Alterations of Leaf Epicuticular Wax of Peanut (*Arachis hypogaea*) by Applications of Herbicides and Adjuvants[1]

J.A. Baysinger, H.A. Melouk, and D.S. Murray[2*]

ABSTRACT

Leaf surface morphology of untreated peanut leaves and peanut leaves treated with herbicides and adjuvants were examined using scanning electron microscopy. Electron micrographs revealed that the adaxial surface of untreated peanut leaves was covered with crystalline wax platelets above an amorphous layer of wax. Electron micrographs revealed that peanut leaves treated with acifluorfen plus nonionic surfactant, bentazon and lactofen with crop oil concentrate, and 2,4-DB, altered the leaf surface morphology when compared to nontreated peanut leaves. Alterations in the leaf epicuticular wax structures appeared amorphous-like rather than normal plate-like structures. Nonionic surfactant and crop oil concentrate applied alone to peanut leaves altered the epicuticular wax structures similarly to that of herbicides plus adjuvants.

Key Words: Leaf surface morphology, scanning electron microscopy.

Postemergence (POST) herbicide applications are important for weed control and for economical peanut production. Surfactants or spray adjuvants are used with many POST herbicide spray solutions to enhance activity on weeds (Hull *et al.* 1982; Wanamarta and Penner, 1989). The role of adjuvants is to aid in the surface spreading and penetration properties of the herbicide through the leaf cuticle of the target species (Price, 1982; Wanamarta and Penner, 1989; Hess and Falk, 1990). However, some spray from topical applications is intercepted by the peanut plant, causing temporary injury to peanut foliage. Contact herbicides that cause this crop injury such as lactofen {(±)-2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate} usually have been reported to have minimal effects on peanut pod yield (Wilcut *et al.*, 1990; Jordan *et al.*, 1993).

Plant cuticles consist of waxes, pectin, cutin, and cellulose material (Eglinton and Hamilton, 1967; Hull *et al.*, 1982; Wanamarta and Penner, 1989). The composition of these cuticular components varies with plant species. The cuticle provides a barrier between the environment and the plant's internal cells, and the cuticle is the first plant structure to be attacked by insects or plant pathogens (Martin and Juniper, 1970). The cuticle surface wax, or epicuticular wax, is an important barrier to ion and water movement across the cuticle (Adams *et al.*, 1990). This wax is made up of crystalline deposits that overlay the cuticle as plate, ribbon, tube, or rod-like structures (Baker, 1982). The amount of epicuticular wax varies with plant species and environment. Plant leaves with thicker deposits of wax tend to be more hydrophobic, thus decreasing water droplet and herbicide spray retention and possibly providing greater resistance to infection by pathogens (Martin and Juniper, 1970; Adams *et al.*, 1990; Hess and Falk, 1990).

Numerous researchers have reported on the effects of herbicide spray formulations and surfactants on leaf surface characteristics (Crafts and Foy, 1962; Jansen, 1964; Franke, 1967; Hull, 1970; Still *et al.*, 1970; Sands and Bachelard, 1973; Hart and Price, 1979; Whitehouse *et al.*, 1982; Kuzych and Meggitt, 1983). Whitehouse *et al.* (1982) suggested that certain herbicides may partition into the epicuticular wax more readily than others causing an alteration in the wax barrier, reducing foliar entry of other herbicides. Several reports (Sands and Bachelard, 1973; Hart and Price, 1979; Whitehouse *et al.*, 1982; Kuzych and Meggitt, 1983) identified leaf surface alterations by herbicides through the use of scanning electron microscopy (SEM). In one report (Sands and Bachelard, 1973), SEM micrographs showed that the surfactant Tween® 20 (formerly a product of ICI Americas, Inc.; currently owned by Zeneca Agric. Corp., Wilmington, DE) dissolved some leaf surface wax of *Eucalyptus polyanthemos* Schau. and altered the physical form of the remaining surface wax to globular appearing formations.

The effect of POST herbicides on peanut leaf surfaces has not been well studied, nor have the leaf surface morphology and epicuticular wax formations been adequately illustrated. Therefore, it is difficult to assess any direct effects herbicides may have on peanut epicuticular wax functions (i.e., barrier to insects and pathogens). The objective of this study was to examine and illustrate the response of several POST-applied herbicides and adjuvants on the adaxial peanut leaf surface topography, specifically the epicuticular wax, with the use of SEM.

Materials and Methods

Plant Material Used. Peanut seed (cv. Okrun) were planted in individual 12-cm diameter containers in the greenhouse with a medium of soil, sand, and finely shredded peat (1:1:2, v/v/v). Greenhouse air temperature during the day was 28 ± 3 C, the night temperature was 22 ± 3 C, and relative humidity was 65 ± 20%. To insure uniformity in epicuticular wax structures, leaf samples were collected

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from different peanut plants at the same vegetative growth stage (i.e., node number).

Herbicides. Four weeks after planting (WAP), 12- to 14-cm tall peanut plants were treated with commercial formulations of herbicides and adjuvants using a laboratory table sprayer equipped with an 8002 even flat fan nozzle delivering 140 L/ha. Herbicide treatments were acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid} at 0.6 kg ai/ha, bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] at 0.8 kg ai/ha, lactofen at 0.2 kg ai/ha, and 2,4-DB [4-(2,4-dichlorophenoxy)butanoic acid] at 0.5 kg ai/ha. The adjuvants used were: crop oil concentrate (COC) (Cornbelt® crop oil concentrate, Cornbelt Chemical Co., McCook, NE) applied at a rate equivalent to 2.3 L/ha and nonionic surfactant (NIS) (Triton AG-98®, Rohm and Haas Co., Philadelphia, PA) applied at 0.25% v/v. Acifluorfen and lactofen treatments were in combination with NIS and bentazon was in combination with COC, herbicide-adjuvant combinations commonly used. No additional adjuvant was used with 2,4-DB. Each adjuvant was applied alone as a treatment for comparison with the other herbicide treatments and to illustrate any leaf surface activity.

Scanning Electron Microscopy. Treated peanut plants were transferred to the Oklahoma State Univ. Electron Microscopy Laboratory for evaluation. Technicians prepared leaf samples using procedures and equipment that were routinely used at the electron microscopy laboratory. Treated peanut leaves were excised 1 wk after herbicide/adjuvant application and placed in 2% gluteraldehyde in 0.1 M sodium carodylate buffer at pH 7.2 for 3 wk. The samples were then given three 20-min buffer washes (0.1 M sodium carodylate buffer pH 7.2) and dehydrated in a graded ethanol series of 50, 70, 90, 95, and 100%. The tissue remained in the alcohol for 20 min each, ending with three changes of 100% for 20 min each. The samples were critical point dried in a liquid CO_2 critical point dryer (Tousimis PVT-3 CPD, Tousimis Res. Corp., Rockville, MD). Specimens were mounted on aluminum stubs with double sticky tape and were coated with 200 / of gold and palladium using a sputter coater (Hummer II. Techniques, Alexandria, VA). All SEM examinations were performed with a JEOL-JSM 35U scanning electron microscope [JSM 35U. JEOL (USA), Peabody, MA] and photographed at accelerating potentials of 25kV.

This experiment was conducted two times with each herbicide treatment replicated four times. Photographs presented in this report were selected for their clarity and are representative of numerous SEM micrographs taken from each treatment and experiment.

Results and Discussion

Scanning Electron Microscopy. The SEM micrographs show that the adaxial surface of nontreated peanut leaflets was covered with well developed crystalline wax platelets above an amorphous layer of wax (Fig. 1A), and resembles that of micrographs of pea (*Pisum sativum* L.) in previous reports (Still *et al.*, 1970; Davis, 1971; Stevens and Baker, 1987; Ruiter *et al.*, 1990). The crystalline wax formations appear to be less abundant on the periclinal walls of guard cells compared to the surrounding area. This similarity has been noted by other researchers with different plant species (Stevens and Baker, 1987; Hess and Falk, 1990). The adaxial surface was found to be stomatous and free of trichomes.

Alterations of peanut leaf epicuticular wax were very evident with applications of acifluorfen plus NIS (Fig. 1B). Areas of herbicide deposition appeared very dark and smooth in texture. The epicuticular wax structures were altered, resulting in an amorphous appearance. Nalewaja *et al.* (1992) reported dark areas below glyphosate [*N*-(phosphonomethyl)glycine] crystal deposits seen on micrographs of common sunflower (*Helianthus annuus* L.) leaves. These areas may represent cuticle injury and phytotoxicity from the herbicide. It is not known whether the original amount of wax was still present in those areas of herbicide deposition or if it was reduced. We do not rule out the possible presence of some epicuticular wax on the leaf surface but it may be in the form of a continuous sheet, with no crystalline, plate-like structures. Acifluorfen applied to soybean [*Glycine max* (L.) Merr.] produced similar results as previously reported (Hess and Falk, 1990).

SEM micrographs of NIS applied without a herbicide (Fig. 1C) illustrate similar results as those with the combination of acifluorfen plus NIS. This suggests that NIS is a major component in the alteration of peanut epicuticular wax. Takeno and Foy (1974) reported that a lipophilic polysorbate surfactant altered the ultrastructure of epicuticular wax on cotton (*Gossypium hirsutum* L.) leaves, but they noticed no erosion of the surface wax. In a more recent report, Falk *et al.* (1994) reported that certain surfactants induced phytotoxicity to several plant species but no morphological changes in surface wax were observed. In other reports, (Kuzych and Meggitt, 1983; Knoche *et al.*, 1992) applications of surfactants altered the leaf wax morphology in *Brassica* species.

Peanut leaves treated with lactofen plus NIS had epicuticular wax alterations along with significant cell damage (Fig. 1D). The loss of cell membrane integrity is the characteristic mode of action of lactofen, a diphenyl-ether herbicide (Weed Science Soc. Amer., 1994). Acifluorfen, another diphenyl-ether, did not damage cell membranes to the extent that lactofen did; therefore, peanut leaf necrosis was visually greater with lactofen treatment (data not shown). Acifluorfen-treated peanut leaves had minimal leaf tissue necrosis and were lightly bronzed in appearance.

Applications of COC alone altered the epicuticular wax of peanut into an amorphous-layered structure (Fig. 2A). The crystalline structures appear to have been altered in the center of the spray deposition areas and the effect gradually lessened toward the outer edges. When bentazon was added to COC, micrographs of treated leaflets showed little difference in epicuticular wax alteration compared to peanut treated with COC alone (Fig. 2B). The only difference observed was more particulate deposits on the leaf surface. This may be due to the nature of the commercial formulation of bentazon in solution.

Peanut treated with 2,4-DB had epicuticular wax alterations similar to the peanut treated with bentazon and COC (Fig. 2C). There were no adjuvants added to 2,4-DB so epicuticular wax alterations were related solely

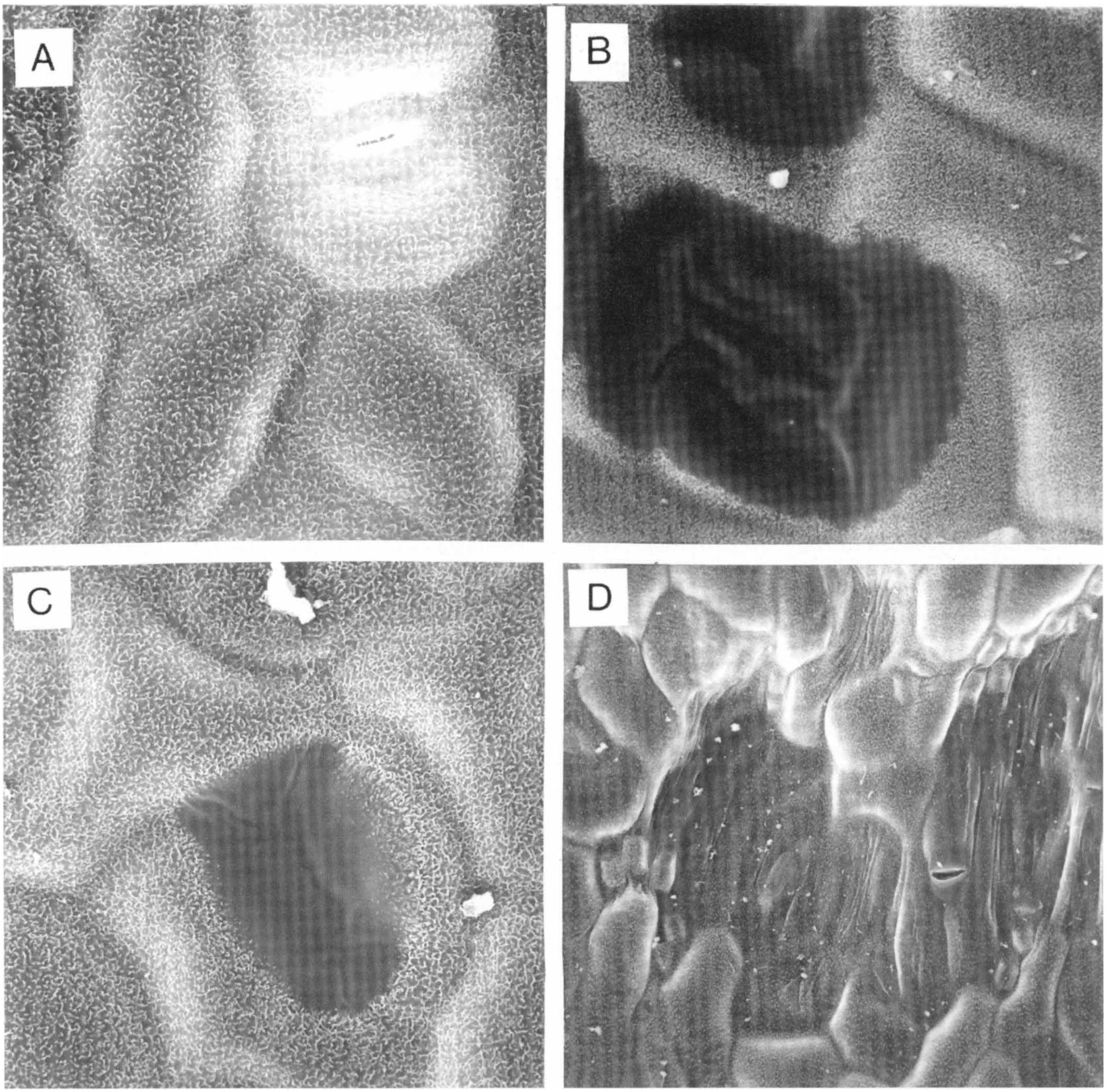

Fig. 1. A. Scanning electron micrograph of the adaxial leaf surface of an untreated peanut. B. Acifluorfen (0.6 kg ai/ha in 140 L/ha water carrier) and nonionic surfactant (0.25% v/v) applied to peanut (the dark areas depict crystalline degradation by herbicide and surfactant droplet). C. Nonionic surfactant (0.25% v/v in 140 L/ha water carrier) applied to peanut. D. Lactofen (0.2 kg ai/ha in 140 L/ha water carrier) and nonionic surfactant (0.25% v/v) applied to peanut (note the ruptured cell membranes). Magnification 1000.

to 2,4-DB. There was no leaf tissue necrosis caused by 2,4-DB; however, plant hormone regulating symptoms were noticed. It is not understood if this injury may be the reason for the leaf wax alterations. One possibility is that the nature of the amine salt formulation of 2,4-DB could have contributed to the wax alterations.

These observations indicate and illustrate that different herbicides and adjuvants, under controlled conditions, cause morphological modifications to peanut leaf epicuticular wax. Peanut plant response to herbicides can be different in greenhouse conditions compared to that in the field. The use of SEM has been very useful in demonstrating the leaf epicuticular wax alterations. This information may be helpful in explaining some of the phytotoxic activity that occurs with the use of these herbicides and possibly the effects this activity may have on other organisms (i.e., pathogens, insects) that share the same environment. The information obtained from this study will be valuable in future research that involve herbicides and peanut leaf cuticles. For example, re-

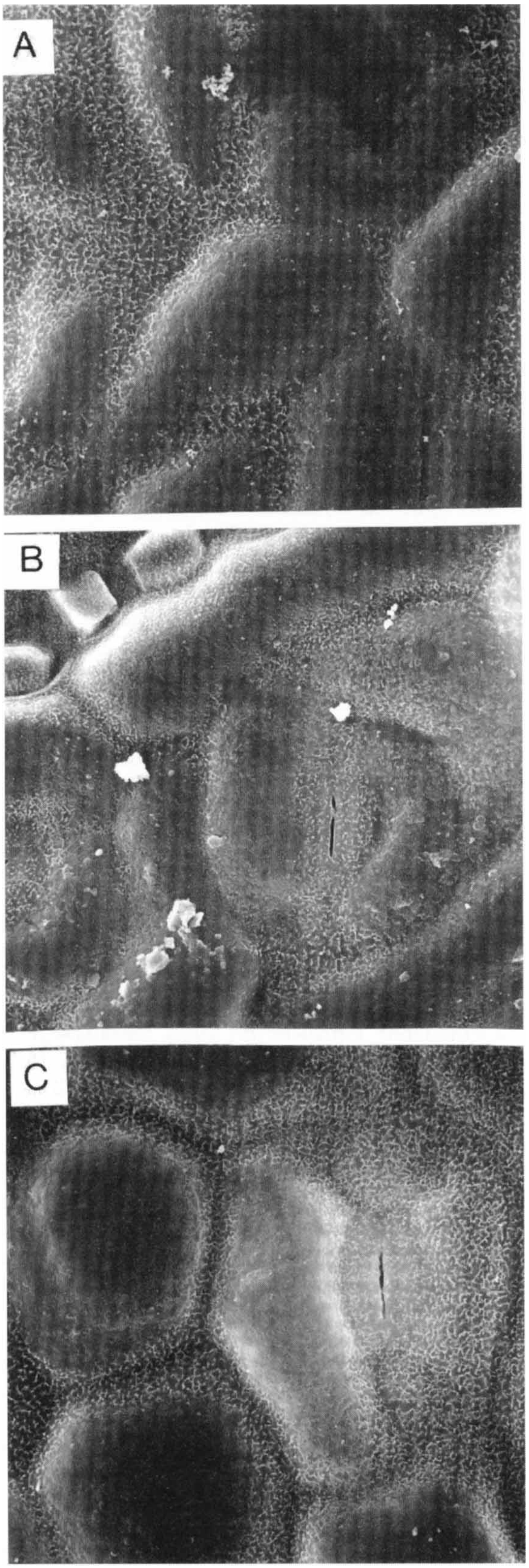

Fig. 2. A. Crop oil concentrate (2.3 L/ha in 140 L/ha water carrier) applied to peanut. B. Bentazon (0.8 kg ai/ha in 140 L/ha water carrier) and crop oil concentrate (2.3 L/ha) applied to peanut. C. 2,4-DB (0.5 kg ai/ha in 140 L/ha water carrier) applied to peanut. Magnification 1000.

searchers may find a benefit of altered peanut leaf cuticles as a means for understanding pest management or understanding why plant responses are so variable at times.

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