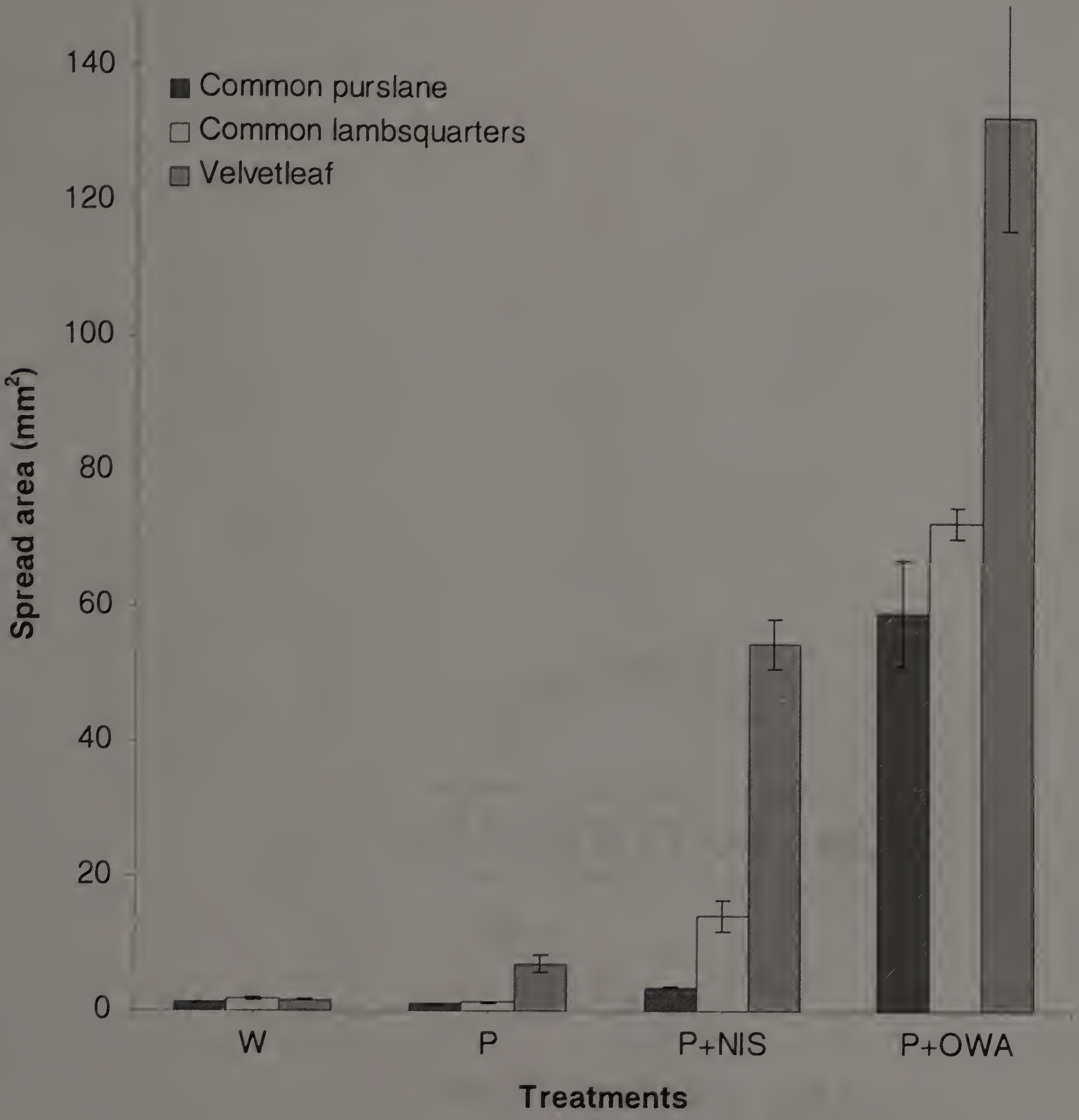

Figure 2.8 Spread area of 1µl droplets of distilled water (W), primisulfuron (P), primisulfuron with a nonionic surfactant (P+NIS), and primisulfuron with an organosilicone surfactant (P+OWA). Primisulfuron was used at 39.5 g ai ha^{-1}, nonionic surfactant at 0.25% (v/v), and the organosilicone surfactant at 0.1% (v/v). LSD (0.05) for species 7.9 and for treatments 9.2.

surfactant. The spread of primisulfuron droplets was higher on the leaf surface of velvetleaf than on leaves of common lambsquarters or common purslane (Figure 2.8). There was no variation in spread of 1 μl droplet of pure distilled water among three weed species (Figure 2.8). 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 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.

The number of stomata in abaxial 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 2.6). 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 in 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 surfactant 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 surfactant 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 a greater weed control.

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CHAPTER III

INFLUENCE OF LEAF SURFACE MICRO-MORPHOLOGY, WAX CONTENT, AND SURFACTANT ON PRIMISULFURON DROPLET SPREAD ON BARNYARDGRASS AND GREEN FOXTAIL[1]

Abstract

Leaf surface micro-morphology and wax content may affect the wetting and absorption behavior of foliar applied herbicides. Laboratory studies were conducted to (a) examine barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and green foxtail (*Setaria viridis* (L.) Beauv.) leaves, (b) quantify wax content per unit of leaf area, and (c) determine the spread area of primisulfuron droplets with and without surfactants on leaf surface of these species. Adaxial and abaxial leaf surfaces were examined using scanning electron microscopy and leaf wax was extracted and quantified. The spread of 1 µl 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 surfactant (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 mm^2 on the adaxial surface as compared to the abaxial, whereas, barnyardgrass had more stomata on the abaxial

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[1] This chapter has been submitted to Weed Science in November, 2005 for publication.

surface than on the adaxial. 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 surface than the abaxial surface of both young and old leaves. In barnyardgrass the number of trichomes was more on abaxial surface than adaxial. The mean values of the wax content per unit of leaf area in barnyardgrass and green foxtail were 35.91 μg cm^{-2} and 19.14 μg cm^{-2}, respectively. On both species primisulfuron droplets when applied with a nonionic surfactant had more spread area than that without a surfactant, and the spread was even larger with organosilicone surfactant. The spread of primisulfuron droplets was greater on the leaf surface of barnyardgrass than the spread on green foxtail when surfactant was added.

Introduction

Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) 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 production (Lopez-Martinez et al. 1999). VanDevender et al. (1997) reported that 20 barnyardgrass plants m^{-2} 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 [*Setaria viridis* (L.) P. Beauv.] is primarily a weed of cultivated cereals, vegetables, pulse crops, and can also be found commonly in pastures, turf, orchards, gardens, and other frequently disturbed sites (Holm et al. 1991; Douglas et al. 1985). 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 (*Oryza sativa* L.), sugar beet (*Beta vulgaris* L.), vineyards, and many other crops (Douglas et al. 1985; Gates 1941; Holm et al. 1991). This weed is also an alternate host for a number of insects, viruses, and nematodes that attack crops (Douglas et al. 1985; Gates 1941; Holm et al. 1991). Understanding the leaf morphology of these weeds is necessary to develop the most ecologically sound and economically sustainable weed management programs.

Leaf surface micro-morphology is an important feature in determining deposition and spread of spray droplets (Boize et al. 1976; Gudin et al. 1976). 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). It was demonstrated that the wetting and penetration behavior of foliar applied herbicides was affected by leaf characteristics (Hull et al. 1982; McWhorter 1985). Herbicide absorption is facilitated by either cuticular or stomatal infiltration (Hess 1985; Wanamarta and Penner 1989). 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 (4-(4-chloro-2-methylphenoxy)butanoic acid) by broad bean (*Vicia faba* L.) leaves (Kirkwood et al. 1982). Leaf surface micro-morphology of various weed species was previously studied by Harr et al. (1991) using the scanning electron micrographs. 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 two studied in this paper. As the leaf surface influences the spreading and subsequent absorption of the herbicidal compounds into the leaf tissue, a sound knowledge of the morphological and physico-chemical characteristics of the leaf surface will definitely help the professionals to better understand the behavior of a given herbicide on various weed species. This knowledge will also help in selection of a specific surfactant to enhance the herbicidal activity in an integrated weed management program.

The importance of postemergence herbicides has risen dramatically in the past decade. Underwood (2001) reported that 70% of U.S.-agrochemical market was occupied by herbicides and 50% of those were postemergence herbicides. Primisulfuron {2-[4,6-bis(difluoromethoxy)pyrimidin-2-ylcarbamoylsulfamoyl]benzoic acid} 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^{-1} of primisulfuron controlled quackgrass over 90% 6 weeks after treatment. With a single early postemergence application of primisulfuron at 40 g ai ha^{-1} was as effective in controlling quackgrass as a split application of 20 g ai ha^{-1} applied at the one- to three-leaf stage followed by a second application of 20 g ai ha^{-1} at four- to six-leaf stage (Bhowmik 1999). According to Tweedy and Kapusta (1995), primisulfuron at 40 g ha^{-1} controlled johnsongrass (*Sorghum halepense* L.) 85 to 100% from 1992 to 1994 and corn yield was more than double as compared to the control plots. According to the commercial label of 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 wirestem muhly (*Muhlenbergia frondosa* (Poir.) Fern.) control with methylated vegetable oil concentrate as compared to nonionic surfactant, whereas, in field experiments, primisulfuron (39 g ai ha^{-1}) 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 surfactants generally increased rimsulfuron [1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonyl)urea] 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. It is apparent from previous literature 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 of five- to six-leaf stage, grown under natural field condition at the USDA Southern Weed Science Research Unit farm, Stoneville, Mississippi. 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. Leaf samples were prepared for scanning electron microscopy using similar procedures as described by McWhorter et al. (1993). Leaf segments of approximately 20 mm^2 were fixed for 12 hours 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 (Balzers CPD 020, Balzers, 8 Sagamore Park Road, Hudson, NH 03051) and were mounted on aluminum stubs. Samples were gold-coated using a Sputter Coater (Hummer X, Anatech, Ltd., 5510 Vine Street, Alexandria, VA 22310) and examined under a scanning electron microscope [JEOL-JSM 840 (USA), 11 Dearborn Road, Peabody, MA 10960]. Leaf surfaces were photographed at 200X magnification for all species. The stomata, glands, and trichomes were studied and counted with four replications for each species and the study was repeated. All data were subjected to ANOVA. Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

Wax Content

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, Mississippi. 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 (Leaf Area Meter, model LI-3100, LI-COR, Inc., Lincoln, NE 68501). Wax extraction was done by immersing approximately 50 leaves for 30 seconds in 500 ml HPLC-grade chloroform in an ultra-sonicator (Branson 2210 Sonicator, Branson Ultrasonic Corporation, 41 Eagle Road, Dunbury, CT 06813-1961) at room temperature. The chloroform-wax solution was filtered using a fritted glass funnel apparatus with Durapore membrane filters (0.22 µm, GV series) (Millipore Corporation, 80 Ashby Road, Bedford, MA 01730), and the volume was reduced to approximately 20 ml in a rotary evaporator (Buchi R-124 Rotavapor, Buchi Analytical Inc. 19 Lukens Drive, New Castle, DE 19720). 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 days before recording the wax mass, to ensure complete dryness of the wax sample. Each treatment had three replications, and the experiment was repeated. All data were subjected to ANOVA. Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. 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 39.5 g ai ha^{-1} (without surfactant), primisulfuron with a nonionic surfactant (blend of alkyl aryl polyoxylkane ethers, free fatty acids, and dimothyl polysiloxane, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017) at 0.25% (v/v), and primisulfuron with an organosilicone surfactant (polyalkyleneoxide-modified heptamethyltrisiloxane 7.5 EO, 100%, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591) 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 minutes 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 horizontal and vertical dimensions of the droplet. Spread area measurements were replicated five times and the experiment was repeated. All data were subjected to ANOVA. Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. 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 3.1 and 3.2). In barnyardgrass, on adaxial leaf surface the epidermal cells are mostly elongated, whereas, the cells are mostly dome shaped on the abaxial surface (Figure 3.1). Epidermal cells of green foxtail are polygonal and on the abaxial surface cells are longer than cells on the adaxial surface (Figure 3.2). 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 3.1). In contrast, green foxtail had short, one celled, hook shaped prickles on both adaxial and abaxial surfaces (Figure 3.2).

In green foxtail the number of stomata per unit area was more on adaxial surface as compared to abaxial, whereas, the number of stomata was more on abaxial surface than adaxial in case of barnyardgrass (Figure 3.3). The analysis of variance showed that the effect of weed species and leaf age (young or old) was significant at 99% level of significance (Appendix, Table A.6). In both weed species the number of stomata per mm^2 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). Recently, it has been shown that uptake of anions (fluorescein) and cations (Fe^{3+}) in *Allium porrum* L., *Commelina communis* L., and *Sedum telephium* L. leaves was increased with stomatal aperture and stomatal density (Eichert and Burkhardt 2001). In *A. porrum* stomatal

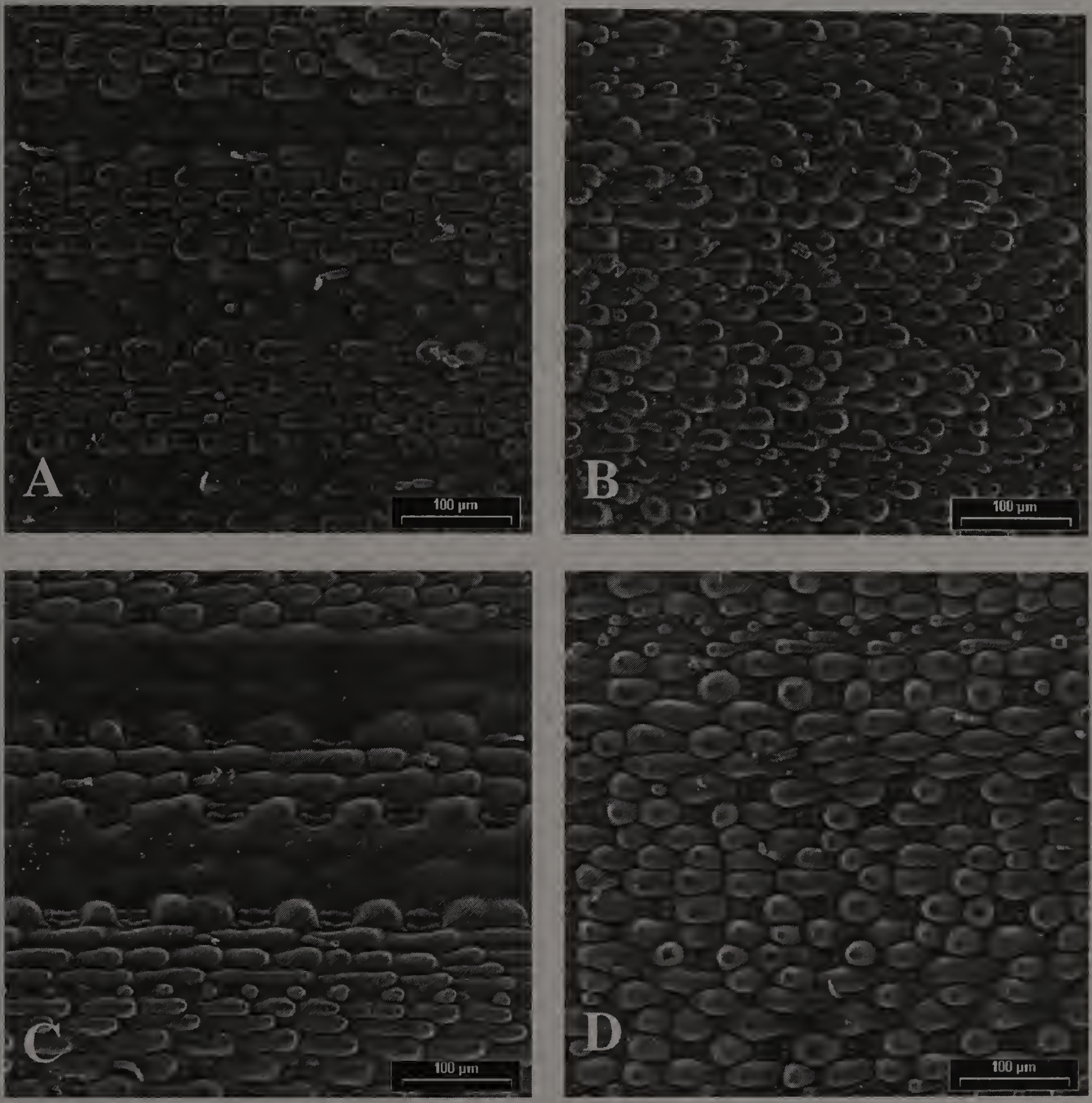

Figure 3.1 Scanning electron micrographs of barnyardgrass leaf surface: (A) adaxial surface of young leaf (first leaf from the tip); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (third or forth leaf from the tip); (D) abaxial surface of old leaf.

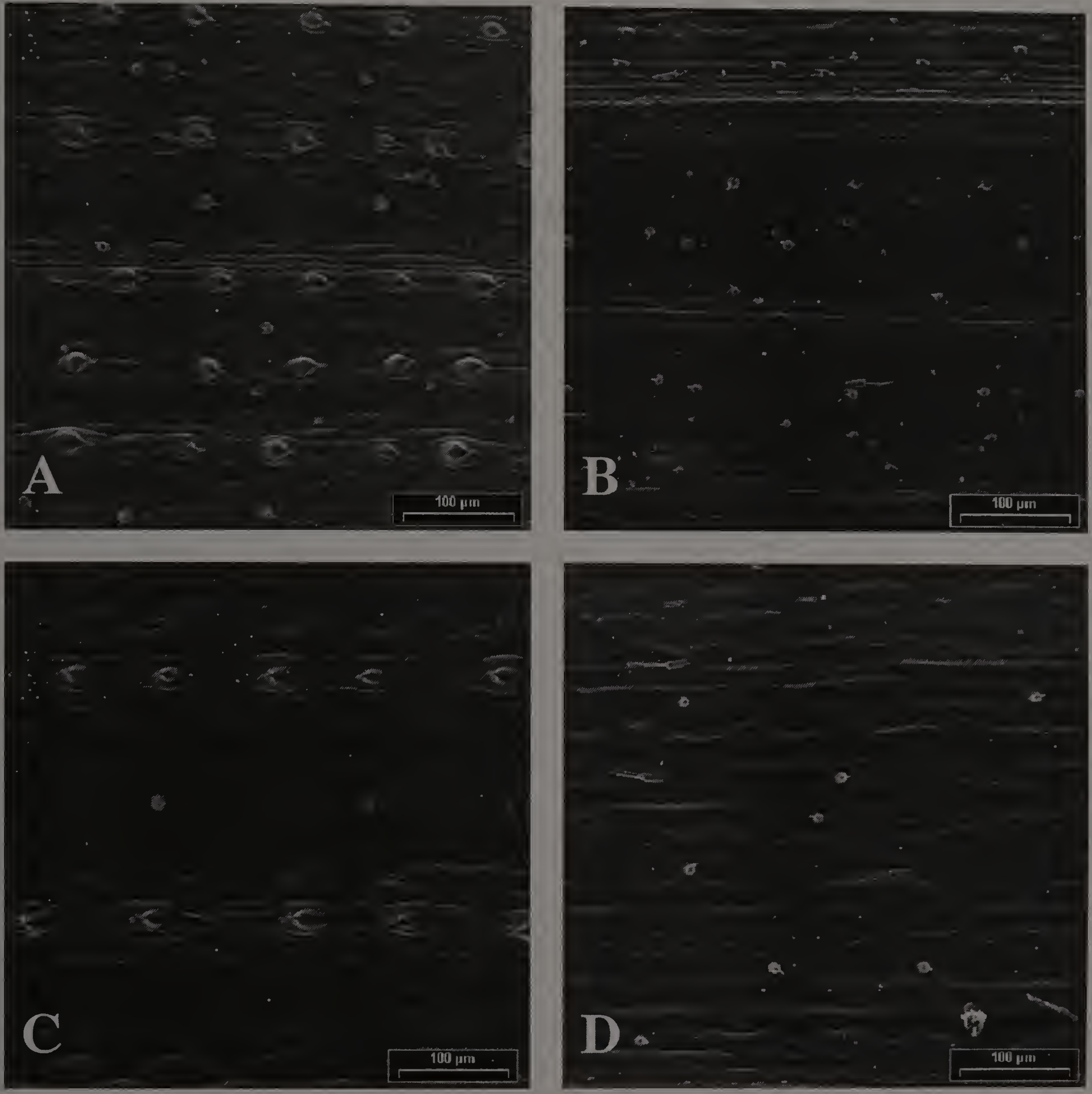

Figure 3.2 Scanning electron micrographs of green foxtail leaf surface: (A) adaxial surface of young leaf (first leaf from the tip); (B) abaxial surface of young leaf; (C) adaxial surface of old leaf (third or forth leaf from the tip); (D) abaxial surface of old leaf.

uptake of uranine was demonstrated for both intact leaves and epidermal peels (Eichert *et al.* 1998). 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 trichomes (Figures 3.1 and 3.2). There was no significant variation in the number of trichomes per unit leaf area of green foxtail (Figure 3.4). In case of barnyardgrass the number of trichomes was more on abaxial surface than adaxial (Figure 3.4) as observed in Johnsongrass leaves by McWhorter et al. (1993). Number of trichomes per mm^2 of leaf area was higher in the younger leaves as compared to the older leaves in both species. Our result showed that the effects of weed species, leaf age, and leaf surface were significant at 99% level of significance (Appendix, Table A.7). Trichomes act in a complex way in relation to spread of herbicide solution and adsorption of herbicide. Trichomes may lead to reduced wetting and spreading of droplets (Hull et al., 1982). Bicellular trichomes discharge a mucilage-type secretion, which contains callose, a carbohydrate component (β1, 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. In contrast, Benzing and Burt (1970) showed by fluorescent dyes that trichomes might provide a site of entry to the foliar applied herbicides.

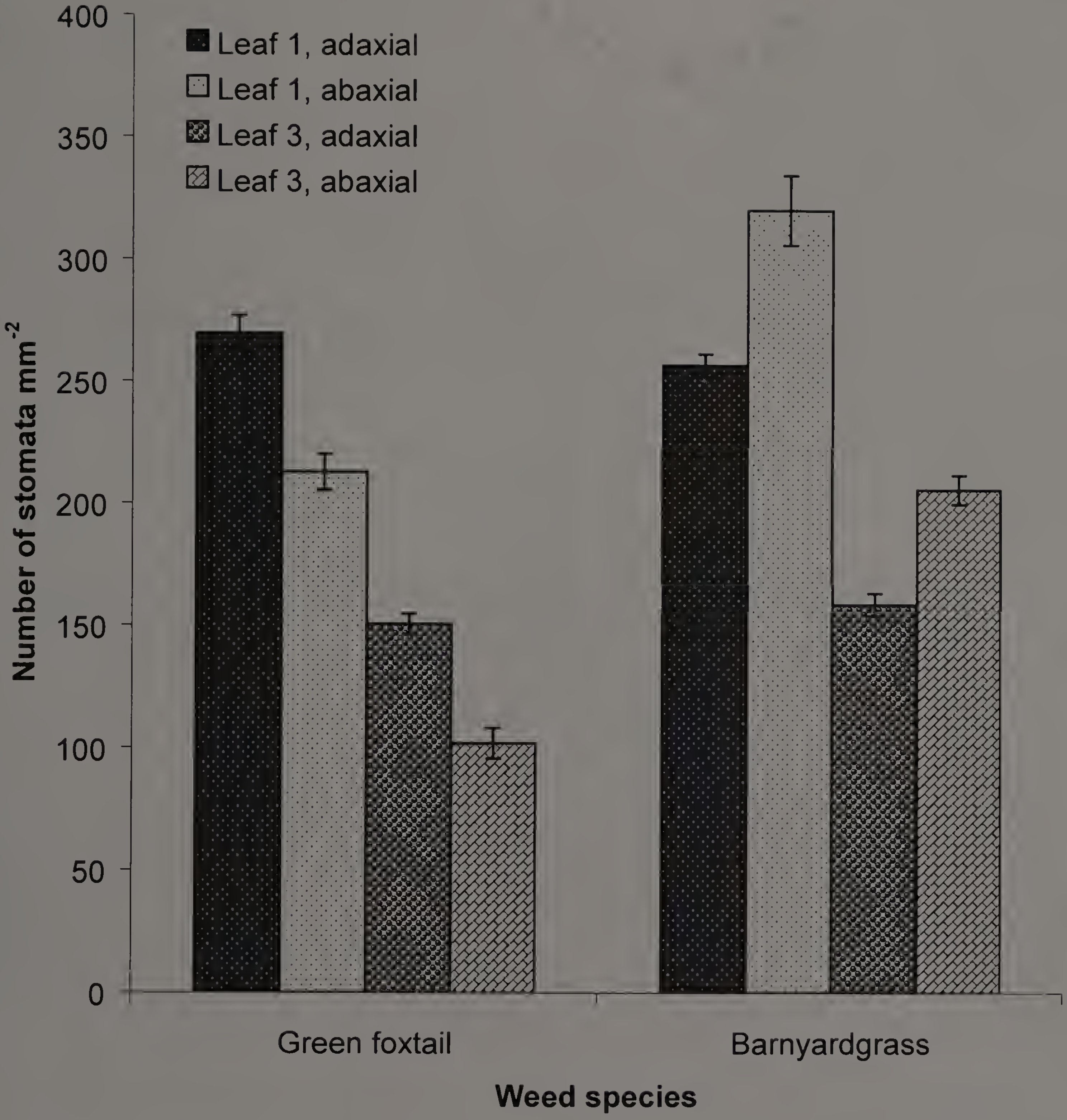

Figure 3.3 Number of stomata per unit area of adaxial and abaxial leaf surfaces of young (first leaf from the tip) and old (third or forth leaf from the tip) leaves of barnyardgrass and green foxtail. LSD (0.05) value was 10.63.

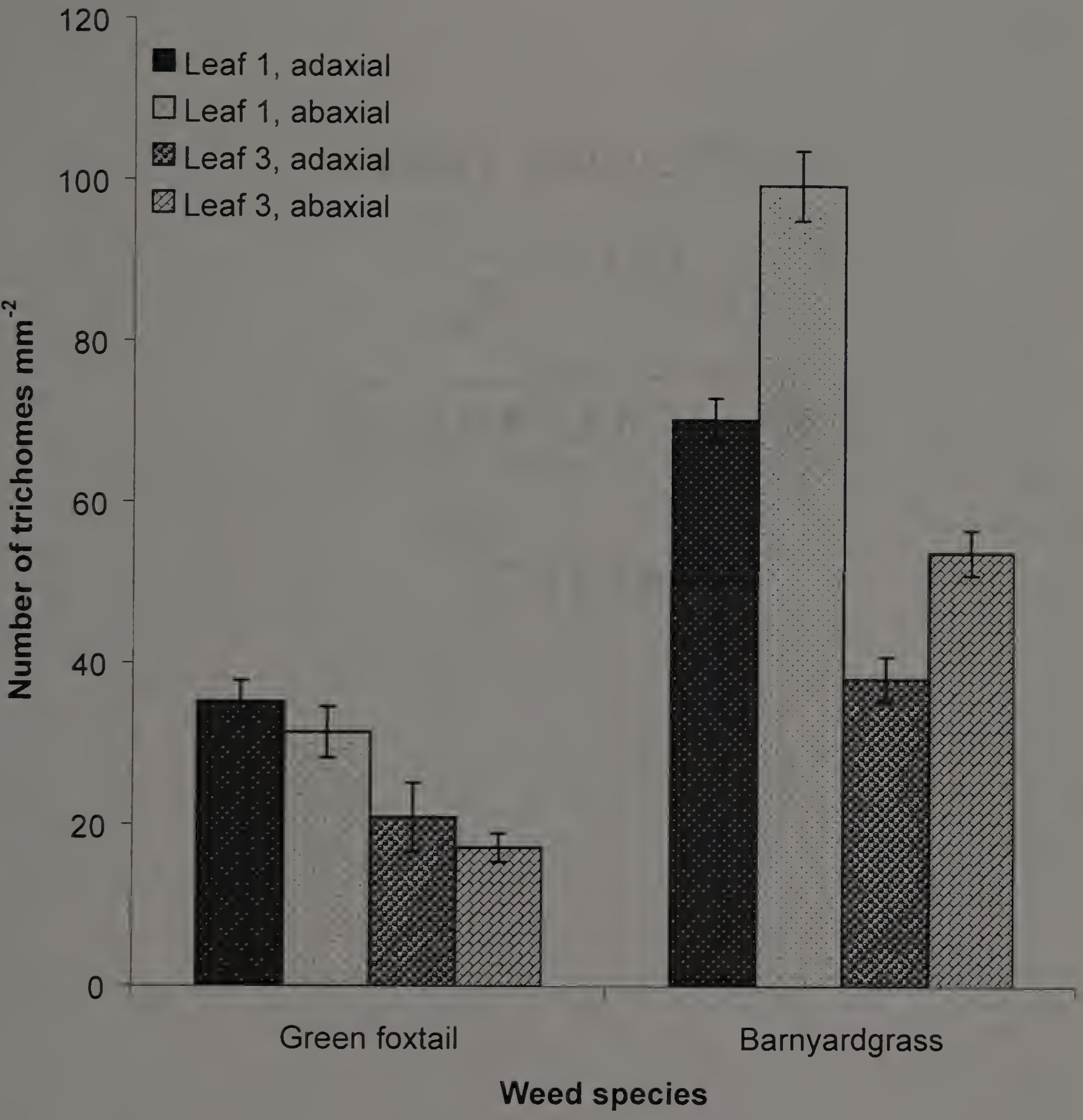

Figure 3.4 Number of trichomes on adaxial and abaxial leaf surfaces of young (first leaf from the tip) and old (third or forth leaf from the tip) leaves of barnyardgrass and green foxtail. LSD (0.05) value was 4.47.

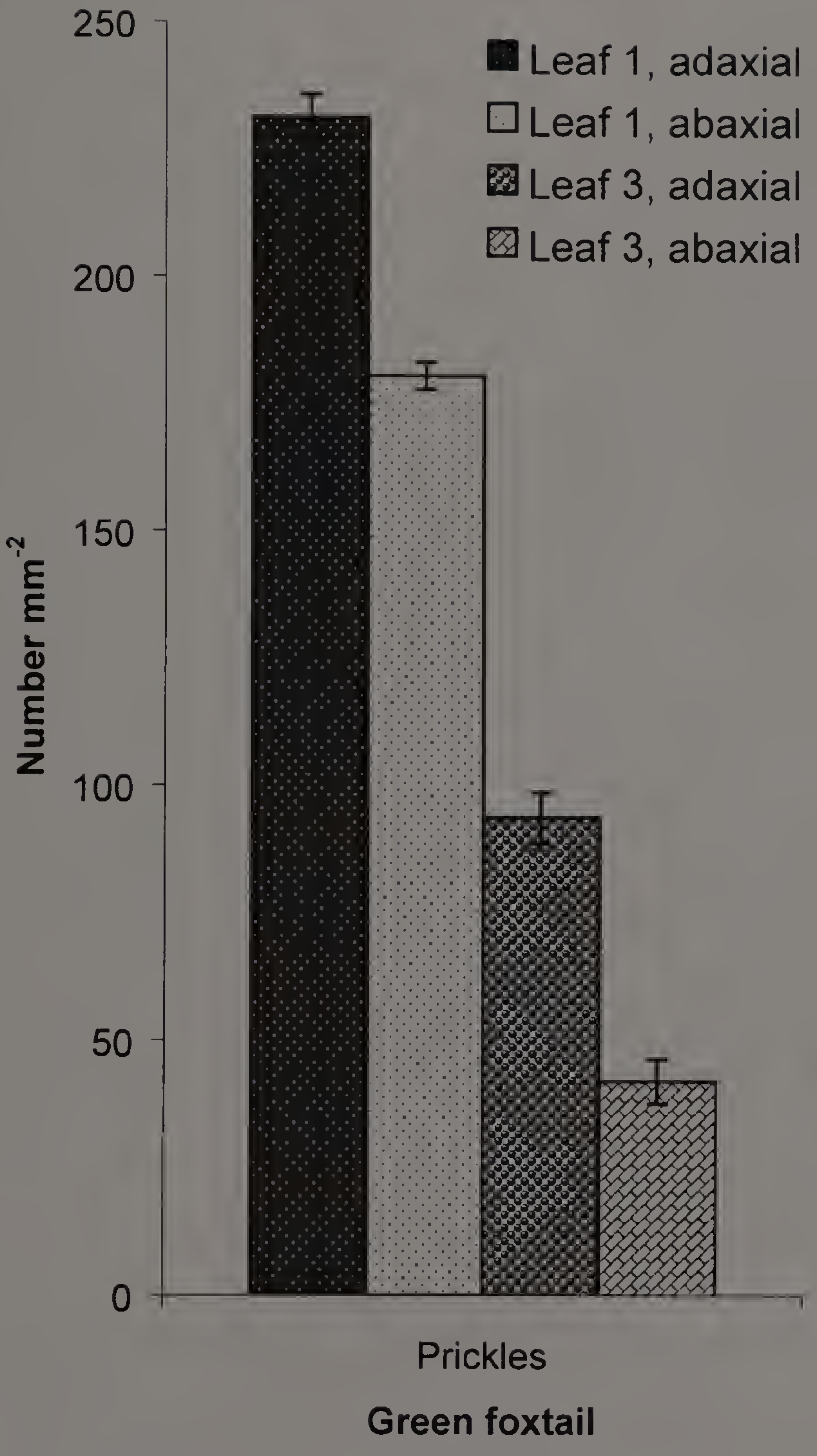

Figure 3.5 Number of prickles on adaxial and abaxial leaf surfaces of young (first leaf from the tip) and old leaves (third or forth leaf from the tip) of green foxtail. LSD (0.05) value was 8.54.

Green foxtail leaves had prickles on both surfaces, which are tough pointed structures that appear to be spines or barbs (Figure 3.2). 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 3.5). The size of individual prickle was also larger on adaxial leaf surface as compared to abaxial. Number of prickles per unit area was higher in young leaves as compared to the old leaves. Effect of leaf age and surface was significant at 99% level as indicated by the *P* values (Appendix, Table A.8). Prickles are heavily silicated structures often have wax crystals around the base which 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 micro-roughness of the leaf surface. Presence of short- and macro-prickles was reported in other grass species by McWhorter et al. (1993). Scanning electron micrographs of leaf surfaces of these weed species were previously demonstrated by Harr et al. (1991), though, quantitative information on number of stomata, trichomes, and prickles and comparative study of abaxial and adaxial surfaces of young and old leaves of the above two weed species were lacking in literature.

Wax Content

The mean value of the wax content per unit of leaf area in barnyardgrass and green foxtail were 35.91 μg cm^{-2} and 19.14 μg cm^{-2}, respectively (Figure 3.6). The *P* value in the analysis of variance shows that the difference was not statistically significant at 95% level of significance (Appendix, Table A.9). For most species wax content varies from 10 to 200 μg cm^{-2} (McWhorter 1993). Wax mass above 300 μg cm^{-2} was reported by Baker (1982). Hull (1970) reported that the amount of wax produced by the plant is

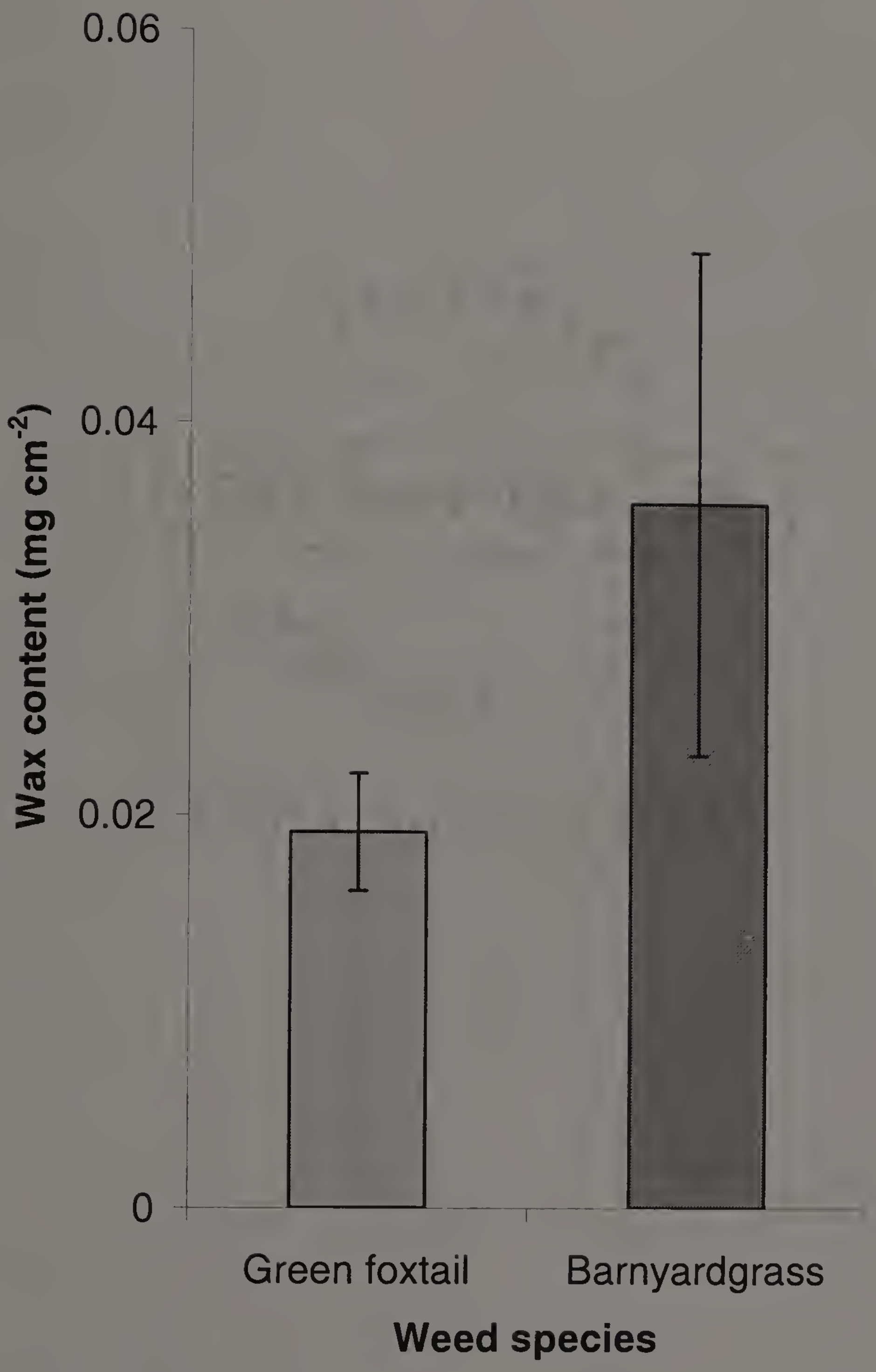

Figure 3.6 Wax content per unit of leaf area in barnyardgrass and green foxtail. LSD (0.05) value was 0.03.

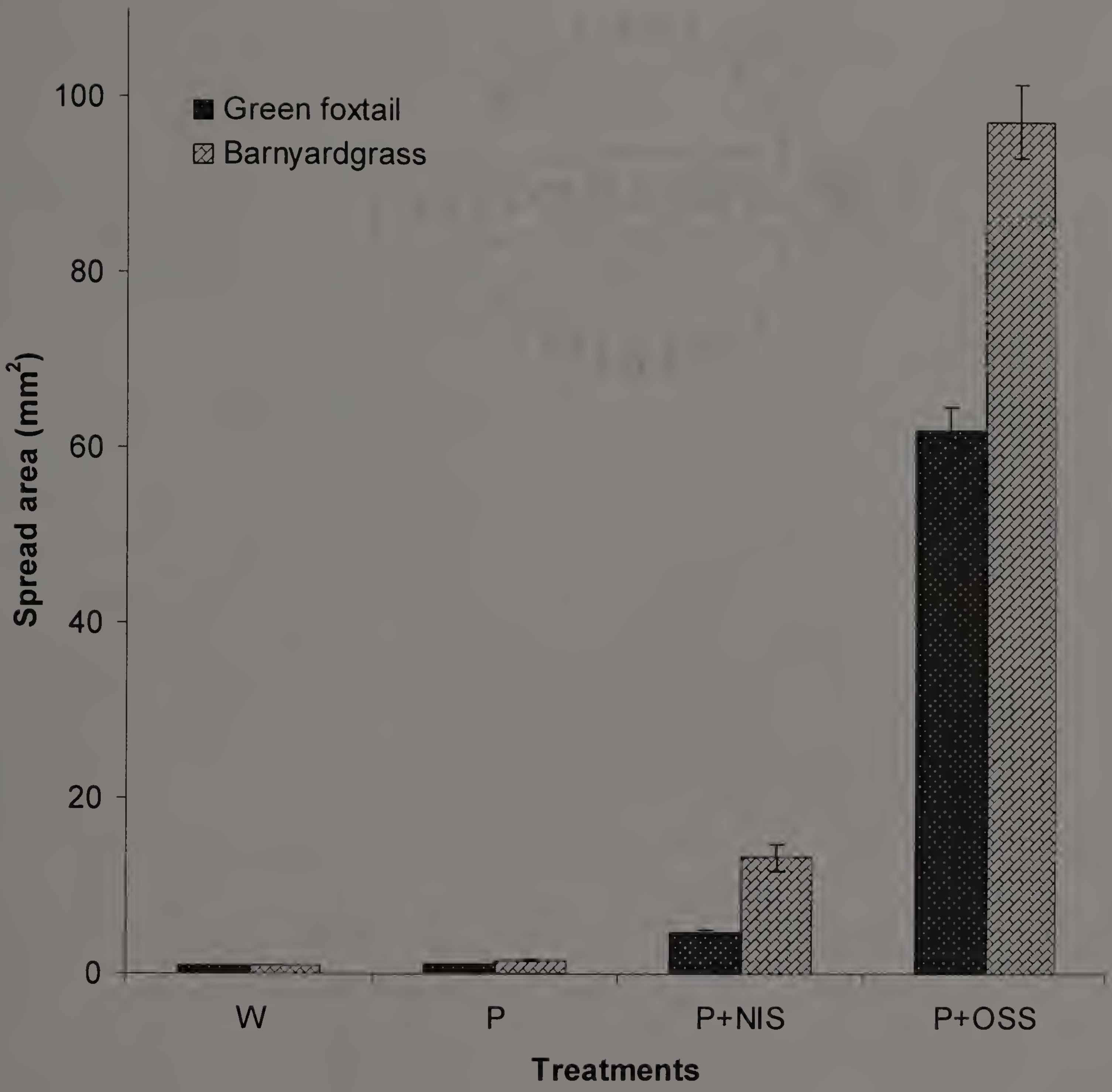

Figure 3.7 Spread area of 1 μl droplets of distilled water (W), primisulfuron (P), primisulfuron with a nonionic surfactant (P+NIS), and primisulfuron with an organosilicone surfactant (P+OSS). Primisulfuron was used at 39.5 g ai ha^{-1}, nonionic surfactant at 0.25% (v/v), and the organosilicone surfactant at 0.1% (v/v). LSD (0.05) for species 2.61 and for treatments 3.69.

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. 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.

Spread Area of Primisulfuron Droplets

There were no significant variations between spread of 1 μl droplet of primisulfuron (without surfactant) and distilled water on both the species (Figure 3.7). Primisulfuron with a nonionic surfactant had more spread area than that without a surfactant, and the spread was even greater with organosilicone surfactant on both barnyardgrass and green foxtail (Figure 3.7). There was no variation in spread of 1 μl 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 3.7). Weed species, surfactant treatments, and their interactions, all significantly affected primisulfuron spread as determined by the *P* values in ANOVA (Appendix, Table A.10). Holloway (1970) also reported that the amount of wax had a positive correlation with herbicide absorption. These results indicate 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). 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).

Our results show that organosilicone with primisulfuron spreads the herbicide droplets better and covers more surface area on leaves, which may lead to higher absorption of primisulfuron into the leaf tissue resulting in effective weed control. These results also give basic support to the concept that the morphological and physico-chemical 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. Herbicide activity can be optimized by using specific surfactant.

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CHAPTER IV

EFFECT OF SURFACTANT ON PRIMISULFURON ACTIVITY ON COMMON LAMBSQUARTERS, COMMON PURSLANE, AND VELVETLEAF

Abstract

Laboratory and greenhouse studies were conducted to determine the contact angle and spread area of primisulfuron droplets (with and without surfactants) on the leaf surface of common lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.), and velvetleaf (*Abutilon theophrasti* Medicus), and to examine the activity of various rates of primisulfuron (with and without surfactants) in controlling these species. A nonionic surfactant (NIS) and an organosilicone surfactant (OSS) were used at 0.25 and 0.1% (v/v), respectively. The contact angles of 1-µl droplets were measured on the leaf surface using a goniometer. Primisulfuron rates were 0, 20, 40, 60 and 80 g ai ha^{-1}, where 40 g ai ha^{-1} was the manufacturer's suggested use rate. The activity of primisulfuron was assessed 3 wk after treatment (WAT) in terms of percent injury and fresh weight. Both NIS and OSS reduced contact angles of 1-µl primisulfuron droplets on the leaf surface of common lambsquarters, common purslane, and velvetleaf as compared to that of without surfactant. However, OSS resulted in lower contact angle than NIS when mixed with primisulfuron, on all weed species. Addition of surfactant increased the spread area on all three species and the increase was more drastic in case of common lambsquarters and velvetleaf as compared to common purslane. Between two surfactants, OSS resulted in a greater spread area as compared to NIS on all species. In general, activity of primisulfuron was higher on all three species when applied with surfactant as compared to the treatment without a surfactant. In common lambsquarters, there was no variation between OSS and NIS treatments applied with primisulfuron. In common purslane, highest injury and lowest fresh weight were obtained when OSS was added to primisulfuron followed by NIS. However, both surfactant treatments resulted in higher injury and lower fresh weight of common purslane as compared to the application of primisulfuron without surfactant. In velvetleaf, addition of surfactant increased injury and reduced fresh weight than the treatment without any surfactant, and primisulfuron at recommended rate (40 g ai ha^{-1}) resulted in lower fresh weight and higher injury when applied with OSS than with NIS. However, at higher rates (60 and 80 g ai ha^{-1}) of primisulfuron, there was no difference in velvetleaf injury between two surfactant treatments. The greater activity of primisulfuron with the surfactants may be related to the lower contact angle and higher spread area of the droplets on the leaf surface of these species.

Introduction

Common lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.), and velvetleaf (*Abutilon theophrasti* Medicus) are three of the most important weeds in the United States and can grow well in a variety of climates and soils. Damages caused by common lambsquarters interference have been well documented in field crops. In field corn (*Zea mays* L.) and sugarbeets (*Beta vulgaris* L.), yield reductions of 11 and 48%, respectively, have been associated with interference by this weed (Beckett et al. 1988; Schweizer 1983). In vegetable crops, season-long interference by common lambsquarters has resulted in 36% yield reductions in tomato (*Lycopersicon esculentum* Mill.) with 64 plants per meter of row (Bhowmik and Reddy 1988) and 65% yield reductions in lettuce (*Lactuca sativa* L.) with 16 plants per 6 meter row (Santos et al. 2004). Common purslane is a common weed in vegetables, annual flowers, field and sweet corn (*Zea mays* L.), and newly planted orchards (Mitich 1997). This weed has become naturalized as a weed in 45 crops in 81 countries (Mitich 1997). Common purslane matures quickly and produces seeds under a wide range of conditions (Zimmerman 1976). If conditions are favorable, seed production can be prolific and may reach over 240,000 seeds per plant (Miyanishi and Cavers 1980). Common purslane, because of its drought tolerance, has been able to survive mechanical cultivation and hand weeding. On moist soil, uprooted plants and plant fragments of common purslane are able to re-root themselves and resume growth (Connard and Zimmerman 1931). Velvetleaf is a major weed of corn, cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], sorghum [*Sorghum bicolor* (L.) Moench], and other crops in the eastern United States (Spencer 1984). This weed causes significant crop yield losses in many parts of the world (Sattin et al. 1992; Spencer 1984; Warwick and Black 1988). The competitive superiority of velvetleaf over crop and other weedy species has been attributed to canopy architecture and photosynthetic characteristics (Akey et al. 1990; Bazzaz et al. 1989; Regnier et al. 1988; Stoller and Wooley 1985). Velvetleaf is a prolific seed producer, and a single velvetleaf plant can produce more than 8,000 seed that may remain viable in the soil for 50 years or more (Spencer 1984).

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 post-emergence herbicides. Primisulfuron {methyl 2-[[[[[4,6-bis(difluromethoxy)-2-pyrimidinyl] amino] carbonyl] amino] sulfonyl] benzoate} is a selective postemergence herbicide for the control of certain broadleaf weeds and grasses. Bhowmik (1995) reported that rates from 15 to 30 g ai ha^{-1} of primisulfuron controlled quackgrass over 90% 6 weeks after treatment. According to Tweedy and Kapusta (1995), primisulfuron at 40 g ha^{-1} controlled johnsongrass (*Sorghum halepense* L.) 85 to 100% from 1992 to 1994 and corn yield was more than doubled as compared to the control plots. Limited information on broadleaf weed control by primisulfuron is available. Sprague et al. (1999) reported that primisulfuron at 40 g ai ha^{-1} rate with 2% (v/v) crop oil concentrate had 73% control of Canada thistle (*Cirsium arvense* (L.) Scop.). The same rate of primisulfuron with a nonionic surfactant and 28% N resulted in 69% control of wild carrot (*Daucus carota* L.) seedling in greenhouse condition (Stachler and Kells 1997). Prostko et al. (1994) reported that primisulfuron reduced horsenettle (*Solanum carolinense* L.) biomass by 68%. Efficacy of primisulfuron (40 g ai ha^{-1}) on velvetleaf control was reduced by 22% when applied in combination with atrazine at 1.7 kg ai ha^{-1} (Hart and Penner 1993). Information on the activity of primisulfuron in controlling common purslane is lacking in the literature.

To achieve weed control by post-emergence herbicides, the herbicides have to come in contact and retain on the leaf surface prior to absorption into plant, able to reach the site of action, and finally induce phytotoxic responses. In general, most postemergence herbicides have been used with one of the surfactant types such as nonionic surfactants (NIS), crop oil concentrates (COC), nitrogen-surfactant blends, esterified seed oils, or organosilicones. Surfactant reduces the contact angle (θ) and increases spread area by reducing surface tension of the spray solution, which ultimately results in more efficient weed control. Nandula et al. (1995) reported that primisulfuron provided greater wirestem muhly (*Muhlenbergia frondosa* (Poir.) Fern.) control with methylated vegetable oil concentrate as compared to nonionic surfactant, whereas, primisulfuron provided greater control to itchgrass (*Rottboellia cochinchinensis* (Lour.) W.D. Clayton) when applied with nonionic surfactant than with an organosilicone or methylated seed oil blend (Strahan et al. 2000).

It was evident from previous literature that the effect of surfactant on herbicidal activity varies with herbicides and with weed species. There is a need for investigating the most effective surfactant for various postemergence herbicides in controlling specific weed species. The objectives of this study were to (1) measure the contact angle and spread area of primisulfuron droplets with and without surfactants on leaf-surface of common lambsquarters, common purslane, and velvetleaf, and (2) examine the activity of primisulfuron in controlling these species.

Materials and Methods

General Greenhouse Procedures

Seeds were purchased from a commercial source (Valley Seed Service, Fresno, CA 93791) and were stored in the refrigerator at 4 C before growing in the greenhouse. Seeds were grown in 10 cm^2 plastic pots having Hadley fine sandy loam (Typic

Udifluvents) with 3.5% organic matter and a pH of 6.5. The greenhouse had an average temperature of 20 ± 2 C with natural sunlight. Plants were thinned to 4 seedlings per pot for common purslane and 5 seedlings per pot for common lambsquarters and velvetleaf. Pots were watered as necessary to prevent wilting and were fertilized weekly with 10 ml solution of a soluble fertilizer (20N-5.2P-6.6K) per pot.

Contact Angle and Spread Area

Contact angle of 1-µl droplets of primisulfuron alone, with a nonionic surfactant (NIS) (Induce, blend of alkyl aryl polyoxylkane ethers, free fatty acids, and dimothyl polysiloxane, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017), and with an organosilicone surfactant (OSS) (Silwet L-77, polyalkyleneoxide-modified heptamethyltrisiloxane, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591) on leaves was measured on the adaxial surface of the fourth to sixth fully expanded leaves from the tip of the plants. NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. The leaves were collected daily just before measurement from five- to six-leaf stage plants. The contact angles of both sides of the 1-µl droplets on the leaf surfaces were measured using a goniometer (Contact Angle Goniometer, Rame-hart, Inc., 43 Bloomfield Avenue, Mountain Lakes, NJ 07046). The experimental design was completely randomized. Contact angle measurements were replicated four times, and the experiment was repeated. All data were subjected to ANOVA using the general linear model procedure (SAS 1992). Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

For the spread area measurement, the leaves of individual weed species were randomly collected daily prior to measurement from five- to six-leaf stage plants. The spread area of a 1 μl droplet of distilled water, primisulfuron at 40 g ai ha^{-1}, primisulfuron with NIS at 0.25% (v/v), and primisulfuron with OSS at 0.1% (v/v) was measured on the adaxial surface of fourth to sixth fully expanded leaves (from the tip of the plants) of common lambsquarters, common purslane and velvetleaf. Spread area was calculated using the formula πr^2, where r was the radius of the droplet. As the spread area of a 1 μl droplet was rarely circular, the radius was estimated as the mean of horizontal and vertical dimensions of the droplet. Spread area measurements were replicated four times and the experiment was repeated. All data were subjected to ANOVA using the general linear model procedure (SAS 1992). Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

Primisulfuron Activity

Commercial formulation of primisulfuron (Beacon, Syngenta Crop Protection, Inc., Greensboro, NC 27419) (75% WG) was used. Primisulfuron rates were used as 2X, 1.5X, 1X, and 0.5X, where 1X represented the manufacturer's suggested use rate (40 g ai ha^{-1}). All rates of primisulfuron were applied alone, with NIS or OSS. NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. An untreated control was included in each experiment for the comparison. Spray solutions were applied using a CO_2-backpack sprayer with Teejet XR 11004 VS nozzles at 152 kPa using a spray volume of 190 L ha^{-1}. Primisulfuron was applied to common purslane at three- to four- pair leaf stage and to common lambsquarters and velvetleaf at five- to six-leaf stage. The activity of primisulfuron was assessed 3 wk after treatment (WAT) in terms of percent injury and fresh weight. Percent injury was assessed by visual rating on a 0 to100% scale, where 0 = no injury and 100 = completely dead plants. At 3 WAT, plant shoots were clipped at the soil surface and the fresh weights were determined. The experimental design was completely randomized. Treatments were replicated three times, and the experiment was repeated. All data were subjected to ANOVA using the general linear model procedure (SAS 1992). Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

Results and Discussion

Contact Angle and Spread Area

Prmimisulfuron droplet had largest contact angle when applied without surfactant on common lambsquarters leaf surface (Figure 4.1). Addition of surfactants had significant effect on contact angle of primisulfuron droplets (Table A.11). Both NIS and OSS reduced contact angles of 1-µl primisulfuron droplets on the leaf surface of common lambsquarters, common purslane, and velvetleaf, as compared to that of without a surfactant (Figure 4.1). However, OSS when mixed with primisulfuron resulted in lower contact angle than NIS on all three weed species.

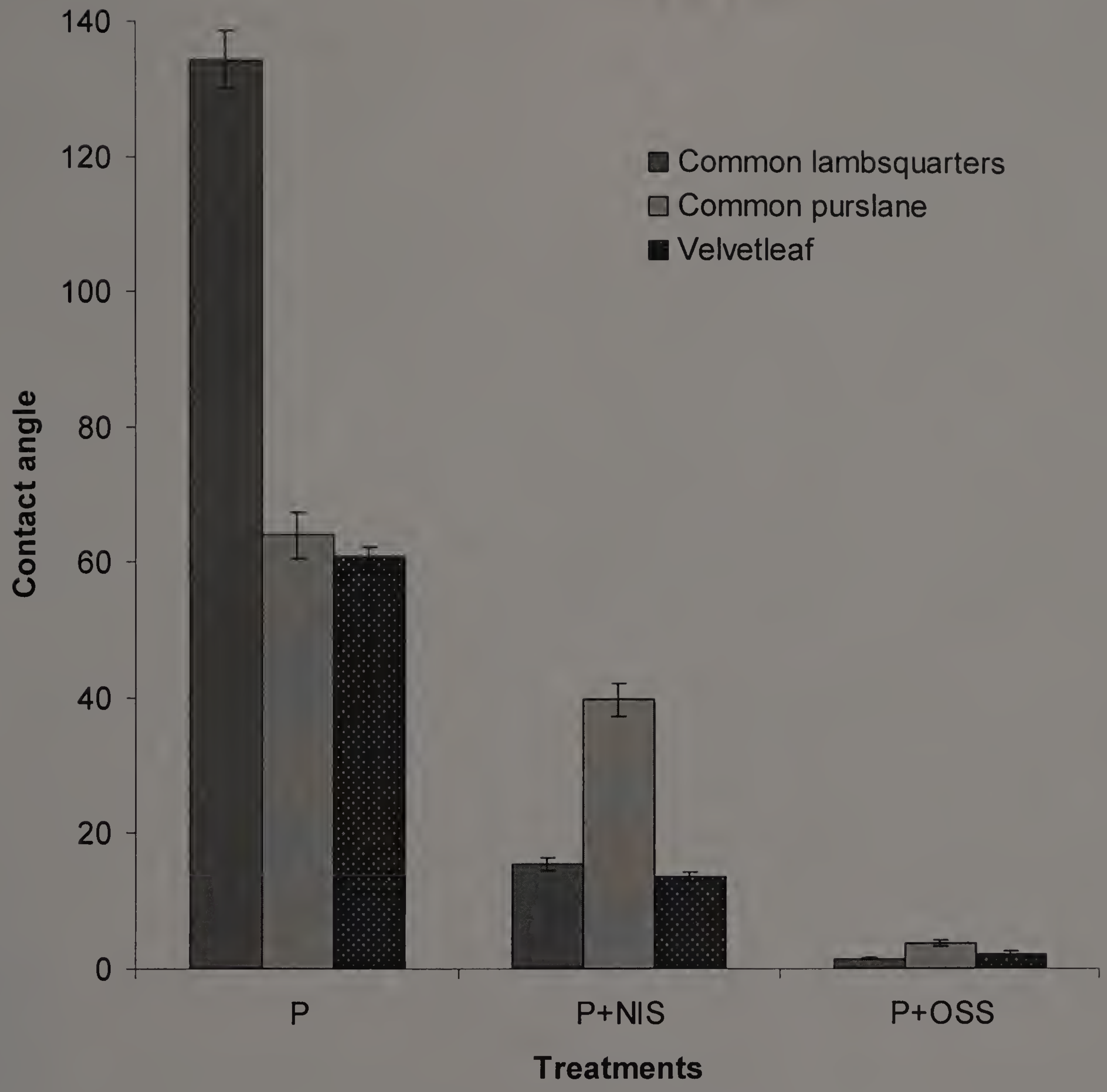

Figure 4.1 Contact angle of 1µl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of common lambsquarters, common purslane, and velvetleaf. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v). LSD value was 3.42.

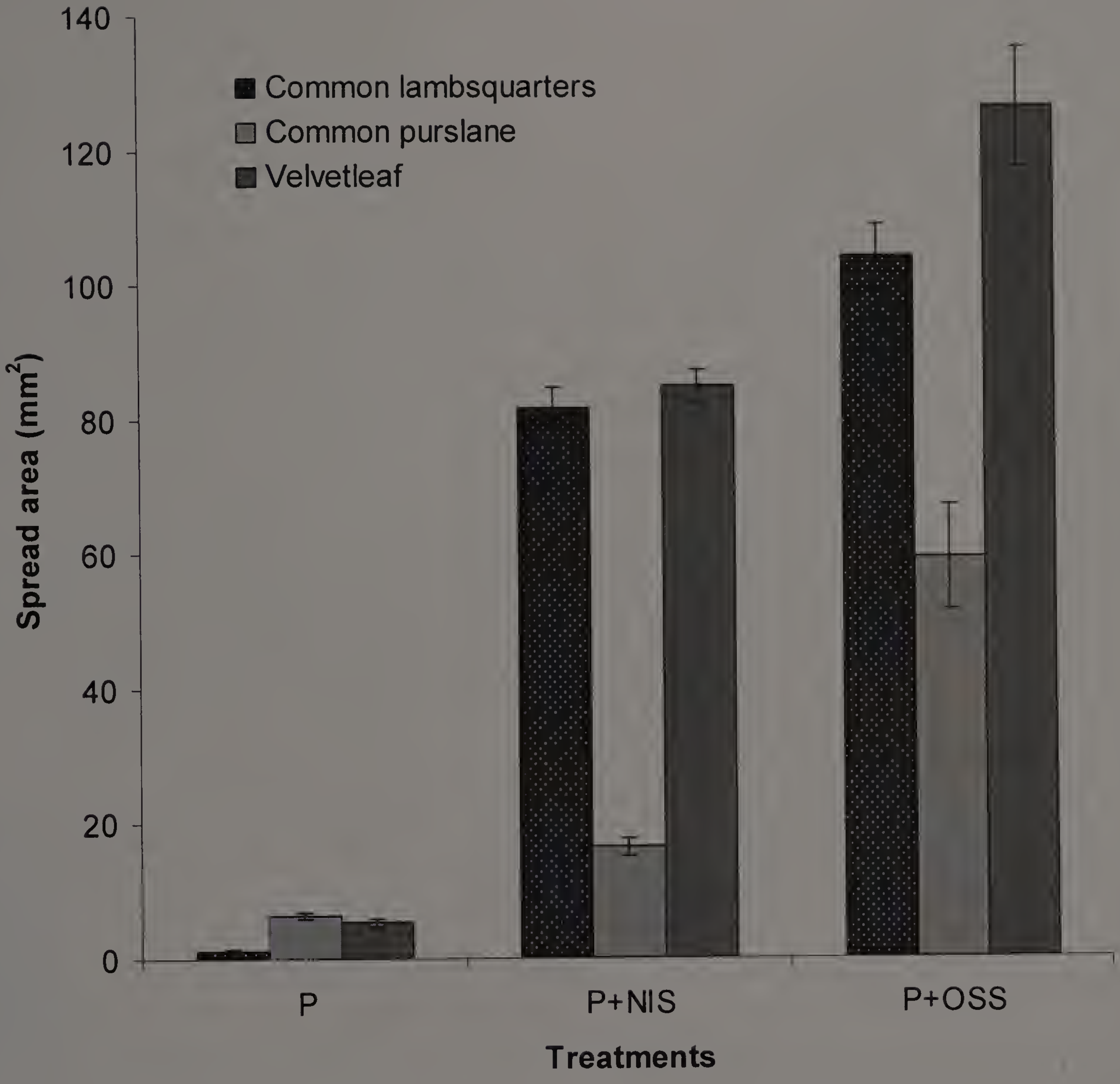

Figure 4.2 Spread area of 1 µl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of common lambsquarters, common purslane, and velvetleaf. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v). LSD value was 7.32.

Surfactant had significant effect on the spread area of primisulfuron droplets on common lambsquarters, common purslane, and velvetleaf (Table A.12). Addition of surfactants increased the spread area on all three weed species and the droplet spread was more in case of common lambsquarters and velvetleaf as compared to common purslane (Figure 4.2). Between two surfactants, OSS resulted in larger spread area as compared to NIS on all species (Figure 4.2).

Our data shows that the organosilicone reduces the contact angle and increases the spread area of herbicide droplets more than NIS, on the weed species studied here, which agrees with the findings of Wells (1989) and Knight and Kirkwood (1991). The addition of surfactants reduces the surface tension and contact angle of herbicide droplets, thereby improving the coverage and increasing the chance for a herbicide to penetrate into the plant tissue (Foy and Smith 1965; Kocher and Kocur 1993; Singh et al. 1984).

Higher leaf wax content may have been a probable reason for higher contact angle and lower spread area of herbicide droplets on the leaf surface. In general, leaf wax content and the spread area of the spray droplet are inversely related, as described by Chachalis et al. (2001a, 2001b). Composition, physical structure, and orientation of leaf wax also play important roles in this regard (Juniper 1960; Whitehouse et al. 1982).

Previous research has shown that trichomes or hairs also cause reduced contact of herbicide droplets with the leaf surface by creating air pocket if it is densely spaced (Hess et al. 1974; Hull et al. 1982). In the present study trichomes in velvetleaf and bladder hairs in common lambsquarters could be probable reason of lower contact angle and higher spread on velvetleaf and common lambsquarters, when applied with a surfactant. Similar results were reported by McWhorter et al. (1993) where the spread of oil droplets was better in abaxial surface of Johnsongrass leaf, which had 17% more trichomes than the adaxial surface. Surfactant reduces the surface tension of the solution and the droplets overcome the air-pocket barrier.

Primisulfuron Activity

Primisulfuron rates and surfactants had significant effect on percent injury and fresh weight of common lambsquarters as determined by the *P* values for each of the variables (Table 4.1). At 40 g ai ha^{-1} primisulfuron injured common lambsquarters only 26% when applied without a surfactant, whereas, 55 and 57% injury occurred when applied with NIS and OSS, respectively (Figure 4.3). There was no difference between two surfactant treatments in terms of percent injury of common lambsquarters at all rates of primisulfuron. Both surfactants reduced the fresh weight of common lambsquarters as compared to the treatment without any surfactant (Figure 4.4). The fresh weight was sharply reduced from 0 to 40 g ai ha^{-1} rate when applied with a surfactant, whereas, from 40 to 80 g ai ha^{-1} fresh weight reduction was not significant (Figure 4.4). No differences in fresh weights were observed between two surfactant treatments at 40 g ai ha^{-1} or higher rates of primisulfuron (Figure 4.4).

Surfactant type and primisulfuron rate significantly affected percent injury and fresh weight of common purslane (Table 4.1). At all rates of primisulfuron (from 20 to 80 g ai ha^{-1}) treatments with OSS resulted in highest injury and lowest fresh weight followed by treatments with NIS (Figure 4.5 and 4.6). There were no differences in fresh weights between the treatment without surfactant and with NIS at 0, 20, and 40 g ai ha^{-1} rate of primisulfuron (Figure 4.6).

Table 4.1 Summary of ANOVA for effect of surfactant types and primisulfuron rates on common lambsquarters, common purslane and velvetleaf.

	Source[1]	Common lambsquarters	Common purslane	Velvetleaf
		P values		
Percent injury	S	<0.0001	<0.0001	<0.0001
	H	<0.0001	<0.0001	<0.0001
	S*H	<0.0001	<0.0001	<0.0001
Fresh weight	S	<0.0001	<0.0001	<0.0001
	H	<0.0001	<0.0001	<0.0001
	S*H	<0.0001	<0.0001	<0.0001

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[1] Source of variations was surfactant types (without surfactant, with nonionic surfactant, and with organosilocone surfactant) and primisulfuron rates (0, 20, 40, 60 and 80 g ai ha^{-1}). Surfactant types, primisulfuron rates, and their interactions were denoted by S, H, and S*H, respectively.

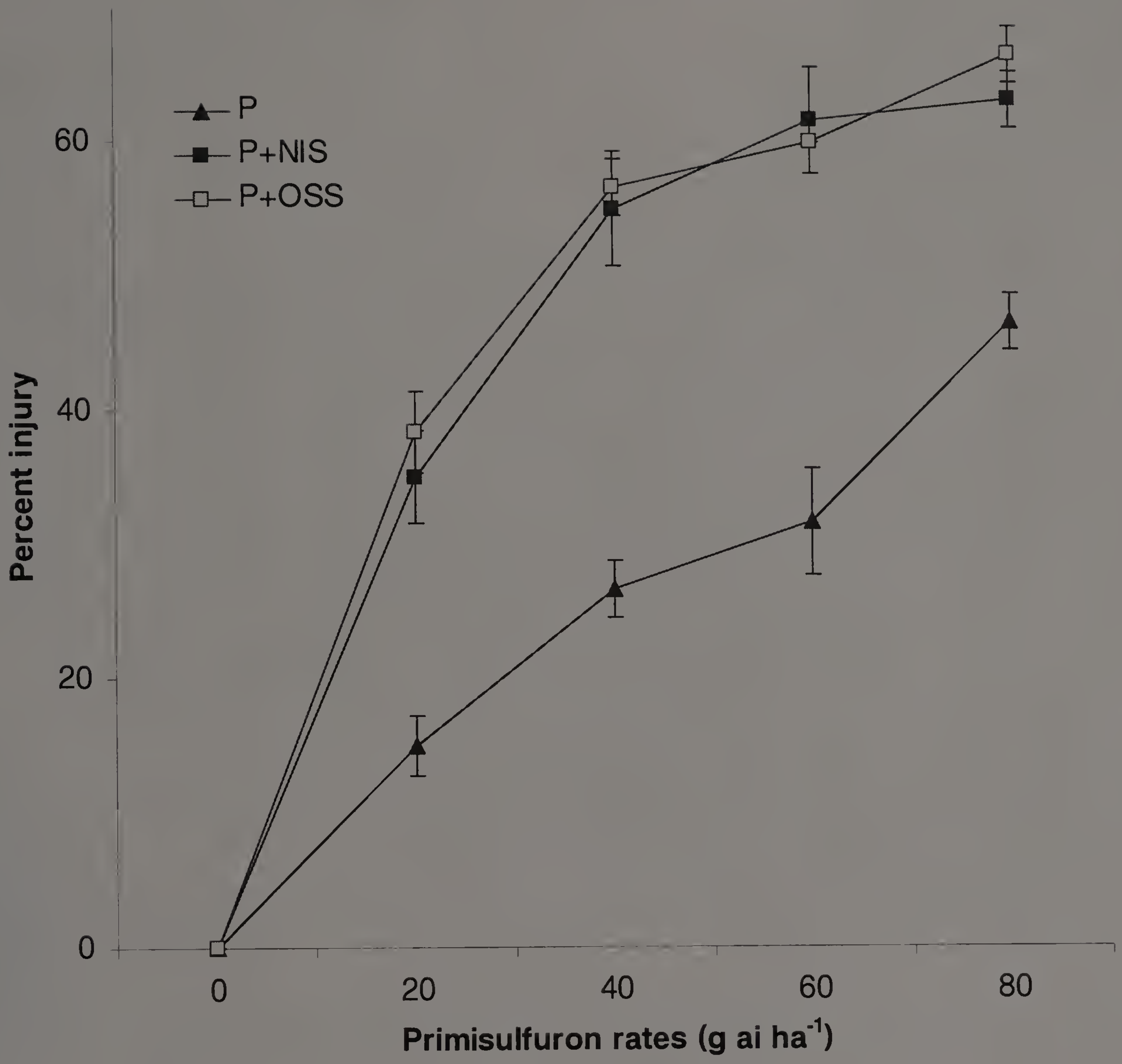

Figure 4.3 Effect of various rates of primisulfuron on common lambsquarters injury. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 4.18 and 3.24, respectively.

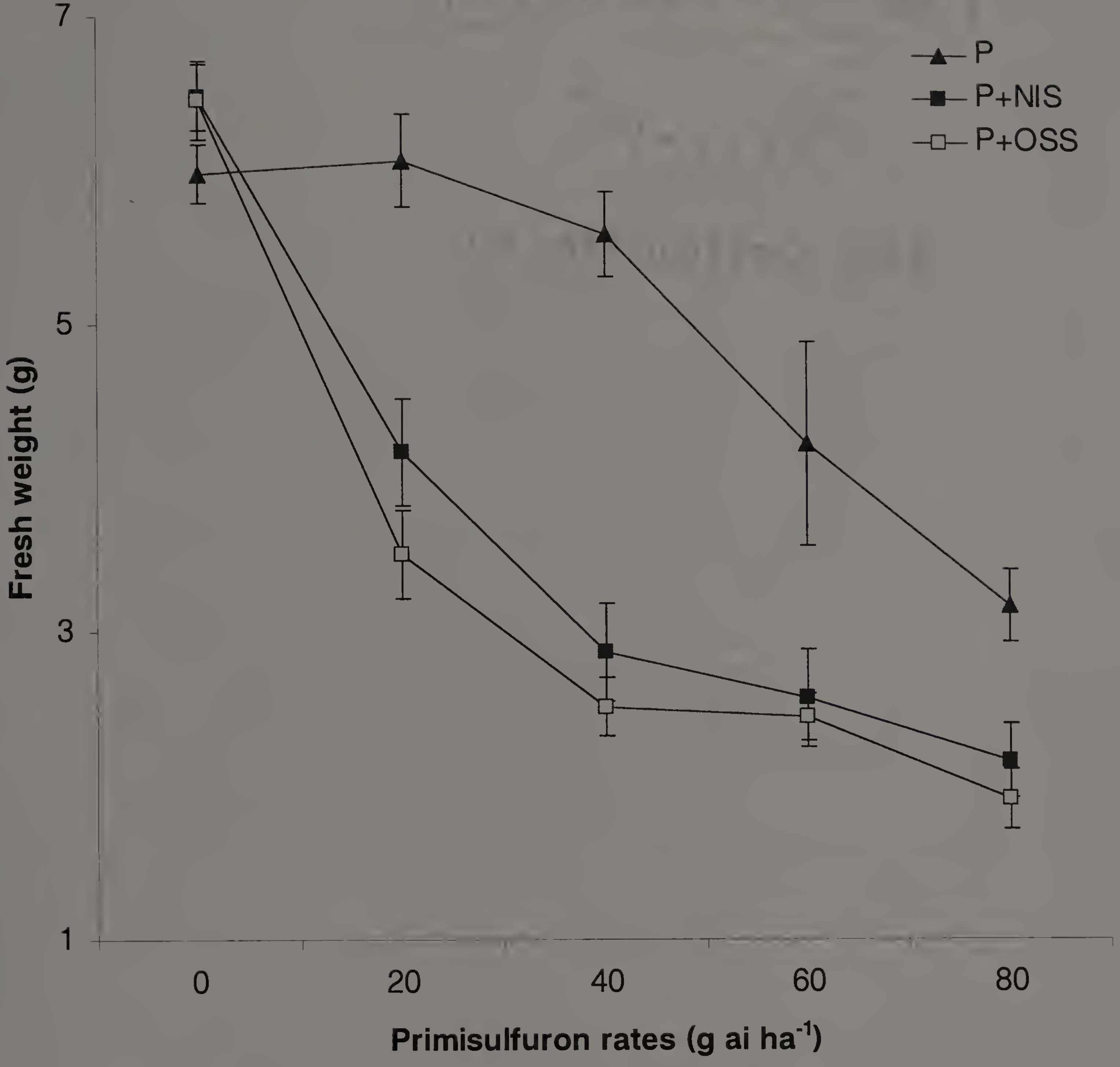

Figure 4.4 Effect of various rates of primisulfuron on common lambsquarters fresh weight. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 0.49 and 0.38, respectively.

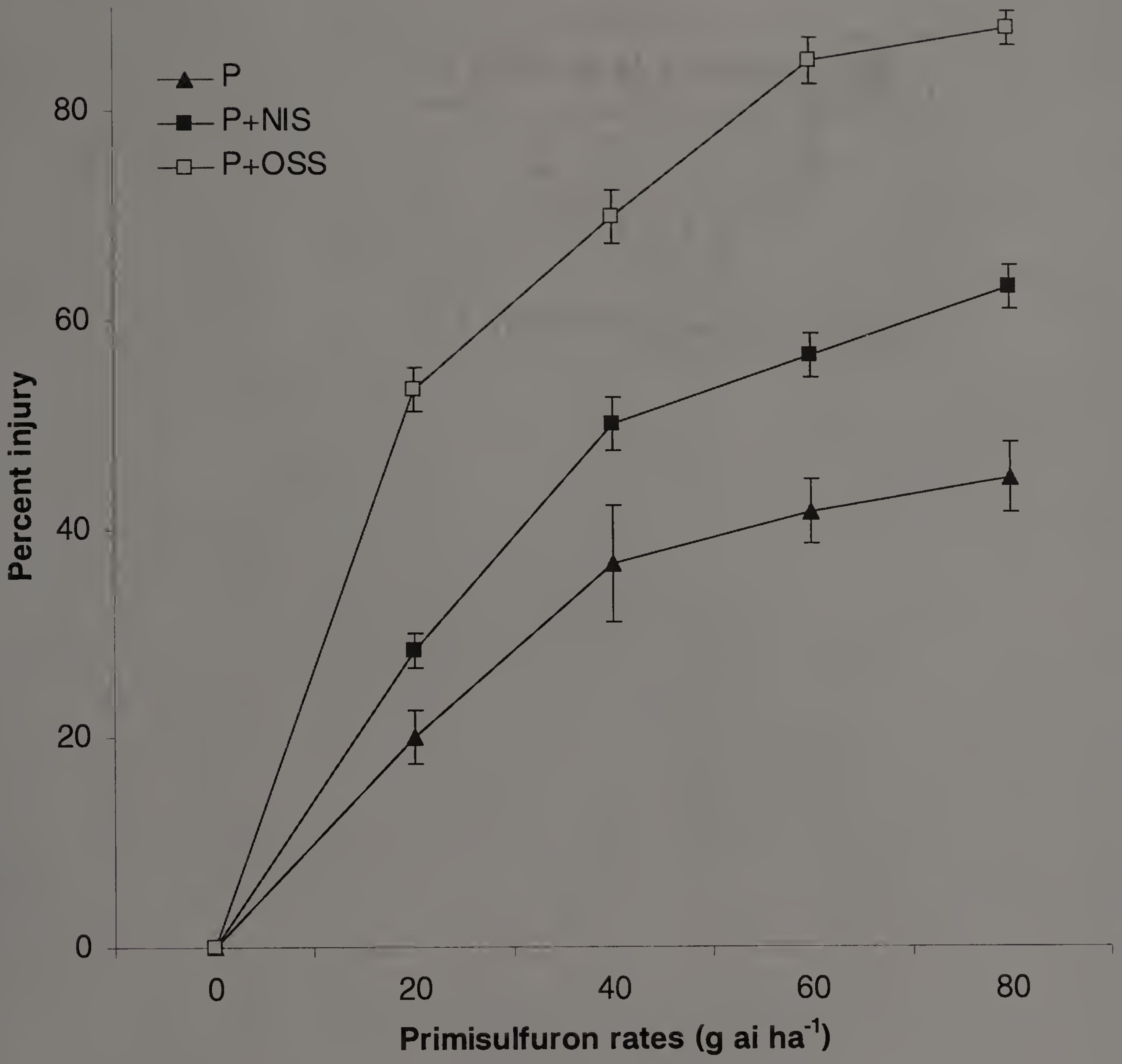

Figure 4.5 Effect of various rates of primisulfuron on common purslane injury. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 4.12 and 3.19, respectively.

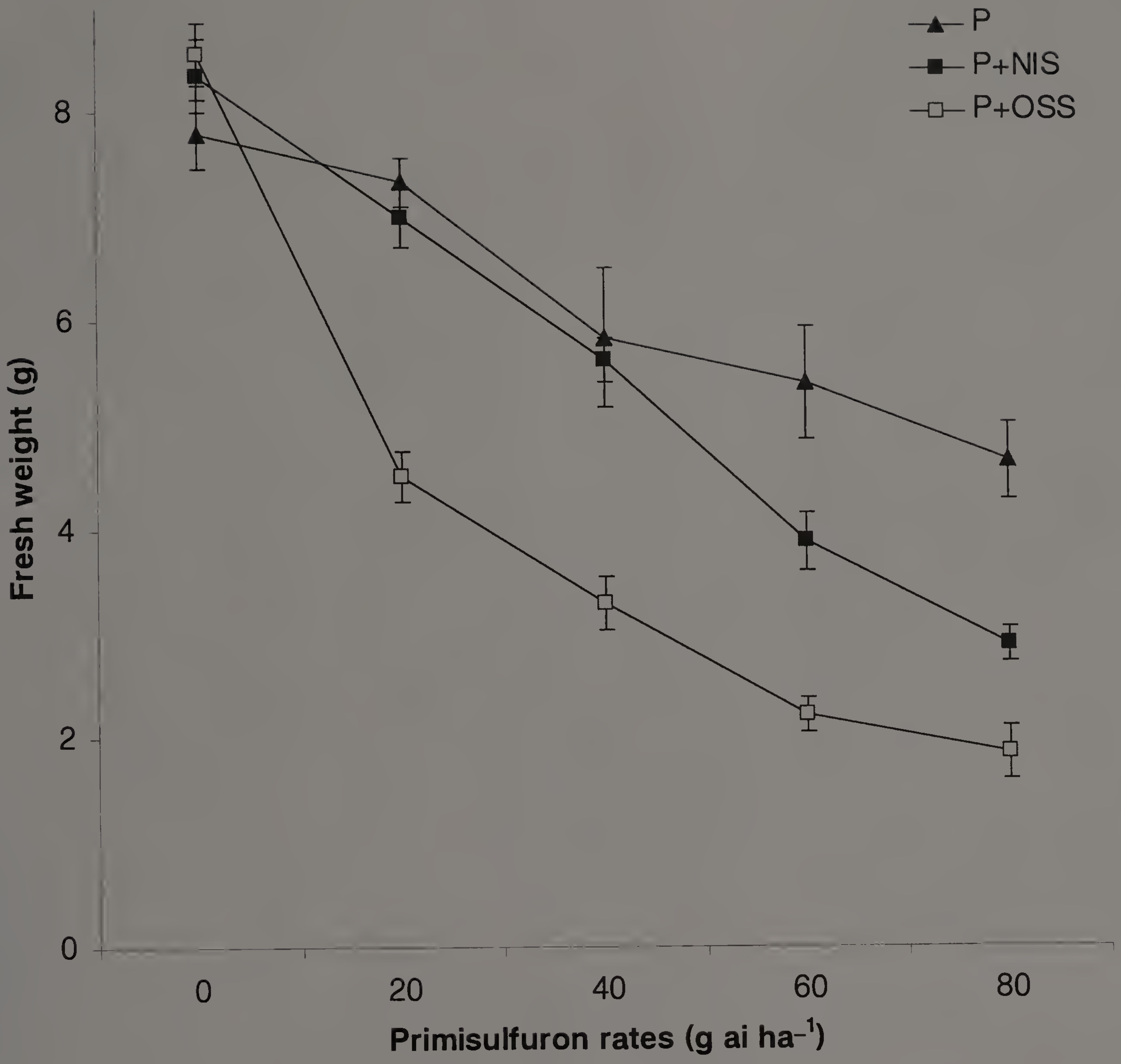

Figure 4.6 Effect of various rates of primisulfuron on common purslane fresh weight. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 0.55 and 0.43, respectively.

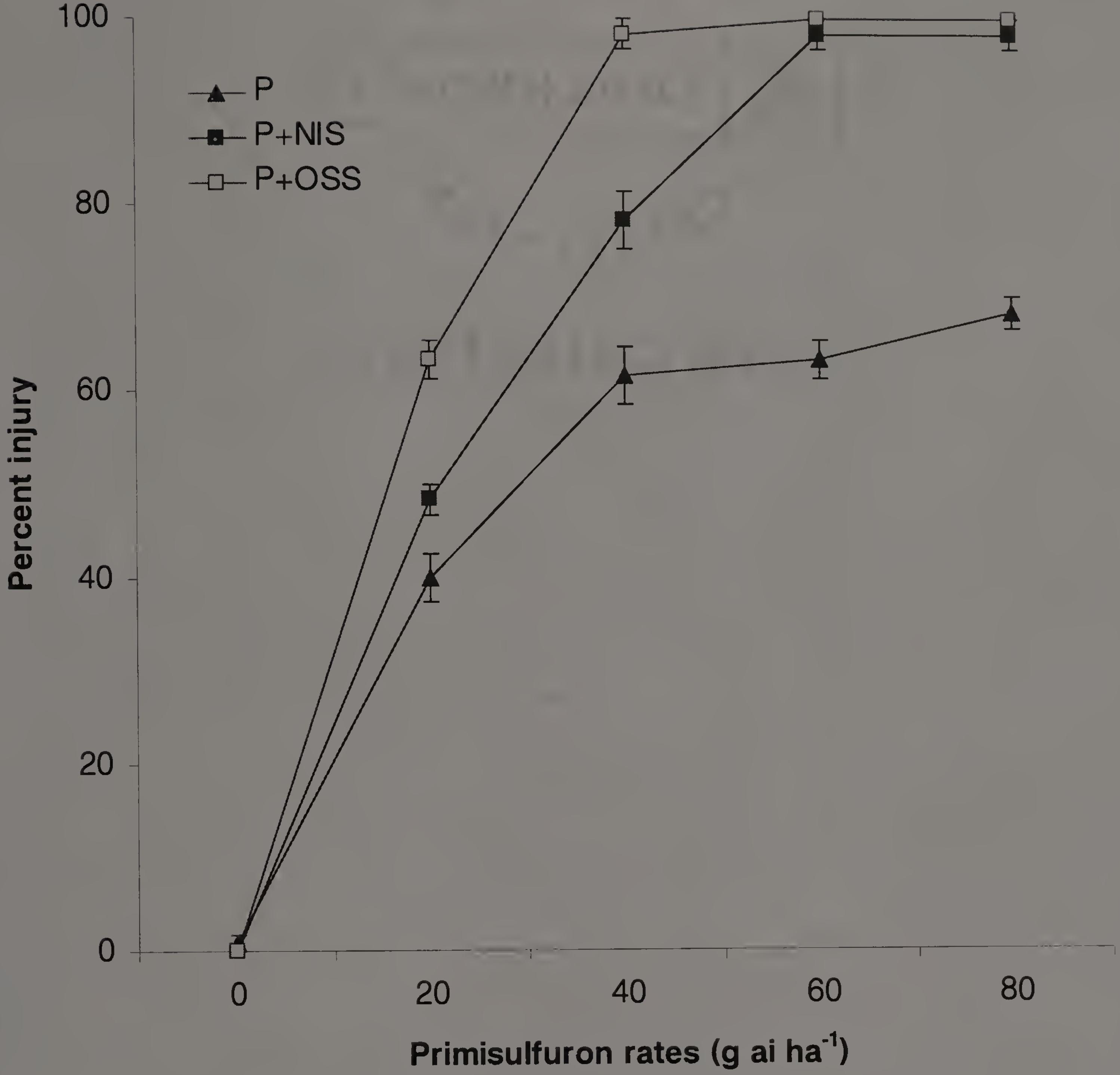

Figure 4.7 Effect of various rates of primisulfuron on velvetleaf injury. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 2.94 and 2.28, respectively.

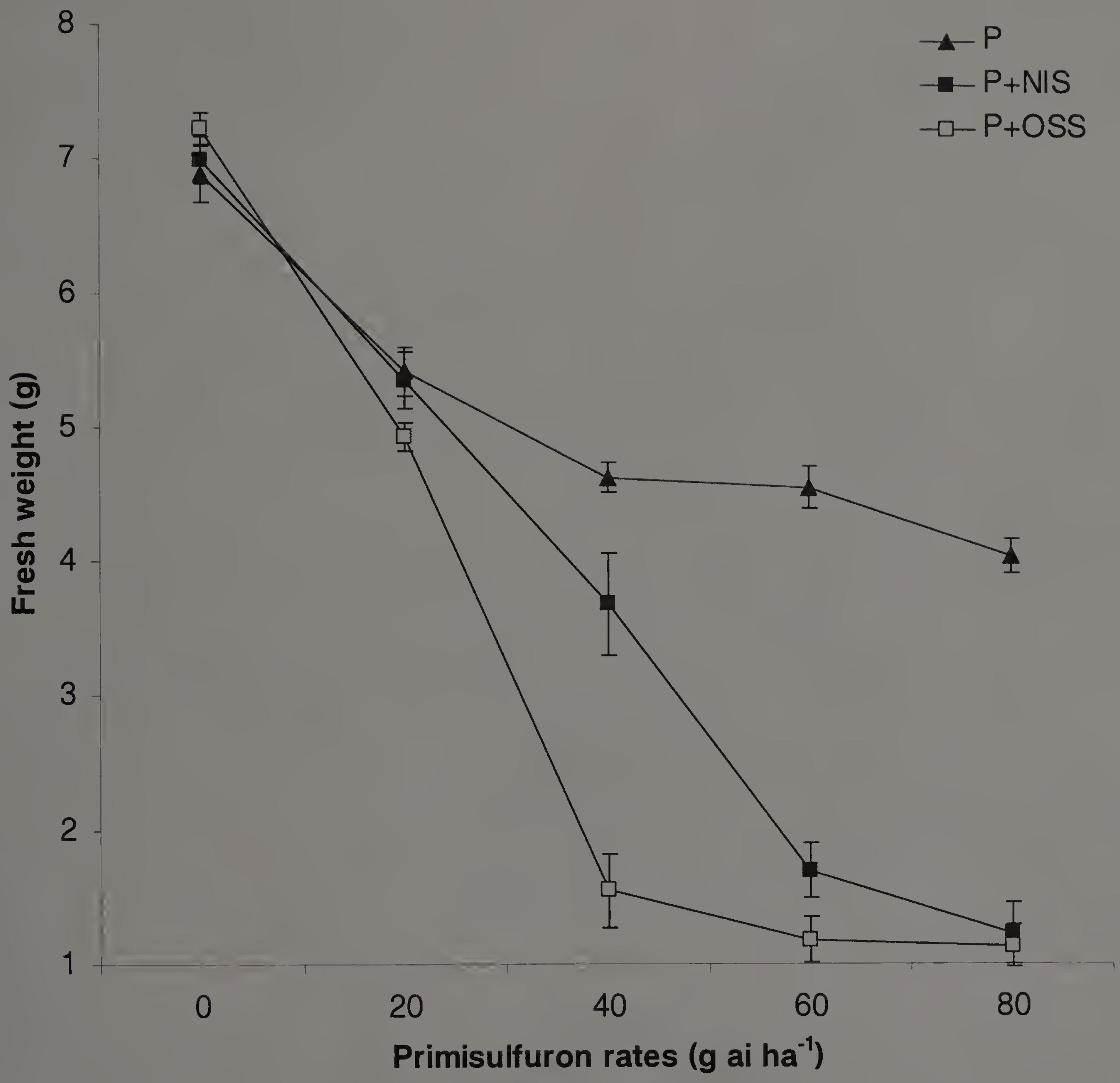

Figure 4.8 Effect of various rates of primisulfuron on velvetleaf fresh weight. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 0.33 and 0.25, respectively.

Similar plant injury and fresh weight reductions of velvetleaf were observed with primisulfuron and surfactants (Table 4.1). Primisulfuron at 40 g ai ha^{-1} resulted in 62% injury to velvetleaf when applied without a surfactant and the injury increased to 78% with NIS and 98% with OSS (Figure 4.7). At 60 g ai ha^{-1} or higher rates of primisulfuron, no differences in plant injury were observed between two surfactant treatments. Both surfactants reduced the fresh weight of velvetleaf plants as compared to the primisulfuron treatment without surfactant (Figure 4.8). At 40, 60, and 80 g ai ha^{-1}rate, fresh weights of velvetleaf plants were lowest when primisulfuron was applied with OSS. Similar trend in fresh weight reduction was observed with NIS.

In general, activity of primisulfuron was higher in controlling all species when applied with surfactant as compared to the treatment without a surfactant. In common lambsquarters, there were no deferences between OSS and NIS treatments, when applied with primisulfuron. Similarly, it was shown by Kown and Penner (1996) that primisulfuron activity on common lambsquarters was enhanced by X-77 (nonionic surfactant) and Sylgard 309 (silicone surfactant). In common purslane, both surfactants treatments resulted in enhanced injury and reduced fresh weight of common purslane as compared to the application of primisulfuron without surfactant. However, highest injury and lowest fresh weight was obtained when OSS was added to primisulfuron. Greater herbicidal activity with organosilicones was also reported previously by others. According to Hart et al. (1992) giant foxtail (*Setaria glauca* (L.) Beauv.) control with primisulfuron consistently increased by methylated seed oil and organosilicone surfactant by increasing foliar absorption and/or spray retention. Mitra et al. (1998) reported 70% quackgrass control by rimsulfuron (1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-

2-pyridylsulfonyl)urea) and the same treatment increased quackgrass control over 90% with Silwet, and 80% with Atplus S-12, Induce, or Renex. In our trial, addition of surfactants enhanced velvetleaf injury resulting in fresh weight reduction than the treatment without surfactant. However, at higher rates (60, 80 g ai ha^{-1}) of primisulfuron, there was no difference in velvetleaf injury between two surfactant treatments. Similarly, it was shown by Kown and Penner (1996) that Sylgard 309 (silicone surfactant) and K-3000 (methylated oil) enhanced velvetleaf control with primisulfuron and thifensulfuron over other surfactants used.

It was evident from our data that surfactants increased primisulfuron activity on commonlambsquarters, common purslane, and velvetleaf and the influence of surfactants on herbicidal activity varie with weed species. These results show that organosilicone reduced the contact angle with increased spread area of the primisulfuron droplets more than the NIS treatments.

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CHAPTER V

EFFECT OF SURFACTANTS ON PRIMISULFURON ACTIVITY ON BARNYARDGRASS AND GREEN FOXTAIL

Abstract

The effect of surfactant on herbicidal activity varies with herbicides and weed species, and therefore, there is a need for investigating the most effective surfactant for various postemergence herbicides in controlling specific weed species. Laboratory and greenhouse studies were conducted to (1) measure the contact angle and spread area of primisulfuron droplets with and without surfactants on leaf-surface of barnyardgrass and green foxtail, (2) determine primisulfuron activity on these weed species, and (3) examine spray deposits of primisulfuron with and without surfactants on the leaf surface of green foxtail by scanning electron microscopy (SEM). A nonionic surfactant (NIS) and an organosilicone surfactant (OSS) were used at 0.25 and 0.1% (v/v), respectively. The contact angles of 1-µl droplets were measured on the leaf surface using a goniometer. The activity of primisulfuron on barnyard grass and green foxtail was assessed 3 wk after treatment (WAT) in terms of percent injury and fresh weight. Contact angles of 1 µl droplet of primisulfuron on the adaxial surface of barnyardgrass and green foxtail leaves was 152^{o} and 127^{o}, respectively, when applied without surfactant. Addition of surfactants resulted in reduction of contact angles on both weed species. Contact angle was smallest when OSS was added to primisulfuron. Without surfactant, spread of 1 µl droplet of primisulfuron on the adaxial surface of barnyardgrass and green foxtail leaves was very small and there was no difference in spread area between these two weed species. Addition of NIS increased the spread area both on barnyardgrass and green foxtail leaves and the spread was even larger when OSS was added to primisulfuron. Percent injury of barnyardgrass was very low (4 to 5%) even at higher rates (80 g ai ha^{-1}) of primisulfuron with an unacceptable weed control. Primisulfuron at 40 g ai ha^{-1} resulted in 43% injury to green foxtail when applied without any surfactant and the plant injury increased to 65% with NIS and 83% with OSS. Both surfactants reduced fresh weight of green foxtail plants as compared to the primisulfuron treatment without any surfactant. The scanning electron micrographs showed that uniform deposit with close contact to the leaf epicuticular surface resulted in higher primisulfuron efficacy. Reduced activity of primisulfuron was related to the treatments with large bulky spray deposit on leaf surface.

Introduction

The first sulfonylurea herbicide, chlorsulfuron, was introduced in 1980 (Appleby 2005), which had a new mechanism of action. The mode of action of sulfonylurea herbicide is to inhibit acetolactate synthase (ALS) activity (Ray 1984; Ray 1985; Matsunaka et al. 1985; Scheel and Casida 1985; Rost and Reynolds 1985; Falco et al. 1987). The sulfonylureas are effective in very low rate of 2 g ha^{-1} (Devine and Vanden Born 1985), and have quite low mammalian toxicity (LD_{50}>5,000 mg kg^{-1} in rats). Sulfonylureas control broadleaf weeds as well as grasses and has excellent crop selectivity. Hageman and Behrens (1984) reported that susceptible velvetleaf was 20,000 times more sensitive to chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) than was eastern black nightshade (*Solanum ptycanthum* Dun.). The most noticeable plant response caused by sulfonylureas, is the strong and rapid inhibition of plant growth. Also, there may be some secondary plant responses like enhanced anthocyanin formation, abscission, terminal bud death, loss of leaf nyctinasty, vein discoloration, chlorosis or necrosis.

There are many sulfonylurea herbicides. Primisulfuron {methyl 2-[[[[[4,6-bis(difluromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate} is a selective herbicide applied postemergence for the control of grasses, especially some difficult to control perennial grasses, and certain broadleaf weeds in field corn for silage or grain and in popcorn (CPR 2005). Bhowmik (1995) reported that rates from 15 to 30 g ai ha^{-1} of primisulfuron controlled quackgrass over 90% 6 weeks after treatment. With a single early postemergence application of primisulfuron at 40 g ai ha^{-1} was as effective in controlling quackgrass as a split application of 20 g ai ha^{-1} applied at the one- to three-leaf stage followed by a second application of 20 g ai ha^{-1} at four- to six-leaf stage (Bhowmik 1999). According to Tweedy and Kapusta (1995), primisulfuron at 40 g ha^{-1} controlled Johnsongrass (*Sorghum halepense* L.) 85 to 100% from 1992 to 1994 and corn yield was more than double as compared to the control plots.

There are several reports showing surfactants improving herbicide uptake into whole plants and isolated leaf tissue (Riederer and Schönherr 1990; Field et al. 1992; Kirkwood et al. 1992; De Ruiter et al. 1992; Gaskin and Holloway 1992; Stock and Holloway 1993; Coret et al. 1993; Coret and Chamel 1993; Coret and Chamel 1994; Coret and Chamel 1995; Holloway and Edgerton 1992). Jansen et al. (1961) noted that inclusion of an appropriate surfactant in herbicidal formulations enhanced retention and penetration only in certain plant species, and thus measurably altered selectivity of the herbicide as compared to its activity without a surfactant. Within a single species, the extent to which a specific surfactant may enhance the activity of a herbicide depends to a considerable extent upon the various components of the formulation and upon the physical properties of the active ingredient. In general, the addition of a surfactant or surfactant tends to equalize the foliar absorption of herbicides (Klingman and Ashton 1975).

Enhanced uptake into the plant system leads to higher activity of herbicides when applied with surfactants. In general, most postemergence herbicides have been used with one of the surfactant types such as nonionic surfactants (NIS), crop oil concentrates (COC), nitrogen-surfactant blends, esterified seed oils, or organo-silicones. Mitra et al. (1998) reported that the addition of an oil to rimsulfuron increased quackgrass (*Elytrigia repens*(L.) Beauv.) control by 40% 5 weeks after treatment, common lambsquarter (*Chenopodium album*(L.)) and yellow foxtail (*Setaria lutescens* (Weigel.) Hubb.) control by 30% 3 weeks after treatment, compared to control achieved by rimsulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide) alone.

As the effect of surfactant on herbicidal activity varies with herbicides and weed species, there is a need for investigating the most effective surfactant for various postemergence herbicides in controlling specific weed species. The objectives of this study were to (1) measure the contact angle and spread area of primisulfuron droplets with and without surfactants on leaf-surface of barnyardgrass and green foxtail, (2) determine primisulfuron activity on these weed species, and (3) examine spray deposits of primisulfuron with and without surfactants on the leaf surface of green foxtail.

Materials and Methods

General Greenhouse Procedures

Seeds were purchased from a commercial source (Valley Seed Service, Fresno, CA 93791) and were stored in the refrigerator at 4 C before growing in the greenhouse. Seeds were grown in plastic pots having Hadley fine sandy loam (Typic Udifluvents) with 3.5% organic matter and a pH of 6.5. The greenhouse had an average temperature of 20 ± 2 C with natural sunlight. Plants were thinned to 12 seedlings per pot for barnyardgrass and 6 seedlings per pot for green foxtail. Pots were watered as necessary to prevent wilting and were fertilized weekly with 10 ml solution of a soluble fertilizer (20N-5.2P-6.6K) per pot.

Contact Angle and Spread Area

Contact angles of 1-μl droplets of primisulfuron alone, with a nonionic surfactant (NIS) [Induce, blend of alkyl aryl polyoxylkane ethers, free fatty acids, and dimothyl polysiloxane, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017], and with an organosilicone surfactant (OSS) [Silwet L-77, polyalkyleneoxide-modified heptamethyltrisiloxane 7.5 EO, 100%, Witco Corporation, Organosilicone Group, 777 Old Saw Mill River Road, Tarrytown, NY 10591] were measured on the adaxial surface of the third or fourth fully expanded leaves from the tip of the plants. NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. The leaves were collected daily just before measurement from four- to five-leaf stage plants. The contact angles of both sides of the 1-μl droplets on the leaf surfaces were measured using a goniometer (Contact Angle Goniometer, Rame-hart, Inc., 43 Bloomfield Avenue, Mountain Lakes, NJ 07046). The experimental design was completely randomized. Contact angle measurements were replicated four times, and each experiment was repeated. All data were subjected to ANOVA using the general linear model procedure (SAS 1992). Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

For the spread area measurement, the leaves of individual weed species were collected daily prior to measurement from four- to five-leaf stage plants. The spread areas of a 1 μl droplets of distilled water, primisulfuron at 40 g ai ha^{-1}, primisulfuron with NIS at 0.25% (v/v), and primisulfuron with OSS at 0.1% (v/v) were measured on the adaxial surface of third or fourth fully expanded leaves (from the tip of the plants) of barnyardgrass and green foxtail. Spread area was calculated using the formula πr^2, where r was the radius of the droplet. As the spread of a 1 μl droplet was rarely circular, the radius was estimated as the mean of horizontal and vertical dimensions of the droplet. The experimental design was completely randomized. Spread area measurements were replicated four times and the experiment was repeated. All data were subjected to ANOVA using the general linear model procedure (SAS 1992). Data from two repeated experiments were tested for homogeneity. All data were combined across two sets of experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

Primisulfuron Activity

Commercial formulation of primisulfuron 75 WG (Beacon, Syngenta Crop Protection, Inc., Greensboro, NC 27419) was used. Primisulfuron rates were 0, 20, 40, and 80 g ai ha^{-1} for barnyardgrass and 0, 20, 40, 60, and 80 g ai ha^{-1} for green foxtail, where 40 g ai ha^{-1} represented the manufacturer's suggested use rate. All rates of primisulfuron were applied alone, with NIS (0.25% v/v) or with OSS (0.1% v/v). An untreated water control was included in each experiment for the comparison. Spray solutions were applied using a CO_2-backpack sprayer with Teejet XR 11004 VS nozzles at 152 kPa using a spray volume of 190 L ha^{-1}. Primisulfuron was applied to barnyardgrass and green foxtail at four- to five-leaf stage. The activity of primisulfuron was estimated 3 wk after treatment (WAT) in terms of percent injury and fresh weight. Percent injury was assessed by visual rating on a 0 to100% scale, where 0 = no injury and 100 = completely dead plants. At 3 WAT plant shoots were clipped at the soil surface and the fresh weights were determined. The experimental designs were completely randomized. For green foxtail, treatments were replicated three times, and the experiment was repeated. For barnyardgrass, treatments were replicated four times and the experiment was repeated three times. All data were subjected to ANOVA using the general linear model procedure (SAS 1992). Data from repeated experiments were tested for homogeneity. All data were combined across the repeated experiments and analyzed. Means were separated using Fisher's Protected LSD test at P=0.05.

Scanning Electron Microscopy

Green foxtail seedlings with four to five leaves were sprayed with primisulfuron at 40 g ai ha^{-1} without any surfactant, with NIS, and with OSS. NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. An untreated water control was included for comparison. Spray solutions were applied using a CO_2-backpack sprayer with Teejet XR 11004 VS nozzles at 152 kPa using a spray volume of 190 L ha^{-1}. Leaves were collected from treated plants 30 minutes after spraying, which allowed the spray solution to get air dried. Plant specimens were prepared for scanning electron microscopy using similar procedures as described by Matysiak and Nalewaja (1999a, 1999b) and Nalewaja and Matysiak (2000). Portion of leaves were removed from sprayed plants and mounted on aluminum stubs using double sticky carbon tape. Silver paint was used to attach the margins of leaves to the aluminum stubs. These leaf specimens were examined under scanning electron microscope (JEOL-JSM 840, USA, 11 Dearborn Road, Peabody, MA 10960). This technique allowed examination of spray droplet residual on cuticle and epidermal surfaces that were unaltered by chemical fixation and dehydration.

Results and Discussion

Contact Angle and Spread Area

Contact angles of 1 µl droplet of primisulfuron on the adaxial surface of barnyardgrass and green foxtail leaves were 152^{O} and 127 ^{O}, respectively, when applied without surfactant (Figure 5.1). *P* values in the analysis of variance showed that surfactant had significant effect on contact angle (see Appendix Table A.13). In general, addition of surfactant resulted in reduction of contact angle on both weed species (Figure 5.1). Primisulfuron with NIS had lower contact angle as compared to that of primisulfuron without any surfactant. OSS resulted in smallest contact angle of primisulfuron droplets.

Spread of 1 μl droplet of primisulfuron on the adaxial surface of barnyardgrass and green foxtail leaves was very small and there was no difference in the spread area between these two weed species, when applied without surfactant (Figure 5.2). Surfactants significantly affected the spread area as determined by the *P* values in ANOVA (see Appendix Table A.14). Addition of NIS enhanced the spread area more than 50 times both on barnyardgrass and green foxtail leaves, and the spread was even higher when OSS was added to primisulfuron (Figure 5.2).

The epicuticular leaf wax may have influenced contact angle and resulted in lower spread area of herbicide droplet on the leaf surface. In general, leaf wax content and the spread area of the spray droplet are inversely related, as reported by Chachalis et al. (2001a, 2001b). However, the spread of herbicide droplets 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 non-repellent 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.

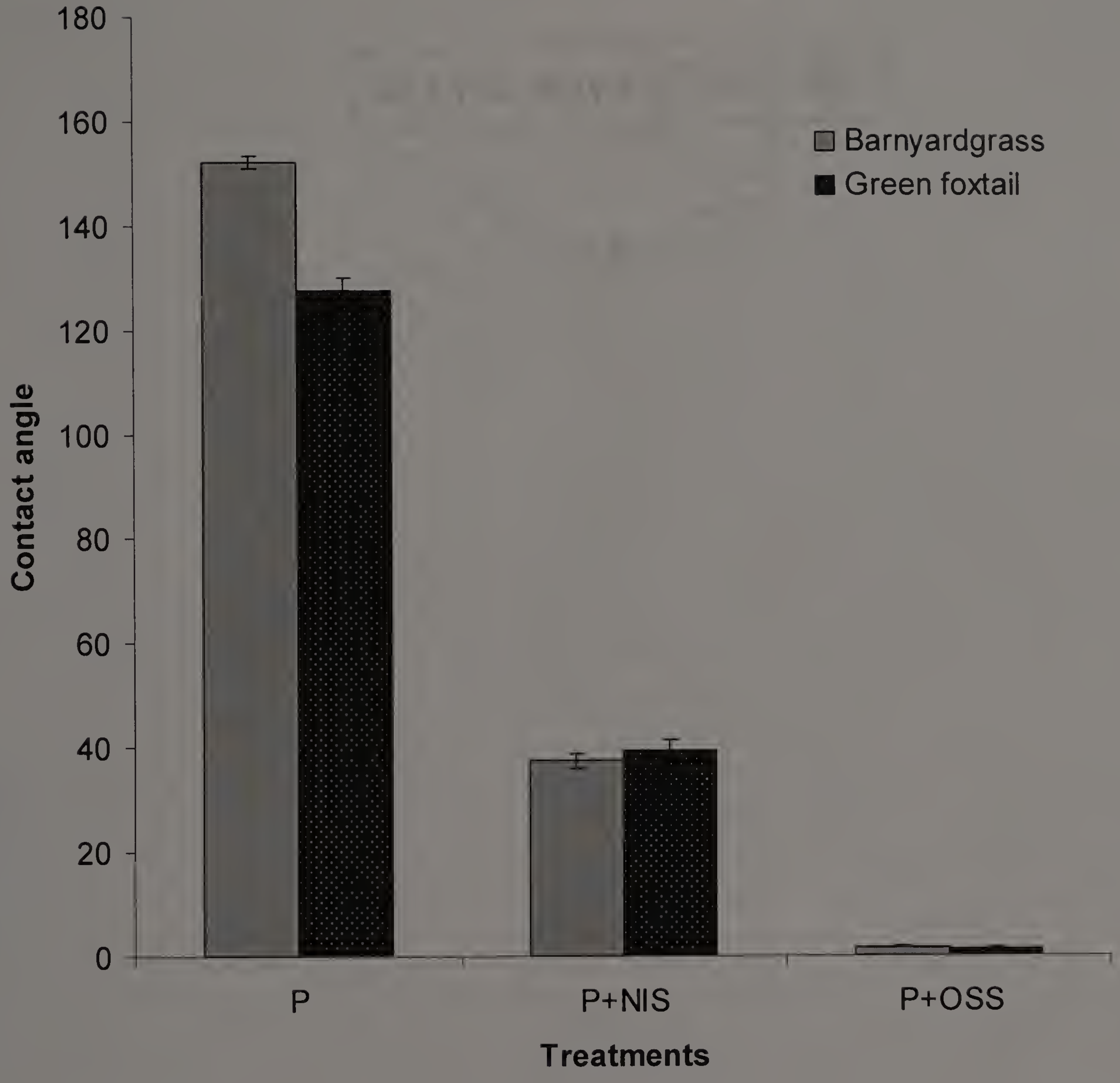

Figure 5.1 Contact angle of 1 μl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of barnyardgrass and green foxtail. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v). LSD values were 2.45 for weed species and 2.99 for treatments, respectively.

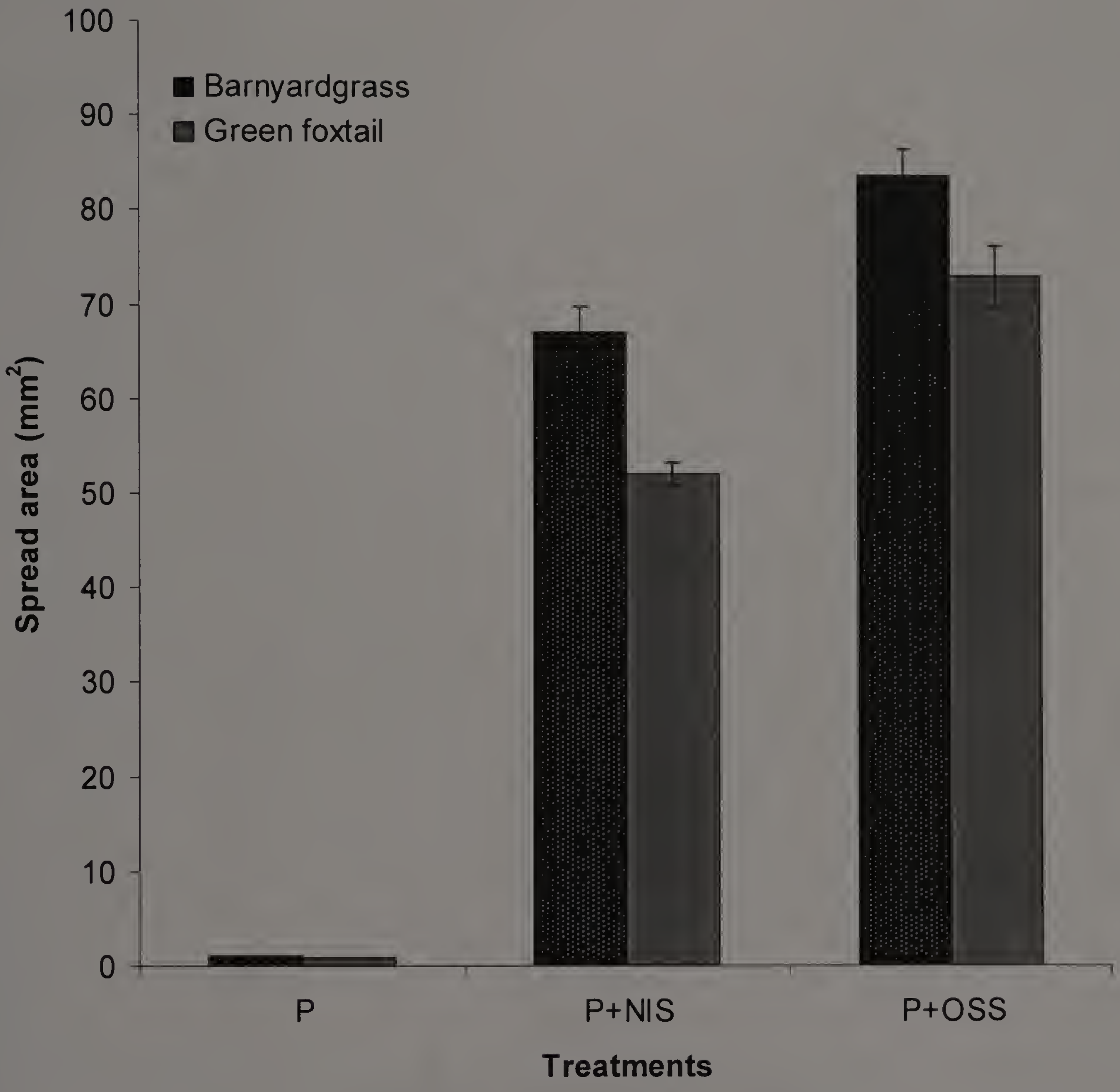

Figure 5.2 Spread area of 1 µl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of barnyardgrass and green foxtail. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v). LSD values were 3.57 for weed species and 4.37 for treatments, respectively.

Surfactant reduces the surface tension and contact angle of herbicide droplets, thereby improving the coverage and increasing the chance for a herbicide to absorb into the plant tissue (Kocher and Kocur 1993; Singh et al. 1984). Addition of surfactant into the spray solution enhances the solution's ability to wet leaf surface, however, there are lots of variations of wetting properties among surfactants (Wells 1989; Knight and Kirkwood 1991). Our data show that the OSS reduces the contact angle and increases the spread area of primisulfuron droplets more than NIS, as reprted by Wells (1989) and Knight and Kirkwood (1991).

Primisulfuron Activity

The analysis of variance showed that primisulfuron rates had significant effect on percent injury of barnyardgrass (Table 5.1). However, the 80 g ai ha^{-1} rate of primisulfuron resulted in unacceptable control (4 to 5%) of barnyardgrass (Figure 5.3). Primisulfuron rate and surfactant type did not have any effect on percent injury and fresh weight of barnyardgrass (Table 5.1, Figure 5.4). Similarly, Webster and Masson (2001) showed that primisulfuron at 40 and 80 g ai ha^{-1} rate resulted in 21 and 20% barnyardgrass control, respectively, 15 days after treatment, and after 30 days the control was 0% for both rates of primisulfuron. Carey et al. (1997) showed that the GR_{50} value of primisulfuron for barnyardgrass was > 480 g ai ha^{-1}.

Surfactant types and primisulfuron rates significantly affected percent injury and fresh weight of green foxtail as determined by the P values for each of the variables (Table 5.1). Primisulfuron when applied with OSS resulted in maximum green foxtail injury with lowest fresh weight (Figure 5.5 and 5.6). Primisulfuron at 40 g ai ha^{-1} resulted

Table 5.1 Summary of ANOVA for effect of primisulfuron rates and surfactant types on barnyardgrass and green foxtail.

	Source[1]	Barnyardgrass	Green foxtail
		P values	
Percent injury	R	0.0078	<0.0001
	S	0.9186	<0.0001
	R*S	0.9919	<0.0001
Fresh weight	R	0.9412	<0.0001
	S	0.7338	<0.0001
	R*S	0.9959	<0.0001

[1] Sources of variations were primisulfuron rates (R), surfactant types (S), and their interactions (R*S).

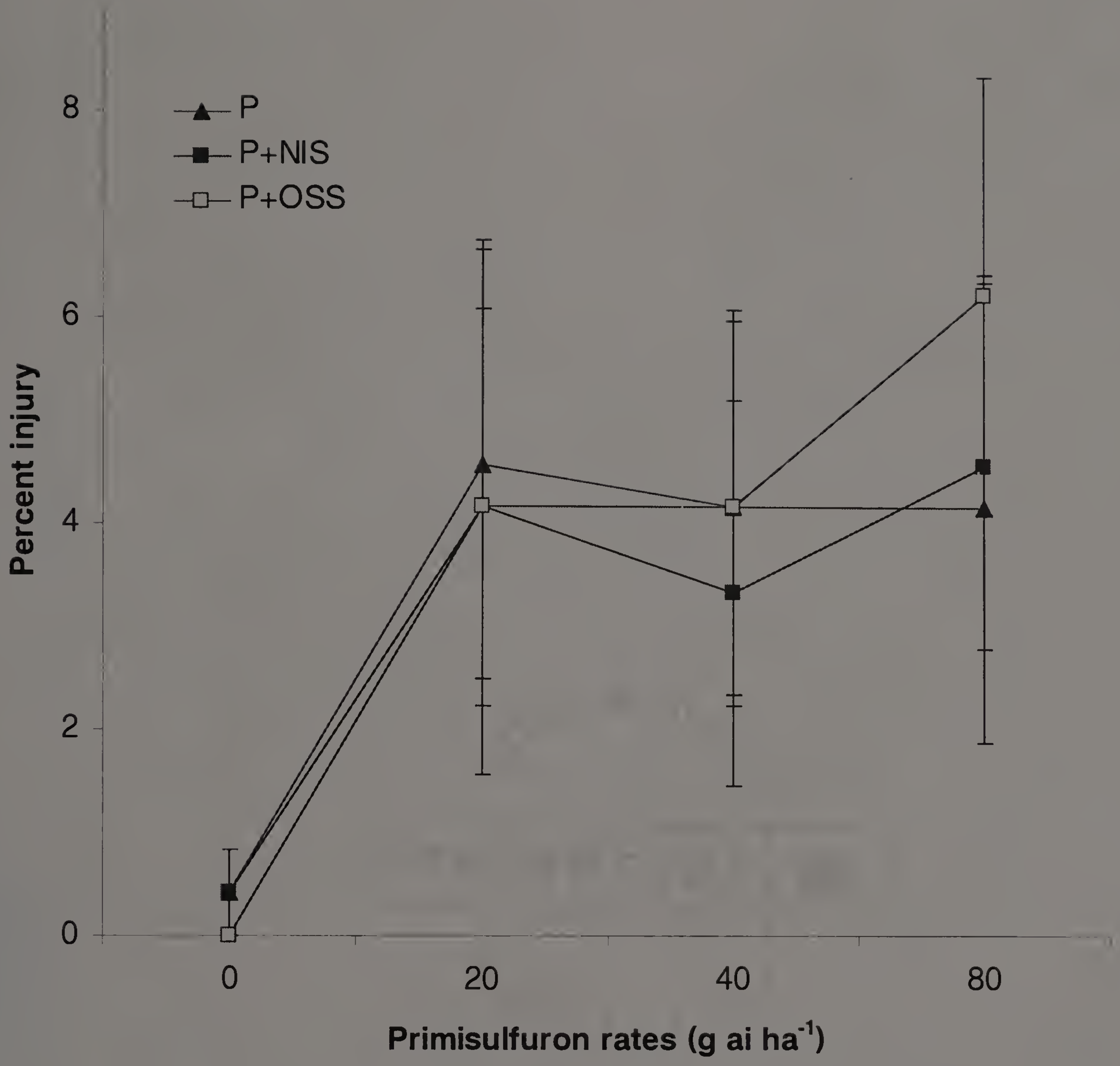

Figure 5.3 Effect of various rates of primisulfuron on percent injury of barnyardgrass. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 2.90 and 2.52, respectively.

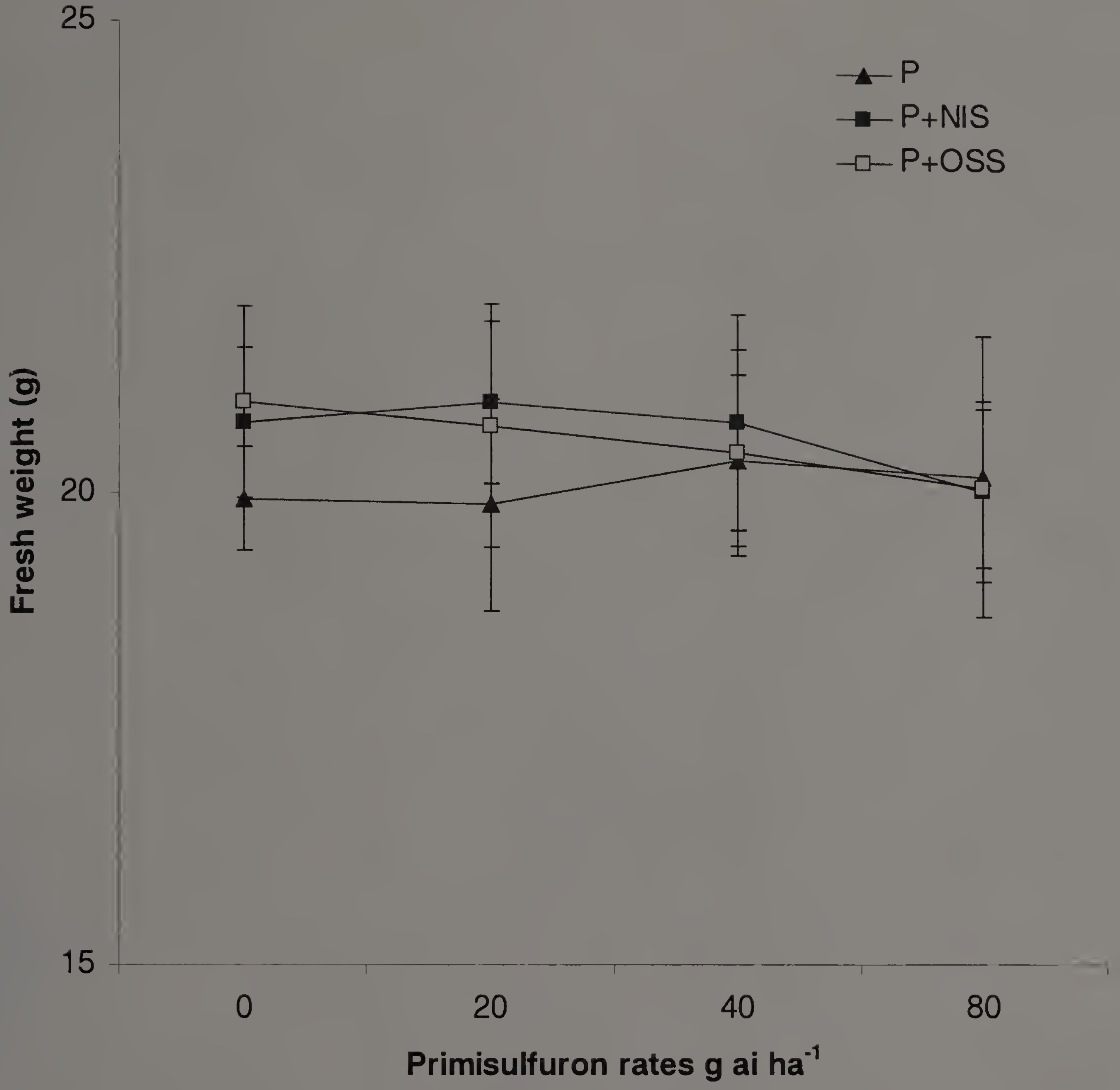

Figure 5.4 Effect of various rates of primisulfuron on fresh wight of barnyardgrass. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 1.67 and 1.45, respectively.

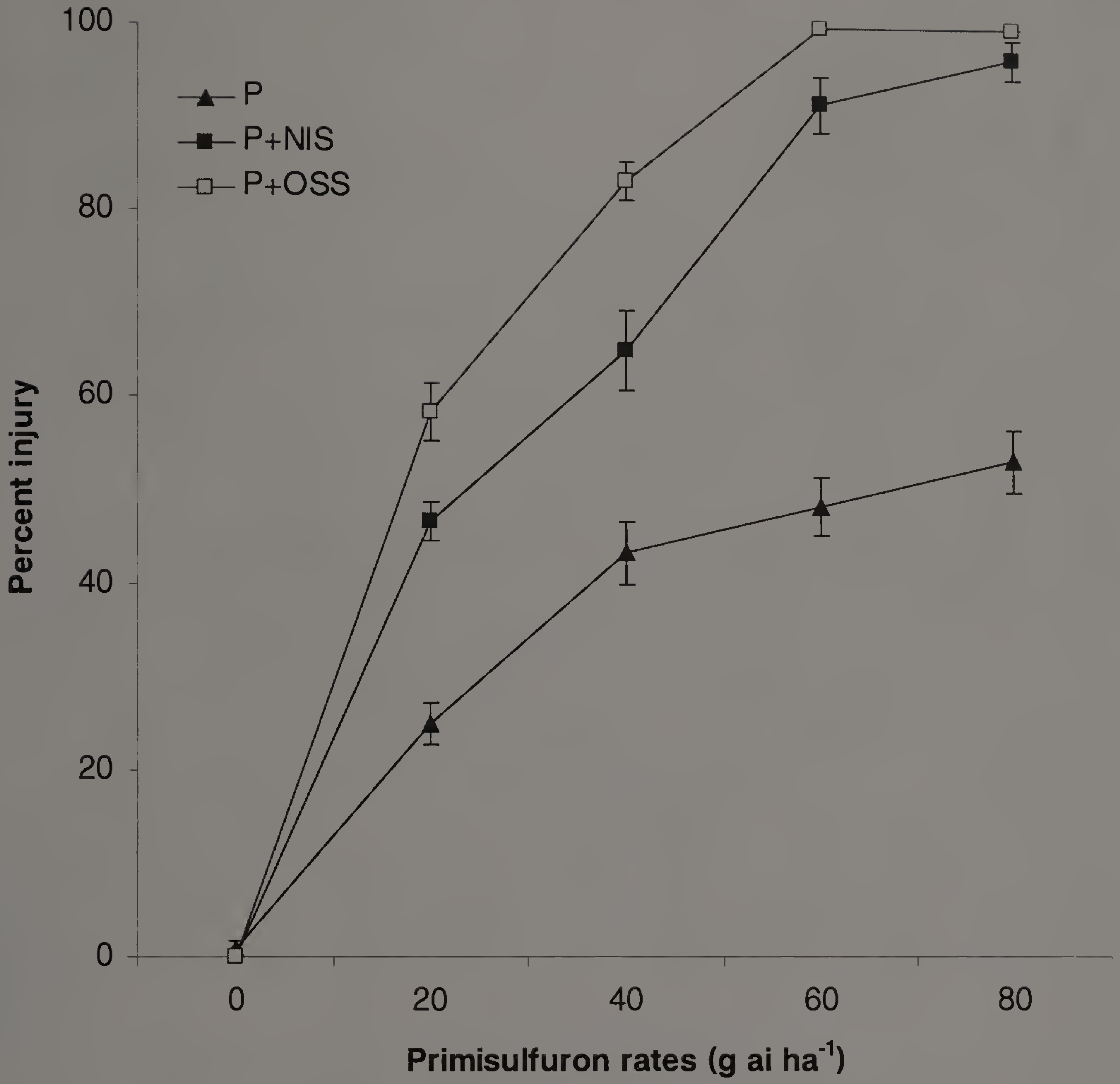

Figure 5.5 Effect of various rates of primisulfuron on percent injury of green foxtail. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 3.94 and 3.05, respectively.

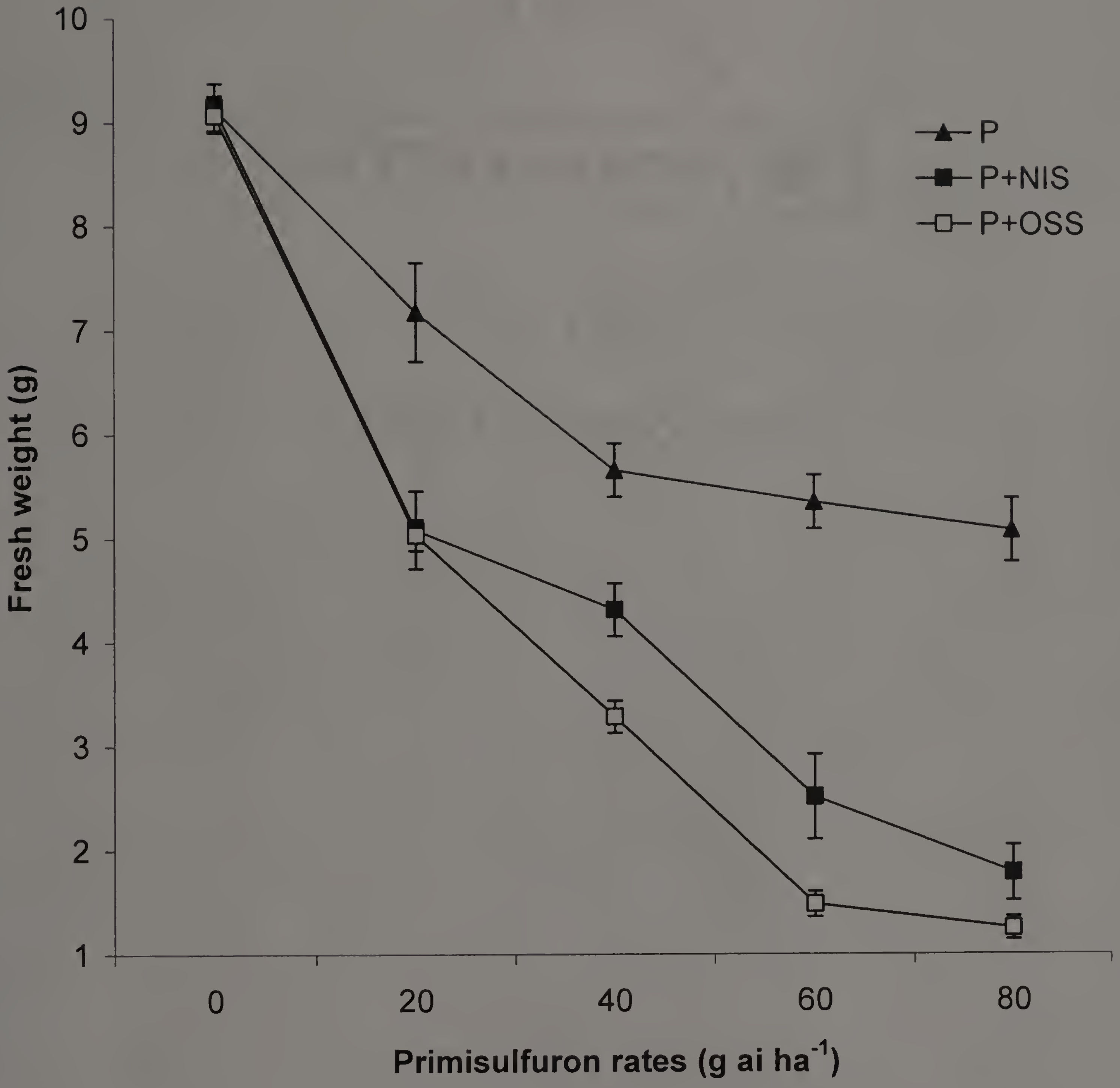

Figure 5.6 Effect of various rates of primisulfuron on fresh wight of green foxtail. All rates of primisulfuron were applied alone (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. LSD values for primisulfuron rates and surfactant types were 0.43 and 0.34, respectively.

in 43% injury to green foxtail when applied without a surfactant and the injury was increased to 65% with NIS and 83% with OSS (Figure 5.5). Both surfactants reduced fresh weights of green foxtail plants as compared to the primisulfuron treatment without surfactant (Figure 5.6). Organosilicone in combination with all rates (40, 60, and 80 g ai ha^{-1}) resulted in lowest fresh weight of geen foxtail with maximum injury. Greater herbicidal activity with organosilicones was also reported previously by others. According to Hart et al. (1992) giant foxtail (*Setaria glauca* (L.) Beauv.) control with primisulfuron consistently increased by methylated seed oil and organosilicone surfactant by increasing foliar absorption and/or spray retention. Mitra et al. (1998) reported 70% quackgrass control by rimsulfuron and the same treatment increased quackgrass control over 90% with Silwet and 80% with Atplus S-12, Induce, or Renex.

It was evident from our data that barnyardgrass was not affected by primisulfuron at rates tested in the greenhouse study and therefore no effect of surfactant was noted. However, surfactants definitely increased primisulfuron activity on green foxtail. Organosilicone surfactant reduced the contact angle and increased the spread area of the primisulfuron droplets more and resulted in greater activity as compared to the NIS.

Scanning Electron Microscopy

The spray droplet deposits from primisulfuron differed greatly, depending upon the accompanying surfactant. Primisulfuron formed more bulky and crusty droplet deposits on the leaf surface when applied without surfactant (Figure 5.7A). The large bulky droplet deposit may trap primisulfuron in nonsoluble surroundings or increase diffusion distance which probably reduced primisulfuron absorption accounting for

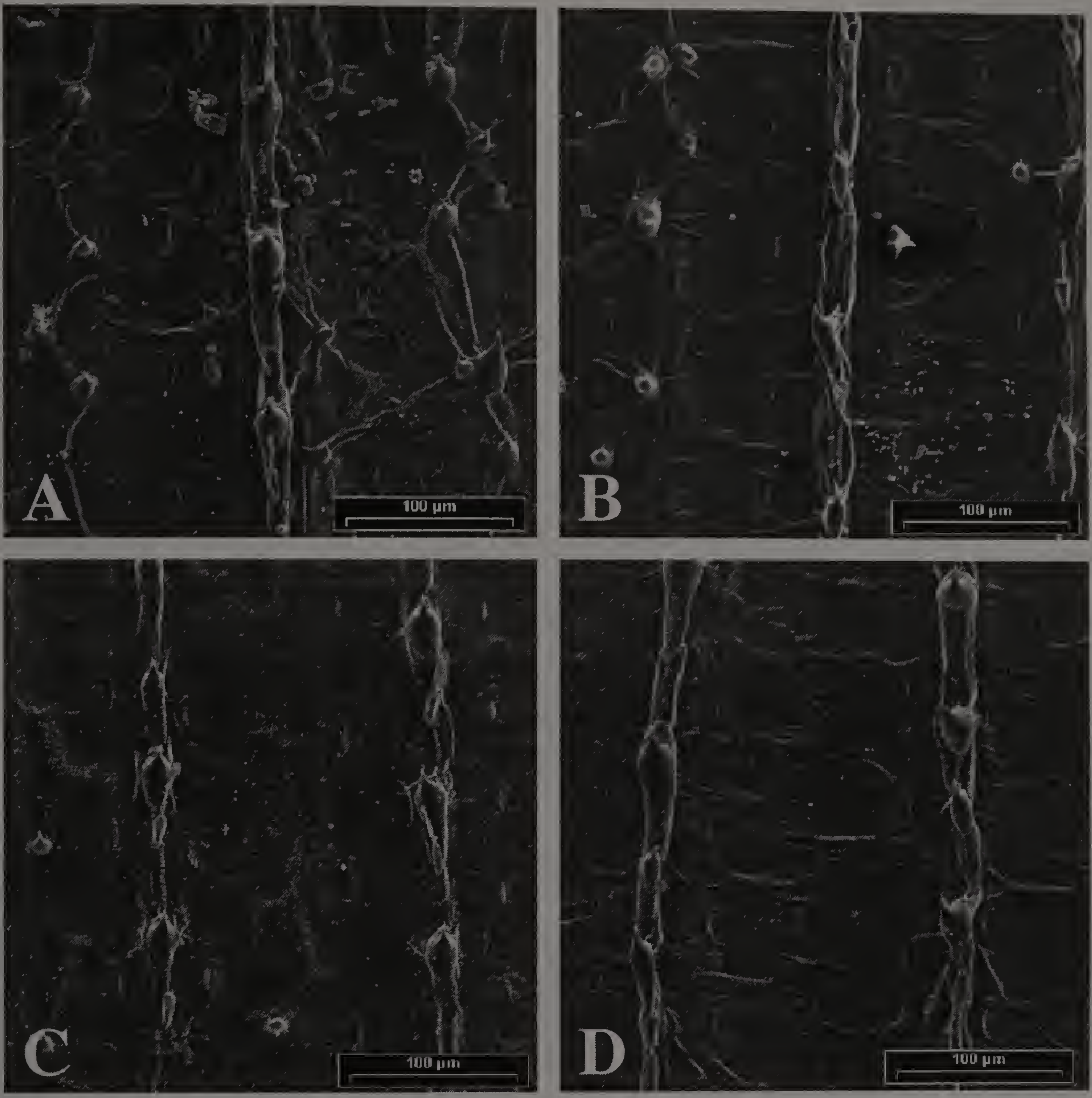

Figure 5.7 Scanning electron micrographs of green foxtail leaves sprayed with primisulfuron at 40 g ai ha^{-1} rate without any surfactant (A), with NIS (B), and with OSS (C). NIS and OSS were used at 0.25 and 0.1% (v/v), respectively. An untreated control was included for comparison (D).

reduced primisulfuron efficacy. With NIS, the dried herbicide droplet deposites are smaller (Figure 5.7B) which allows a closer leaf contact for primisulfuron as compared to the larger deposites in Figure 5.7A, and increased the chance of herbicide absorption. Primisulfuron when applied with OSS, the deposits appeared to blend uniformly into the cuticle (Figure 5.7C) which probably facilitated primisulfuron absorption into the leaf tissue resulting in enhanced activity.

Our data showed that the bulky and crusty deposits formed by primisulfuron when applied without surfactant, resulted in lower injury to green foxtail. In contrast, primisulfuron with organosilicone resulted in most uniform and fine deposites, and had highest green foxtail injury. These data support the findings of Nalewaja and Matysiak (2000) who reported that uniform deposit with close contact to the leaf epicuticular surface resulted in higher nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide) efficacy and reduced activity was related to the treatments with large bulky spray deposit on leaf surface. Previous research showed that higher sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) phytotoxicity was related to the spray deposit that are uniform, nonbulky, and in close contact with the leaf epicuticular surface (Matysiak and Nalewaza 1999a, 1999b).

These scanning electron micrographs indicate that the physical characteristics of the spray droplet deposits are important to the activity of applied herbicide. These results also support the concept that specific surfactant may optimize primisulfuron activity.

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CHAPTER VI

EFFECT OF SURFACTANTS ON BIOHERBICIDAL ACTIVITY OF *Alternaria helianthi* ON MULTIPLE-SEEDED COCKLEBUR

Abstract

Greenhouse studies were conducted to investigate the bioherbicidal activity of *Alternaria helianthi* (Hansf.) Tubaki & Nishih. on multiple-seeded cocklebur as affected by various rates of Tenkoz COC (crop oil concentrate), Activator 90 (nonionic surfactant), BAS 9050 0 S (methylated oil), Silwet L-77 (organosilicone surfactant), and Top film (natural based surfactant). Taking "X" as the recommended rate for each surfactant, 0-X, ¼-X, ½-X, X, and 2-X rates were used for each of the surfactants. Each surfactant rate was applied without and with *A. helianthi*. Surfactants were added to the conidial suspension right before spraying. Treated plants were subjected to two sets of immediate dew periods of 6 and 12 h before transferring to the greenhouse for 14 days. Plants were watered and fertilized (with N:P:K, 20:20:20) as needed. At the end of the experiment (two weeks after treatment), plant shoots were clipped at the soil surface and the fresh weights were determined. *A. helianthi* resulted in significant reduction in fresh weight of multiple-seeded cocklebur as compared to the plants treated without the fungus. The bioherbicidal activity of *A. helianthi* was higher when exposed to a 12 h dew period as compared to a 6 h dew. Among five surfactants, Activator 90 and Silwet L-77 had significant effects at various rates on the bioherbicidal activity of *A. helianthi* in

reducing fresh weight of multiple-seeded cocklebur. With 12 h of dew exposure, addition of 0.05 or 0.06% rates of Activator 90 or Silwet L-77, respectively, resulted in lower fresh weight of multiple-seeded cockleburs as compared to the treatment without surfactant. But in case of 6 h dew, fresh weight gradually reduced with increase in surfactant rates. Our data demonstrate that even under lower dew period (6 h) greater control of multiple-seeded cocklebur can be achieved using higher rates of Activator 90 and Silwet L-77.

Introduction

Cocklebur (*Xanthium strumarium* L.) is an economically important weed of soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), and peanut (*Arachis hypogaea* L.) (Byrd and Coble 1991; Royal et al. 1997; Rushing and Oliver 1998). Bloomberg et al. (1982) showed that heavy infestation of cocklebur resulted in yield reduction of 50 to 75% in soybean. In addition to the substantial yield reduction, this weed is becoming a concern because several biotypes are resistant to some conventional herbicides. Abbas et al. (1996) showed 12 different biotypes of cocklebur, in which the biotypes from Bolivar county, Mississippi, are resistant to imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid), and the biotypes from Duck Hill county, Mississippi, are resistant to MSMA (monosodium methylarsonate). A common cocklebur biotype resistant to imidazolinone herbicides was found by Barrentine (1994). Haigler et al. (1988) identified a biotype resistant to the organic arsenical herbicides. Biotypes were identified on the basis of their morphological or phenological characteristics like leaf size and shape, bur mass, number of seedling produced per bur, blooming time (early or late) etc (Abbas et al. 1996).

Multiple-seeded cocklebur is a particular biotype of cocklebur, which has a different leaf and stem morphology as compared to the common cocklebur. The burs of the multiple-seeded cocklebur are large, round, covered with hairy spines or prickles, with each seed terminated by a beak (Abbas et al. 1999). This biotype has up to 25 seeds per bur, usually producing up to nine seedlings, whereas common cocklebur has two seeds per bur and usually produces only one seedling (Abbas et al. 1999). Higher seedling production ability increases the weediness of this particular biotype (Abbas et al. 1999; Barrentine 1974; Bloomberg et al. 1982; Buchanan and Burns 1971; Snipes et al. 1982).

As some biotypes of cocklebur have become resistant to the conventional herbicides (Barrentine 1994, Haigler et al. 1988 and 1994), the need for an alternative to the chemical control methods like biological control has become obvious. *Alternaria helianthi* (Hansf.) Tubaki & Nishih. has been well documented as a potential bio-control agent for cockleburs (Abbas and Egley 1996; Abbas and Barrentine 1995; Quimby, 1989). This is a pathogen of sunflower (*Helianthus annus* L.) and can infect other plants in compositae family (Allen et al. 1983).

The role of surfactants in activating the conidia or spores of bio-control agents (pathogens) to germinate and infect the weed hosts, has often been recognized (Boyette et al. 1996; Connick et al. 1990; Diagle and Connick 1990; Watson and Wymore 1990). Surface active agents play important role by modifying leaf wettability to improve spore deposition and retention on sprayed leaves and also by prolonging water retention to overcome dew period requirements (Green et al. 1998). However there are no guidelines for selecting the compatible surfactants for bio-control agents. Thus, there is a need to investigate the compatibility of surfactants for different pathogen-weed systems.

In the present study, the objective was to determine the effect of five surfactants at various rates on bioherbicidal activity of *Alternaria helianthi* on multiple-seeded cocklebur when followed by two dew periods.

Materials and methods

General

Seeds of multiple-seeded cocklebur were collected from plants growing at experimental plots at the Southern Weed Science Research Nursery, Stoneville, Mississippi, established from the seeds from Bell County, Temple, Texas, collected in 1995. The burs were soaked in water for a week before planting in a 1:1 potting mix of jiffy mix and soil, (Jiffy Mix, Jiffy Products of America, Inc., Batavia, IL 60510). Germinated seeds were transplanted to 10-cm^2 pots and grown in the greenhouse (28 to 33 C, 40 to 78% relative humidity and 12 h day length) until 6 to 8 leaf stage.

Preparation of fungal culture

Fungal cultures were taken from a stock culture of *A. helianthi* and inoculated in fresh sunflower leaf-agar medium prepared by the procedure described by Abbas et al. (1995). Dried sunflower leaves (25 g) were homogenized with 1 liter of distilled water in a blender at high speed for 5 min. The homogenate was centrifuged for 10 min. at 10,000 x g, and the supernatant was filtered through a double-layer cheesecloth. The filtrate was combined with 20 g of agar, autoclaved, and poured into 9-cm plates, 20 ml each. The plates were inoculated with *A. helianthi* and incubated at 18 C in alternating regimes of 14 h of fluorescent light at 165 E m^{-2} s^{-1} and 10 h of dark period. After 10 to 14 days of incubation, 5 ml of autoclaved distilled water was added to each plate and the conidia of *A. helianthi* were scraped from each plate and collected in autoclaved distilled water. Conidial suspension was homogenized by a polytron PT3000 (Brinkmann Instruments, Inc., Westbury, NY) and counted in a haemocytometer. The concentration of the conidial suspension was 1 x 10^5 per ml.

Effect of surfactants on bioherbicidal activity

Effect of five surfactants on the bioherbicidal activity of *A. helianthi* were studied on multiple-seeded cocklebur. The surfactants used in the present study were Tenkoz COC (crop oil concentrate), Activator 90 (nonionic surfactant), BAS 9050 O S (methylated oil), Silwet L-77 (organosilicone surfactant), and Top film (natural based). Taking "X" as the recommended rate for each surfactant, we used 0-X, ¼-X, ½-X, X, and 2-X rates for each of the surfactants. The actual rates used were 0, 0.25, 0.5, 1, and 2% (v/v) for Tenkoz COC; 0, 0.06, 0.125, 0.25, and 0.5% (v/v) for Activator 90; 0, 0.25, 0.5, 1, and 2% (v/v) for BAS 9050 O S; 0, 0.05, 0.1, 0.2, and 0.4% (v/v) for Silwet L-77; and 0, 0.06, 0.125, 0.25, and 0.5% (v/v) for Top film. Each surfactant rate was applied without and with *A. helianthi*. Surfactants were added to the conidial suspension right before spraying. The six- to eight-leaf seedlings were sprayed using a Spra-Tool (Crown Industrial Products Co., Hebron, IL, USA). The treated plants were subjected to two sets of immediate dew periods of 6 and 12 h before transferring to the greenhouse for 14 days. Plants were watered and fertilized (with N:P:K, 20:20:20) as needed. At the end of the experiment (two weeks after treatment), plant shoots were clipped at the soil surface and the fresh weights were determined. Experimental designs were completely randomized for all experiments and each treatment had three replications. Data were subjected to analysis of variance (ANOVA) using the general linear model procedures of the Statistical Analysis System (SAS Institute, Inc. 1999).

Results and Discussion

In all experiments, *A. helianthi* resulted in severe damage and fresh weight reduction of multiple-seeded cocklebur plants as compared to the treatments without the fungus and the fresh weight reduction was significant at 99% level of significance as indicated by the P values in the analysis of variance (Table 6.1). The disease symptoms appeared on infected plants as soft necrotic lesions on leaves and stems within 24 h of treatment. Necrotic lesions became dry and larger with time, resulting in death of some plants within 1 week depending on severity of symptoms. These data confirms the effectiveness of *A. helianthi* as a bioherbicidal pathogen for multiple-seeded cockleburs. This agrees with the findings of Abbas et al. (2004) where *A. helianthi* caused severe disease infestation and growth reduction of multiple-seeded cockleburs.

Duration of dew also had significant effect in all experiments as demonstrated by the *P* values at 99% level (Table 6.1). *Alternaria helianthi* resulted in reduction of fresh

Table 6.1 Summary of ANOVA on the effect of various surfactants with *A. helianthi* on multiple-seeded cocklebur under 6 and 12 h of dew periods.

	P Values				
Source[a]	Tenkoz COC	Activator 90	BAS 9050	Silwet L-77	Top Film
S	0.6569	0.0091	0.1418	<.0001	0.4008
A	<.0001	<.0001	<.0001	<.0001	<.0001
S X A	0.0945	0.0002	0.0830	<.0001	0.0512
D	<.0001	<.0001	<.0001	<.0001	<.0001
S X D	0.9535	0.0774	0.5287	0.0052	0.6419
A X D	<.0001	<.0001	<.0037	<.0001	<.0001
S X A X D	0.6883	0.8960	0.4941	0.3129	0.0399

[a] S = Surfactants at various rates; A = *A. helianthi* (with and without); and D = dew periods (6 h and 12 h).

weight under 12 h dew period as compared to 6 h dew at all rates and types of surfactants (Table 6.1). This agrees with previously published literature where it has been well documented that longer dew period increases the weed control activity of bioherbicidal plant pathogens (Abbas et al. 2004; Walker and Tilley 1997; Pitelli and Amorim 2003). Abbas et al. (2004) showed that irrespective of formulation used, *A. helianthi* resulted in 75 to 100% mortality of multiple-seeded and normal cockleburs under 24 h of dew period as compared to no mortality under 4 h of dew. Walker and Tilley (1997) reported that mortality of sicklepod (*Senna obtusifolia* L.) caused by *Myrothecium verrucaria* increased to 94% following a 6 h dew period as opposed to 0 h dew. Our results confirms the fact that certain duration of dew is a major requirement for effective biological control of weed species using plant pathogens.

P values in the analysis of variance shows that among five surfactants, Activator 90 and Silwet L-77 had significant effects on the bioherbicidal activity of *A. helianthi* in reducing fresh weight of multiple-seeded cocklebur, whereas, Tenkoz COC, BAS 9050, and Top Film did not have any effect (Table 6.1). Under 6 h dew period, *A. helianthi* reduced the fresh weight of cocklebur plants more at higher rates (0.5%) of Activator 90 as compared to treatments with lower rates (0.06%) or without surfactant (Figure 6.1). With a 12 h dew exposure, addition of Activator 90 reduced fresh weights as compared to the treatments without surfactant, and there is no variation in fresh weight among other rates of Activator 90. At all rates of Activator 90, *A. helianthi* reduced fresh weight of the multiple-seeded cocklebur plants more under 12 h dew period as compared to the 6 h dew. With Silwet L-77, *A. helianthi* resulted in gradual reduction of fresh weight of

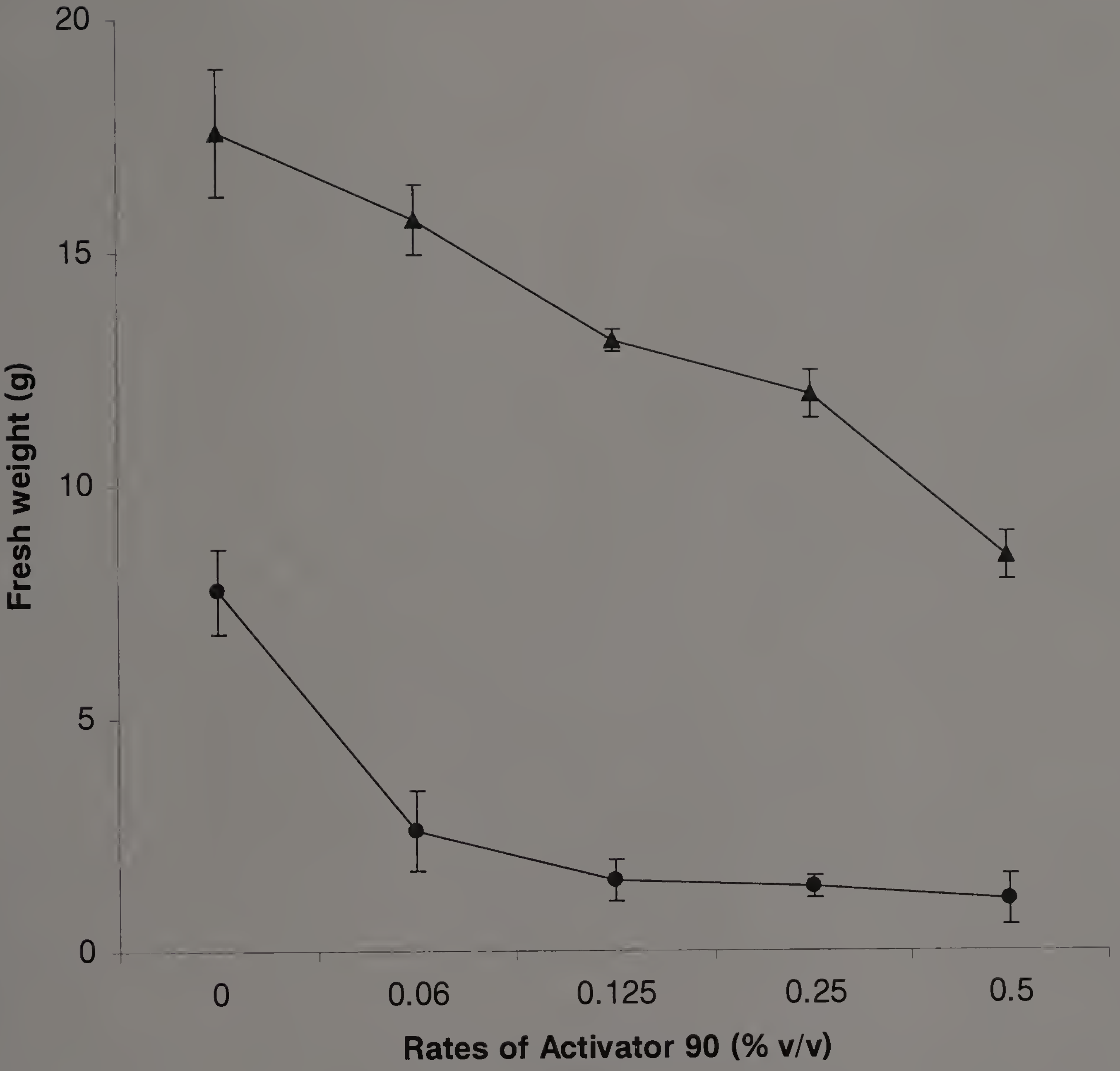

Figure 6.1 Effect of various rates of Activator 90 (% v/v) on fresh weight of multiple-seeded cocklebur treated with *Alternaria helianthi* followed by 6 h (▲) and 12 h (●) of dew exposure.

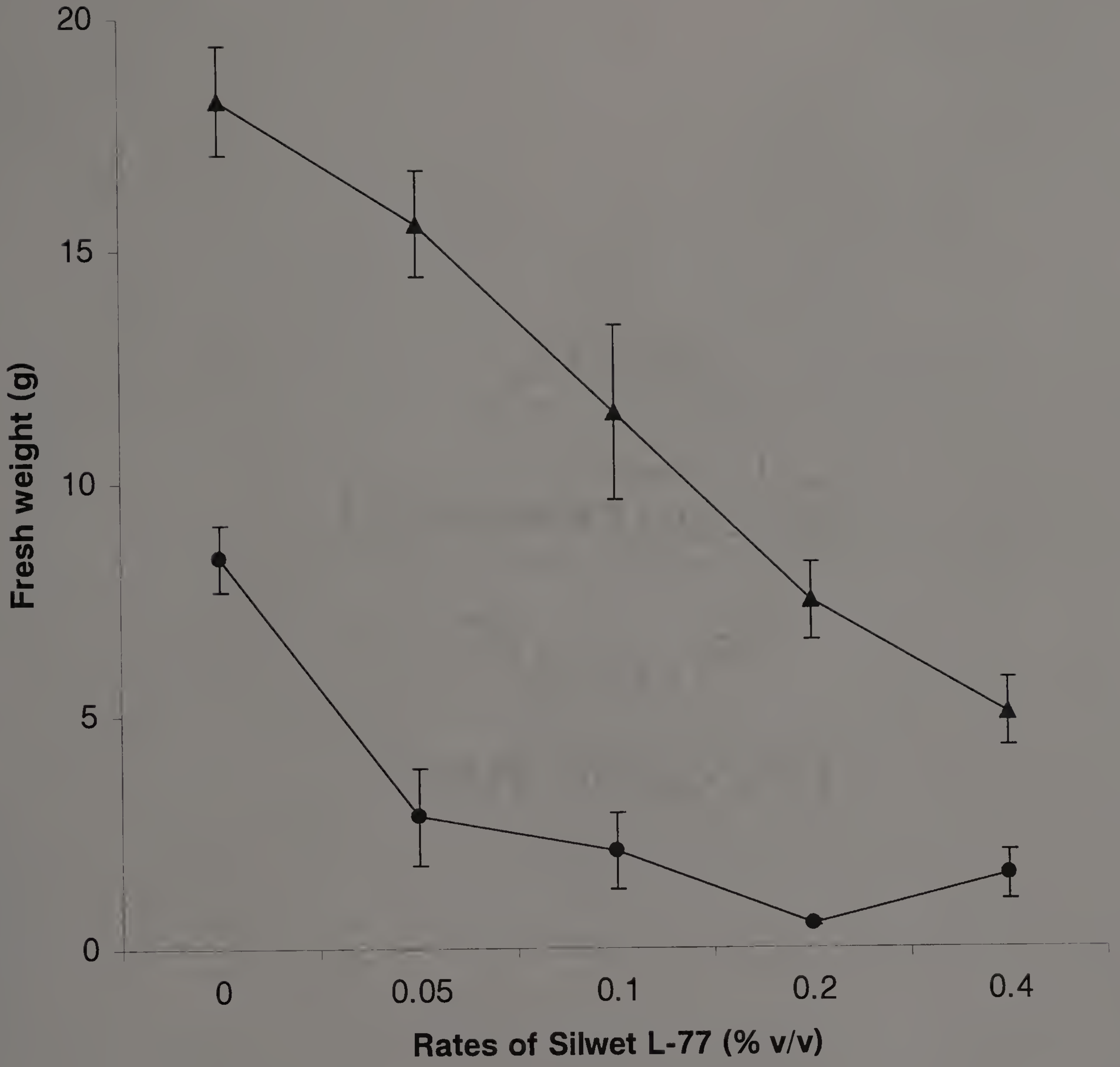

Figure 6.2 Effect of various rates of Silwet L-77 (% v/v) on fresh weight of multiple-seeded cocklebur treated with *Alternaria helianthi* followed by 6 h (▲) and 12 h (●) dew exposure.

multiple-seeded cocklebur plants with increased surfactant rates when followed by a 6 h dew period (Figure 6.2). Fresh weights were significantly lower at 0.2 and 0.4% rate of Silwet L-77, as compared to 0.05% rates. Silwet L-77, at 0.4% rate caused some injury to the leaves of control plants (plants treated with 0.4% Silwet L-77 in sterilized water without any fungal conidia) within 24 h of treatment (Figure 6.3). Walker and Tilley (1997) observed similar phytotoxicity due to 0.4% ratte of Silwet L-77 on sicklepopd. Under 12 h of dew exposure, Silwet L-77 at all rates (0.05, 0.1, 0.2, and 0.4%) reduced fresh weights as compared to the treatment without surfactant. At the two highest rates (0.2 and 0.4%) cocklebur plants were completely dead two weeks after treatment when followed by12 h dew period (data not shown). Previous research also showed that *A. helianthi* when applied with Silwet L-77 caused upto 100% mortality of normal and multiple-seeded cockleburs (Abbas et al. 2004).

Overall, *A. helianthi* resulted in higher control of multiple-seeded cockleburs in terms of fresh weight when followed by a 12 h dew period as compared to a 6 h dew. With 12 h of dew exposure, the addition of even 0.05 or 0.06% rates of Activator 90 or Silwet L-77, respectively, resulted in lower fresh weight of multiple-seeded cocklebur as compared to the treatment without a surfactant. But in case of 6 h dew, fresh weight gradually reduced with increase in surfactant rates. Our data demonstrates that even under less dew period (6 h) better control of multiple-seeded cocklebur can be achieved using higher rates of Activator 90 and Silwet L-77 with *A. helianthi*. Enhanced bioherbicidal activity by specific adjuvants was previously reported by Babu et al. (2003). They showed that the application of *Alternaria alternata* in an oil emulsion

Figure 6.3 Effect of Silwet L-77 at 0.4 and 0.2% rates (v/v) on multiple-seeded cocklebur 24 h after treatment.

enhanced disease incidence on waterhyacinth (*Eichhornia crassipes*) as compared to a 0.1% solution of Tween 80.

Activator 90 and Silwet L-77 have shown potential for achieving effective biological control of multiple-seeded cockleburs by *A. helianthi*. The precise mode by which these surfactants enhance the activity of *A. helianthi* is yet to be determined. Surfactants may modify leaf wettability causing fungal conidia to adhere more closely to leaf tissues than fungal conidia in water. Specific surfactants may prolong water retention to overcome dew period requirements as suggested by Green et al. (1998). Silwet L-77 may stimulate spore germination of *A. helianthi*, allowing conidia to produce multiple germ tubes to penetrate stomata or possibly woonds caused by Silwet L-77 as well. Abbas and Egley (1996) showed that germination and germ tube production of *A. helianthi* on cocklebur leaves increased with addition of Silwet L-77 or corn oil. Previous research demonstrated that Silwet L-77 promoted the activity of fungi and bacteria on the leaves of their host kudzu (*Pueraria lobata*) (Boyette et al. 2002; Zidak et al. 1992).

Multiple-seeded cocklebur has characteristics that suggests that it might be difficult to control, particularly the fact that it produces several seedlings per bur, giving possibility of faster spread. However, the results of the current study demonstrate that multiple-seeded cocklebur is highly susceptible to *A. helianthi*, and its effectiveness can be definitely enhanced by use of appropriate surfactants in proper rates with a minimum dew period. As these experiments were tested in the greenhouse, more research is needed in field situation for better understanding of the feasibility of this approach. Research to enhance the activity of *A. helianthi* against other biotypes of cocklebur is also needed.

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CHAPTER VII

SUMMARY

In this research, the micro-morphology of three broadleaf and two grass species was studied and the leaf wax content was quantified. The contact angle and spread area of primisulfuron droplets were measured and the activity was measured without and with various surfactants. Influence of various surfactants on bioherbicidal activity of *Alternaria helianthi* was also examined.

The number of stomata on abaxial surface was more than on adaxial leaf surface of common lambsquarters and velvetleaf, whereas, common purslane had more stomata on adaxial surface than abaxial. The younger leaves of three broadleaf species had higher number of stomata per unit area of leaf surface as compared to the older leaves. "Bladder hairs" were present in common lambsquarters and the abaxial surface had dense bladder hairs. Velvetleaf had glands and star shaped trichomes. The number of the trichomes was higher on adaxial surfaces, although trichomes on the abaxial surface were more branched and larger in size. Common lambsquarters had the highest wax content per unit of leaf area (274.5 $\mu g\ cm^{-2}$) and velvetleaf had the lowest (7.4 $\mu g\ cm^{-2}$). Wax content of common purslane was 153.4 $\mu g\ cm^{-2}$. Organosilicone surfactant enhanced the spread of primisulfuron droplets significantly more than the nonionic surfactant, and the spread area was larger in case of velvetleaf as compared to common lambsquarters or common purslane.

In green foxtail, the number of stomata per unit area was more on adaxial surface as compared to abaxial, whereas, barnyardgrass had more stomata per unit area on abaxial surface than adaxial. The younger leaves of green foxtail and barnyardgrass had higher number of stomata per mm^2 of leaf area as compared to the older leaves. There were no significant differences in the number of trichomes per unit leaf area on both sides of green foxtail. In case of barnyardgrass, more trichomes were present on abaxial surface as compared to adaxial surface.

The younger leaves of green foxtail and barnyardgrass had higher number of trichomes per mm^2 of leaf area as compared to the older leaves. Green foxtail leaves had tough pointed prickles on both surfaces and the number was significantly higher in adaxial surfaces than the abaxial. The mean values of wax content per unit of leaf area in barnyardgrass and green foxtail were 35.91 $\mu g\ cm^{-2}$ and 19.14 $\mu g\ cm^{-2}$, respectively, and the difference was not statistically significant. On both barnyardgrass and green foxtail, primisulfuron droplets with a nonionic surfactant had more spread area than that without a surfactant. Organosilicone surfactant resulted in maximum spread of primisulfuron droplets. The spread of primisulfuron droplets when applied with surfactants was higher on the leaf surface of barnyardgrass than the spread on green foxtail leaves. These results indicate that herbicide spread is not dependent solely on the wax content per unit area of leaf surface.

Addition of surfactants had significant effect on contact angle of primisulfuron droplets on the leaf surface of common lambsquarters, common purslane, and velvetleaf. Both nonionic surfactant and organosilicone surfactant reduced contact angles of spray droplets as compared to that of without a surfactant. However, organosilicone surfactant when mixed with primisulfuron resulted in lower contact angle than nonionic surfactant on all three species. Addition of surfactants increased the spread area on all three weed species and the droplet spread was more in case of common lambsquarters and velvetleaf as compared to common purslane. On all species, organosilicone resulted in larger spread area as compared to nonionic surfactant. In general, activity of primisulfuron was higher in controlling common lambsquarters, common purslane, and velvetleaf when applied with surfactant as compared to the treatments without any surfactant. In common lambsquarters, there were no differences between organosilicone and nonionic surfactant treatments, when applied with primisulfuron. When organosilicone surfactant was added to primisulfuron, plant injury was severe with lowest fresh weight of common purslane. At higher rates (60 and 80 g ai ha^{-1}) of primisulfuron, there was no difference in velvetleaf injury between two surfactant treatments.

Organosilicone reduced the contact angle and increased the spread area of the primisulfuron droplets more on barnyardgrass and green foxtail. It was evident from our data that primisulfuron had no effect on barnyardgrass at the rates tested in this study and therefore no effect of surfactant was noted. Surfactants increased primisulfuron activity on green foxtail. The scanning electron micrographs revealed that primisulfuron when applied without surfactant formed more bulky and crusty deposits on the leaf surface which may trap primisulfuron in nonsoluble surroundings or increase diffusion distance accounting for reduced primisulfuron absorption and efficacy. With nonionic surfactant, the dried herbicide deposits were smaller which allowed a closer leaf contact and increased the chance of herbicide absorption. With organosilicone surfactant, the spray deposits appeared to blend uniformly into the cuticle, which probably facilitate primisulfuron absorption into the leaf tissue resulting in enhanced activity.

It was evident from our data that surfactants increased primisulfuron activity on common lambsquarters, common purslane, velvetleaf, and green foxtail. The influence of various surfactants on herbicidal activity varied with weed species. In general, organosilicone reduced the contact angle with increased spread area of primisulfuron droplets more than the nonionic surfactant treatments and resulted in greater activity.

The results of the current study demonstrate that multiple-seeded cocklebur is susceptible to *Alternaria helianthi*, and its effectiveness can be enhanced by use of appropriate surfactants in proper rates with a minimum dew period. *A. helianthi* resulted in significant reduction of fresh weight of multiple-seeded cocklebur when followed by a 12 h dew period as compared to a 6 h dew. Even under short dew period (6 h), better control of multiple-seeded cocklebur was achieved using higher rates of Activator 90 and Silwet L-77 and has potential for achieving effective biological control.

These results support the concept that leaf morphological characteristics of weed species influence the behavior of herbicide on leaf surface. The differential activity of a given herbicide can be optimized by using specific surfactant. Addition of organosilicone surfactant 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. Addition of surfactant also enhanced the bioherbicidal activity of *A. helianthi* on multiple-seeded cocklebur.

APPENDIX

ANOVA TABLES

Table A.1 Summary of ANOVA for number of bladder hairs per unit area of 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.

Source[1]	*P* values
L	<.0001
F	<.0001
L*F	0.7163

[1] Sources of variations were leaf age (young and old) and leaf surface (adaxial and abaxial). Leaf age, leaf surface, and their interactions were denoted by L, F, and L*F, respectively.

Table A.2 Summary of ANOVA for number of glands and trichomes 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 velvetleaf.

	Source[1]	*P* values
Glands	L	<.0001
	F	0.0824
	L*F	0.6559
Trichomes	L	<.0001
	F	<.0001
	L*F	<.0001

[1] Sources of variations were leaf age (young and old) and leaf surface (adaxial and abaxial). Leaf age, leaf surface, and their interactions were denoted by L, F, and L*F, respectively.

Table A.3 Summary of ANOVA for 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.

Source[1]	*P* values
S	<.0001
L	<.0001
S*L	<.0001
F	<.0001
S*F	<.0001
L*F	0.0775
S*L*F	<.0001

[1] Sources of variations were weed species (common lambsquarters, common purslane, and velvetleaf), leaf age (young and old) and leaf surface (adaxial and abaxial). Weed species, leaf age, and leaf surface were denoted by S, L, and F, respectively.

Table A.4 Summary of ANOVA for wax content per unit of leaf area in common lambsquarters, common purslane, and velvetleaf.

Source[1]	*P* values
S	0.0023

[1] Source of variation was weed species (S).

Table A.5 Summary of ANOVA for spread area of 1µl droplets of distilled water (W), primisulfuron (P), primisulfuron with a nonionic surfactant (P+NIS), and primisulfuron with an organosilicone surfactant (P+OWA). Primisulfuron was used at 39.5 g ai ha^{-1}, nonionic surfactant at 0.25% (v/v), and the organosilicone surfactant at 0.1% (v/v).

Source[1]	*P* values
S	<.0001
T	<.0001
S*T	<.0001

[1] Sources of variations were weed species (common lambsquarters, common purslane, and velvetleaf) and treatments (distilled water, primisulfuron, primisulfuron with a nonionic surfactant, and primisulfuron with an organosilicone wetting agent). Weed species, treatments, and their interactions were denoted by S, T, and S*T, respectively.

Table A.6 Summary of ANOVA for number of stomata per unit area of adaxial and abaxial leaf surfaces of young (first leaf from the tip) and old (third or forth leaf from the tip) leaves of barnyardgrass and green foxtail.

Source[1]	*P* values
S	<.0001
L	<.0001
S*L	0.4208
F	0.8059
S*F	<.0001
L*F	0.6996
S*L*F	0.2495

[1] Sources of variations were weed species (barnyardgrass and green foxtail), leaf age (young and old) and leaf surface (adaxial and abaxial). Weed species, leaf age, and leaf surface were denoted by S, L, and F, respectively.

Table A.7 Summary of ANOVA for number of trichomes on adaxial and abaxial leaf surfaces of young (first leaf from the tip) and old (third or forth leaf from the tip) leaves of barnyardgrass and green foxtail.

Source[1]	*P* values
S	<.0001
L	<.0001
S*L	<.0001
F	<.0001
S*F	<.0001
L*F	0.1369
S*L*F	0.1369

[1] Sources of variations were weed species (barnyardgrass and green foxtail), leaf age (young and old) and leaf surface (adaxial and abaxial). Weed species, leaf age, and leaf surface were denoted by S, L, and F, respectively.

Table A.8 Summary of ANOVA for number of prickles on adaxial and abaxial leaf surfaces of young (first leaf from the tip) and old leaves (third or forth leaf from the tip) of green foxtail.

Source[1]	*P* values
L	<.0001
F	<.0001
L*F	0.9291

[1] Sources of variations were leaf age (young and old) and leaf surface (adaxial and abaxial). Leaf age, leaf surface, and their interactions were denoted by L, F, and L*F, respectively.

Table A.9 Summary of ANOVA for wax content per unit of leaf area in barnyardgrass and green foxtail.

Source[1]	*P* values
S	0.2325

[1] Source of variation was weed species (S).

Table A.10 Summary of ANOVA for spread area of 1μl droplets of distilled water (W), primisulfuron (P), primisulfuron with a nonionic surfactant (P+NIS), and primisulfuron with an organosilicone surfactant (P+OSS) on leaf surface of barnyardgrass and green foxtail. Primisulfuron was used at 39.5 g ai ha^{-1}, nonionic surfactant at 0.25% (v/v), and the organosilicone surfactant at 0.1% (v/v).

Source[1]	*P* values
S	<.0001
T	<.0001
S*T	<.0001

[1] Sources of variations were weed species (barnyardgrass and green foxtail) and treatments (distilled water, primisulfuron, primisulfuron with a nonionic surfactant, and primisulfuron with an organosilicone surfactant). Weed species, treatments, and their interactions were denoted by S, T, and S*T, respectively.

Table A.11 Summary of ANOVA for contact angle of 1μl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of common lambsquarters, common purslane, and velvetleaf. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v).

Source[1]	*P* values
S	<.0001
T	<.0001
S*T	<.0001

[1] Sources of variations were weed species (common lambsquarters, common purslane, and velvetleaf) and treatments (distilled water, primisulfuron, primisulfuron with a nonionic surfactant, and primisulfuron with an organosilicone surfactant). Weed species, treatments, and their interactions were denoted by S, T, and S*T, respectively.

Table A.12 Summary of ANOVA for spread area of 1μl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of common lambsquarters, common purslane, and velvetleaf. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v).

Source[1]	*P* values
S	<.0001
T	<.0001
S*T	<.0001

[1] Sources of variations were weed species (common lambsquarters, common purslane, and velvetleaf) and treatments (distilled water, primisulfuron, primisulfuron with a nonionic surfactant, and primisulfuron with an organosilicone surfactant). Weed species, treatments, and their interactions were denoted by S, T, and S*T, respectively.

Table A.13 Summary of ANOVA for contact angle of 1μl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of barnyardgrass and green foxtail. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v).

Source[1]	*P* values
S	<.0001
T	<.0001
S*T	<.0001

[1] Sources of variations were weed species (barnyardgrass and green foxtail) and treatments (distilled water, primisulfuron, primisulfuron with a nonionic surfactant, and primisulfuron with an organosilicone surfactant). Weed species, treatments, and their interactions were denoted by S, T, and S*T, respectively.

Table A.14 Summary of ANOVA for spread area of 1μl droplets of primisulfuron without any surfactant (P), with nonionic surfactant (P+NIS), and with organosilicone surfactant (P+OSS) on the leaf surface of barnyardgrass and green foxtail. Primisulfuron was used at 40 g ai ha^{-1}, NIS at 0.25% (v/v), and OSS at 0.1% (v/v).

Source[1]	*P* values
S	<.0001
T	<.0001
S*T	0.0042

[1] Sources of variations were weed species (barnyardgrass and green foxtail) and treatments (distilled water, primisulfuron, primisulfuron with a nonionic surfactant, and primisulfuron with an organosilicone surfactant). Weed species, treatments, and their interactions were denoted by S, T, and S*T, respectively.

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