INTERACTIONS OF SURFACTANTS WITH PLANT LEAVES: INDUCTION OF PHYTOTOXICITY AND ETHYLENE PRODUCTION IN RELATION TO SURFACTANT CHEMISTRY

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ABSTRACT

INTERACTIONS OF SURFACTANTS WITH PLANT LEAVES: INDUCTION OF PHYTOTOXICITY AND ETHYLENE PRODUCTION IN RELATION TO SURFACTANT CHEMISTRY

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Surfactant-induced phytotoxicity and ethylene production were studied using cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Dixielee') seedlings. Phytotoxicity appeared as discolored and sunken areas at the periphery of the droplet:leaf interface area resulting from the loss of structural integrity and collapse of epidermal, palisade and mesophyll cells. Phytotoxicity was localized beneath the treatment site and there was no evidence of tissue recovery. Selected spray application factors (surfactant concentration, drop volume, temperature and humidity) affected both the rate of development and the degree of phytotoxicity. Ethylene production was induced in cowpea leaves by selected surfactants of each ionic class (nonionic, anionic and cationic). The rate of ethylene production increased significantly the first 6 to 12 hr after treatment and then decreased for the next 12 to 36 hr, returning to control levels within 48 hr. Surfactant activity was affected by chemistry and concentration. There was a close relationship between ethylene oxide (EO) chain length and ethylene production. For octylphenol (OP) surfactants, ethylene production decreased log linearly with increasing EO. In contrast, for linear alcohol surfactants the relationship between EO chain length and ethylene production was nonlinear with greatest biological activity at intermediate (8-12) EO content. Activity of a short chain (OP+1EO) non-water soluble surfactant was significantly increased when combined with a long chain (OP+40EO) non ethylene producing surfactant. Surfactant-induced ethlyene production was also induced in a variety of annuals and selected perennial tree crops. In all studies there was a strong positive relationship between phytotoxicity and ethylene production. Surfactants affected the production of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, and its conversion to ethylene. Triton X-100 increased ACC production, and when foliar-applied with 1-naphthaleneacetic acid or ACC, significantly increased ethylene production. The apparent rate constant for conversion of endogenous ACC to ethylene was increased, most notably following treatment with NAA. The treated tissue contained significantly higher ACC levels, but the ACC was not translocated from the treatment site and its conversion to ethylene remained localized as well.

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Guidance Committee:

The journal-article format was adopted for this dissertation in accordance with departmental and university requirements. Sections I, II and IV were prepared and styled for publication in the *Journal of the American Society for Horticultural Science*. Section III was prepared and styled for publication in the *Journal of Agriculture and Food Chemistry*.

INTRODUCTION

Surfactants are frequently applied to plants as components of foliar sprays. Therefore, surfactant inter-actions with the active ingredient and/or plant may play a significant role in determining the overall performance of foliar applied compounds (5). Studies on these interactions, however, have been limited.

Most agriculturally useful surfactants are composed of hydrophilic and hydrophobic moieties in a single molecule. The hydrophobic moiety may consist of a long chain hydrocarbon or aromatic ring structures while the hydrophilic moiety is derived from ionizable groups or, in the case of nonionic surfactants, often by ether oxygens contained in a polyoxyethylene (EO) chain (28). Most nonionic surfactants are complex mixtures of ethoxylated analogs whose EO chain lengths follow a Poisson distribution (10) and the number of EO units per molecule represents an average value.

Due to this unique chemical structure, surfactants impart characteristic properties to aqueous solutions. First, surfactants form oriented layers at interfaces. The hydrophilic moieties associate with the aqueous phase while the lipophilic portions associate with the non-aqueous phase. This property results in the reduction of surface and interfacial tensions. These reductions increase with increasing surfactant concentration until a maximum is reached which corresponds to the critical micelle concentration (CMC) (14,18). At and above the CMC, surfactants exhibit a second property, forming thermodynamically stable colloidal aggregates or micelles. In aqueous solutions micelles are oriented with their hydrophilic moieties associated with the aqueous phase while the lipophilic moieties are aggregated towards the interior of the micelle. Through micelle formation, compounds only slightly soluble in water can be solubilized by partitioning into the hydrophobic region (core) of the micelle. Similarly, micelle formation is utilized in the development of emulsifiable concentrates (34).

Surfactants are used in the formulation of agrochemicals because of their ability to reduce surface tension and thereby increasing wetting (7,11,12), effects on solubilization associated with micelle formation (3) and a wide variety of other reasons (29) with the overall goal of increasing performance. Increased activity of herbicides and growth regulators from the addition of surfactants is well documented (9,11,19,20,23), however, surfactants may also have no effect or depress herbicide activity (19,20). Proposed modes of action are numerous (7,13) ranging from increased coverage to complex interactions among surfactant, pesticide and plant. In all cases, surfactant chemistry plays a key role (3) especially in interactions at the plant surface (5,31).

Numerous surfactant-pesticide-plant interactions are conceivable and probable, but because of complexity, have not been studied extensively. Some reports are available on the effects of surfactants on plant processes including photophosphorylation (26), protoplasmic streaming (15), mitosis (25), elongation of root hairs (4), permeability of cell walls (17,32) and foliar phytotoxicity (14,33). Few, however, relate these effects to surfactant chemistry and properties. In the studies relating physiological effects to surfactant chemistry, there was decreased phytotoxicity to soybean cells (8) and apple leaves (14) and less inhibition of root elongation (4) with increasing EO content. These effects may be related to surfactant penetration (30,31) but conclusive data are not available. The effects of surfactant interactions on whole plant and pesticide performance have not been stressed. Their potential importance, however, must not be overlooked. Phytotoxicity, for example, could markedly affect penetration and translocation of the applied compound as well as the long term physiological functioning of the target plant. Such effects may also be economically important.

Recently, surfactant-induced ethylene production in cowpea leaves has been reported (21). Because ethylene is an active plant growth regulator at low concentrations, it may induce physiological responses in plants and these responses may affect pesticide performance. However, the extent of these effects will depend on the magnitude and duration of ethylene production, tissues and/or pesticide response and other factors. Currently there are no data available on these parameters.

Surfactant effects on ethylene biosynthesis have been studied in selected systems. In mung bean hypocotyl segments (27), apple protoplasts (1) and apple tissue discs (2) pretreated with 1-aminocyclopropane-1-carboxylic acid (ACC), surfactants blocked the final step in ethylene biosynthesis, conversion of ACC to ethylene. In contrast, ethylene production from ACC treated isolated microsomal membrane fractions increased with the addition of surfactant (22). Thus, it appears that surfactants may affect ethylene biosynthesis in more than one way and these processes and their potential importance will require further study.

Based on the widespread use of surfactants in foliar sprays (24), their reported biological activity (3) and the limited information on their interactions with plant leaves, studies were initiated to investigate the interactions of surfactants with cowpea leaves (6). The objectives were: a) to characterize surfactant-induced phytotoxicity on cowpea leaves and the effects of selected spray application factors, b) to characterize surfactant-plant interactions in terms of ethylene production and its relationship to surfactant chemistry and c) to examine the effects of surfactants on various aspects of ethylene biosynthesis.

SECTION I

SURFACTANT-INDUCED PHYTOTOXICITY AND EFFECTS OF SPRAY APPLICATION FACTORS

SURFACTANT-INDUCED PHYTOTOXICITY AND EFFECTS OF SPRAY APPLICATION FACTORS

Abstract

The development of surfactant-induced phytotoxicity was examined following application of octylphenol (Triton X-100) and linear alcohol (Neodol 25-9) surfactants to the adaxial surface of 10-day-old cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Dixielee') leaves. Phytotoxicity first appeared as isolated discolored areas at the periphery of the droplet:leaf interface, developed toward the center of the treatment area and when maximum the entire treatment area was necrotic. Epidermal cells beneath the treatment site became discolored, lost structural integrity, collapsed and became necrotic. Similar changes were observed in the palisade layer and spongy parenchyma. In addition, walls of damaged cells were preferentially stained with Safranin O. For a given surfactant dose, phytotoxicity increased with increasing concentration, droplet volume and temperature and decreased with increasing humidity. There was, in general, an inverse relationship between the length of the ethylene oxide (EO) chain and phytotoxicity for the octylphenol (Triton X) series and a similar relationship for the C_{12-15} linear alcohol (Neodol 25) series. Tissue did not recovery after injury.

Introduction

Surfactants are widely used in the formulation of pesticides for foliar sprays (18,21). Hence, in the course of crop production they are applied frequently to a variety of crops with the assumption that they are innocuous. However, surface active chemicals may induce pronounced effects in numerous plant systems (3,7,12,24).

Surfactants can disrupt cell membranes (5) and be phytotoxic to cells in culture (3,4), isolated cells (24,26), roots (9,12) and leaves (7,8,14). Limited data are available on surfactant-induced phytotoxicity in relation to the chemistry and physico-chemical properties of surfactant solutions, namely, critical micelle concentration (3,4,7,8), surface tension (4,12,14) and wetting (10). However, no detailed data are available on the development of surfactant-induced phytotoxicity on plant leaves or the effects of spray application factors.

Relationships between surfactant properties and phytotoxicity are complex (6,8,22,25). Further, spray application factors such as concentration, drop size, spray coverage, temperature, humidity, leaf age and surface properties and others may modify surfactant action (13) and thus influence phytotoxicity.

In this paper we report on the nature of surfactant-induced phytotoxicity on cowpea leaves and the effects of selected spray application factors on surfactant-induced phytotoxicity.

Materials and Methods

Plant material and growing conditions. Cowpea seeds were pregerminated (24 hr) in the dark at 30°C on moist paper towels. Healthy seeds of uniform size and radicle length were selected, seed coats removed to facilitate epicotyl emergence and planted into disposable AC-4-8 "Cell Paks" (Geo. J. Ball Co., W. Chicago, IL) using PROMIX BX (Premier Brands Inc., New Rochelle, NY) as a growing medium. Germination was completed and seedlings were held in a growth chamber at a day/night temperature of 25/20°C. Light was provided for 16 hr daily at 150-200 μmol m^{-2} s^{-1} (400-700 nm; cool-white fluorescent, GE F48T12 CW-1500 supplemented with 15% incandescent). Relative humidity during the light period varied from 45-55% and 65-75% during the dark period.

Surfactant chemistry. Octylphenol (OP) was condensed with 3 (OP+3EO), 5 (OP+5EO), 9.5 (OP+9.5EO), 30 (OP+30EO) and 40 (OP+40EO) moles ethylene oxide (EO) (Triton X surfactants; Rohm and Haas Co., Philadelphia, PA). A linear alcohol (LA) hydrophobe containing a mixture of 12-15 carbon atoms (17) was condensed with 3 (LA+3EO), 7 (LA+7EO), 9 (LA+9EO), 12 (LA+12EO) and 30 (LA+30EO) moles EO (Neodol 25 surfactants; Shell Chemical Co., Houston, TX). All surfactants were mixtures of oligomers where the listed EO number represents an average value and the ethoxymer mole ratio distribution follows a Poisson distribution (17,27).

General procedures. Primary leaves of 10-day-old cowpea, selected for uniformity and freedom from defects, were treated by applying the appropriate surfactant solution as discrete droplets to the adaxial surface (avoiding the veins) using a microsyringe fitted with an automatic dispenser. All treatments were made 2.5 to 3.5 hr after the beginning of the light period.

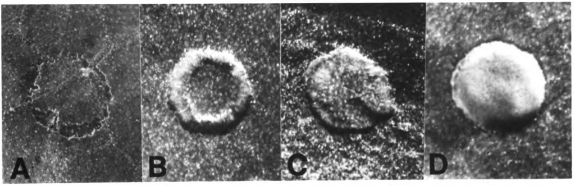
Phytotoxicity. Leaves were visually inspected at specified times after treatment and each treatment site was assigned a phytotoxicity rating (1 to 5) based on degree of tissue discoloration and/or necrosis as follows (see Fig. 1):

Rating = 1 - No discoloration or necrosis.
2 - Isolated areas of discolored, sunken and/or necrotic tissue.
3 - Discolored, sunken and/or necrotic tissue at the entire periphery of the droplet:leaf interface.
4 - Entire droplet:leaf interface area discolored and/or sunken, less than 50% necrotic.
5 - Entire droplet:leaf interface area discolored and/or sunken, greater than 50% necrotic.

Ten individual droplets on a single leaf were averaged to obtain the rating for each replication.

Scanning electron microscopy. At selected times discs containing the treatment site were excised and washed with distilled water (5 ml) to remove residual surfactant. Fresh tissue sections for surface viewing were mounted on aluminum stubs with O.C.T. compound (Miles Laboratories Inc., Naperville, IL), quick frozen in slush nitrogen, etched at -80°C, coated with gold (100 A) and observed on a cold stage using a JSM-35C SEM (JEOL) operating at 15 kV. Fresh tissue for cross-section viewing was prepared in the same manner but freeze-fractured before etching. Additional tissue for surface viewing was frozen by immersion in liquid nitrogen, freeze-dried at -60°C for 24 hr, mounted on aluminum stubs with a suspension of carbon (Television Tube Koat, G.C. Electronics, Rockford, IL), coated and viewed as above. Between 5 and 10 randomly selected leaf discs were prepared for SEM viewing from each experiment. Of those, 3 to 5 were viewed and photographed. Scanning electron micrographs were taken on positive/negative 665

Figure 1. Photographs illustrating phytotoxicity rating scale on primary leaves of cowpea. A: isolated areas of discolored, sunken and/or necrotic tissue (rating = 2), B: discolored, sunken and/or necrotic tissue at the entire periphery of droplet:leaf interface (rating = 3), C: entire droplet:leaf interface area discolored and/or sunken, less than 50% necrotic (rating = 4), D: entire droplet:leaf interface area discolored and/or sunken, greater than 50% necrotic (rating = 5).


A
B
C
D

Polaroid film.

Microtechnique. Leaves were sampled 1, 2, 3, 6, 9, 12, 24, and 48 hr after treatment, washed with distilled water to remove residual surfactant and the treated areas excised. Tissue was fixed in formalin, acetic acid and alcohol, dehydrated, embedded in paraffin and sectioned (12 μm) by standard microtechnique procedures (15). Sections were stained with Safranin O and Fast Green (15) and photographed using a Wild M 20 research microscope equipped with a 35 mm film carrier and a photoautomat exposure control unit.

Effect of spray application factors. The effect of surfactant concentration was examined by holding droplet number and size constant (10 and 1 μl, respectively) and applying OP+9.5EO at 0.01, 0.05, 0.1, 0.25, 0.5, and 1.0% to the adaxial surface of cowpea leaves as previously described. The effect of droplet size was examined by applying OP+9.5EO (0.5%) as 0.2, 0.5, 1.0, 2.0, and 5.0 μl droplets. In both studies plants were held in the growth chamber under previously defined growing conditions following treatment.

Effects of temperature were determined by holding the plants at a constant 20, 25 or 32^{o}C ($\pm$ 1.5^{o}C). Plants were placed at the desired temperature at the beginning of the light cycle (3 hr pre-treatment), primary leaves were treated (10, 1 μl droplets) with OP+9.5EO (0.5%) applied to the adaxial surface and the plants then held at the designated temperature for the duration of the study. Relative humidity was in the range of 45-55%/65-75% during the light/dark periods, respectively.

Similarly, the effects of relative humidity were determined at approximately 40 (38-42), 60 (58-62) and 80 (77-82)%. Briefly, primary leaves of cowpea were enclosed (3 hr pre-treatment) in environmentally controlled plexiglass chambers (25°C, 200 μmol m^{-2} s^{-1}) and vapor pressure deficit regulated using a controlled temperature radiator which condensed water from the incoming air (20). Dew point of the incoming and outgoing air was monitored using 2 General Eastern 1100 dew point hygrometers. Relative humidity was calculated from dew point readings from the output side of the chamber. Temperature was held constant (25 ± 0.5°C) as described elsewhere (20). Each treatment was replicated 4 times and the experiment was performed twice. Primary leaves were treated and rated as previously described.

Effect of moles EO. The effect of the hydrophile was examined by applying OP+3EO, OP+5EO, OP+9.5EO, OP+30EO and OP+40EO or LA+3EO, LA+7EO, LA+9EO, LA+12EO and LA+30EO as 10, 1 μl droplets. Plants were maintained under previously described conditions.

Experimental design. Randomized complete block designs were used with 10 replications (leaves) for each treatment.

Results

Phytotoxicity. Both surfactants induced phytotoxicity on cowpea leaves at concentrations of 0.1% and greater (Figs. 1-3). Phytotoxicity first appeared as isolated discolored areas at the periphery of the droplet:leaf interface (Figs. 1A, 1B and 2A). Phytotoxicity continued to develop toward the center of the droplet:leaf interface area (Fig. 1C) and when maximum caused necrosis of the entire droplet:leaf interface (Fig. 1D). Damaged tissue was characterized by collapse of epidermal cells with deformation and/or collapse of underlying palisade cells (Fig. 3). No visual changes were

Figure 2. Scanning electron micrographs of the adaxial surface of the primary leaf of cowpea illustrating the effects of Triton X-100 (0.5%) 24 hr after treatment. A: collapsed tissue at the periphery of the droplet:leaf interface, B and C: epicuticular wax and surface structure in a non-damaged and damaged area, respectively.

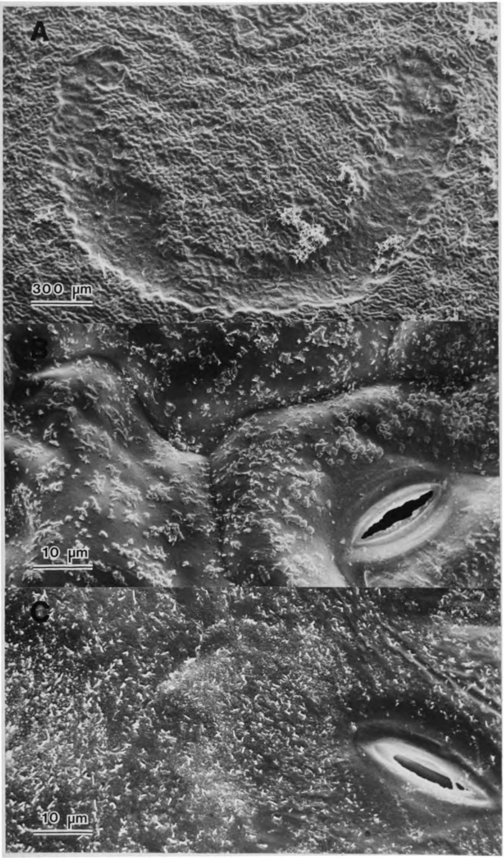
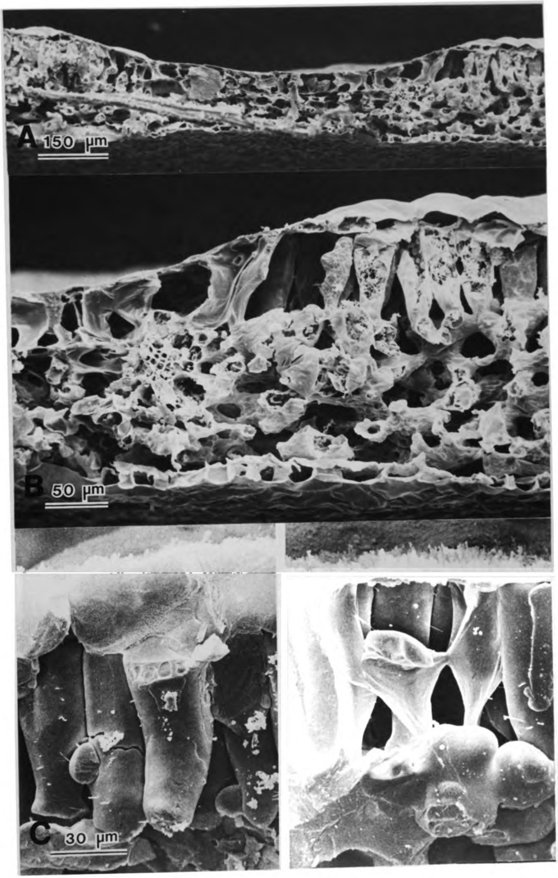

A
300 µm
B
10 µm
C
10 µm

Figure 3. Scanning electron micrographs of freeze-fractured cross-sections of primary leaves of cowpea illustrating Triton X-100 (0.5%) damage 24 hr after treatment. A: beneath treatment site, B: beneath edge of droplet, C: non-damaged (left) and damaged (right) cells.


A
150 µm
B
50 µm
C
30 µm

apparent in non-damaged cells within the treated area (Fig. 2B) and the epicuticular wax fine-structure did not appear to be altered (Figs. 2B and C). Damage was localized within the treated area and there was no evidence of tissue recovery after injury.

Damaged and non-damaged tissue had different affinities for the histochemical stains Safranin O and Fast Green. Non-damaged epidermal, palisade and mesophyll cells stained green with red counterstain in chloroplasts (Fig. 4A). In contrast, damaged tissues stained bright red (Figs. 4B and C).

Effects of spray application factors. Surfactant-induced phytotoxicity was concentration dependent (Fig. 5). At 0.01%, OP+9.5EO did not induce phytotoxicity. Higher concentrations induced a two stage response. First, symptoms appeared earlier, being evident, 12, 9, 3, 3, and 2 hr after treatment with 0.05, 0.1, 0.25, 0.5 and 1.0%, respectively. Second, the magnitude of the phytotoxicity response increased with increasing concentration (Fig. 5). At 24 hr phytotoxicity was greatest for 1.0%, intermediate for 0.5 and 0.25% and least for 0.1 and 0.05%. Tissue damage occured most rapidly during the first 12 hr with little additional injury between 12 and 48 hr.

The phytotoxicity rating generally increased with increasing droplet volume (Fig. 6). Symptoms developed most rapidly the first 12 hr after treatment.

Phytotoxicity symptoms appeared more rapidly and developed to a greater degree with increasing temperature (Fig. 7A). At 24 hr phytotoxicity was 2.0, 2.9 and 4.1 for 20, 25 and 32°C, respectively ($LSD_{.05} = 0.75$).

Surfactant-induced phytotoxicity was inversely related to relative

Figure 4. Photomicrographs of cross-sections of non-damaged and damaged cowpea leaves stained with Safranin O and Fast Green. A: non-damaged, B and C: damaged tissues (beneath treatment site) following 3 and 24 hr, respectively.

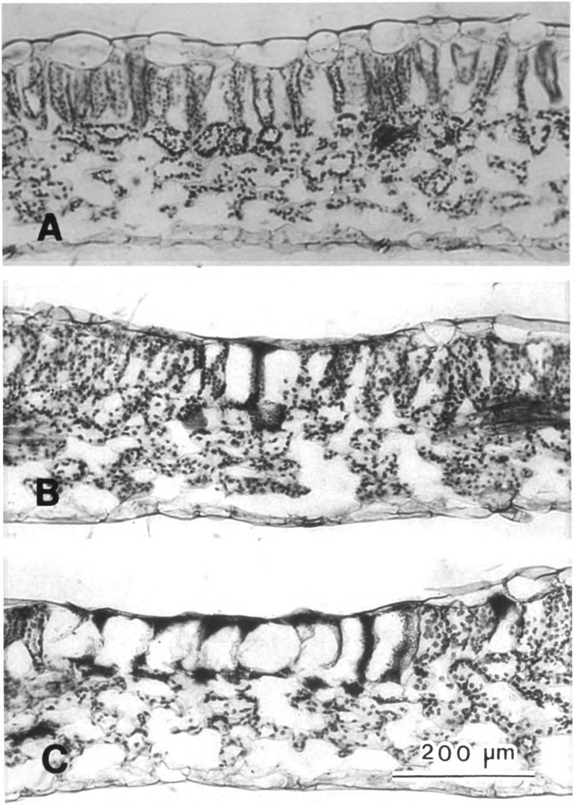
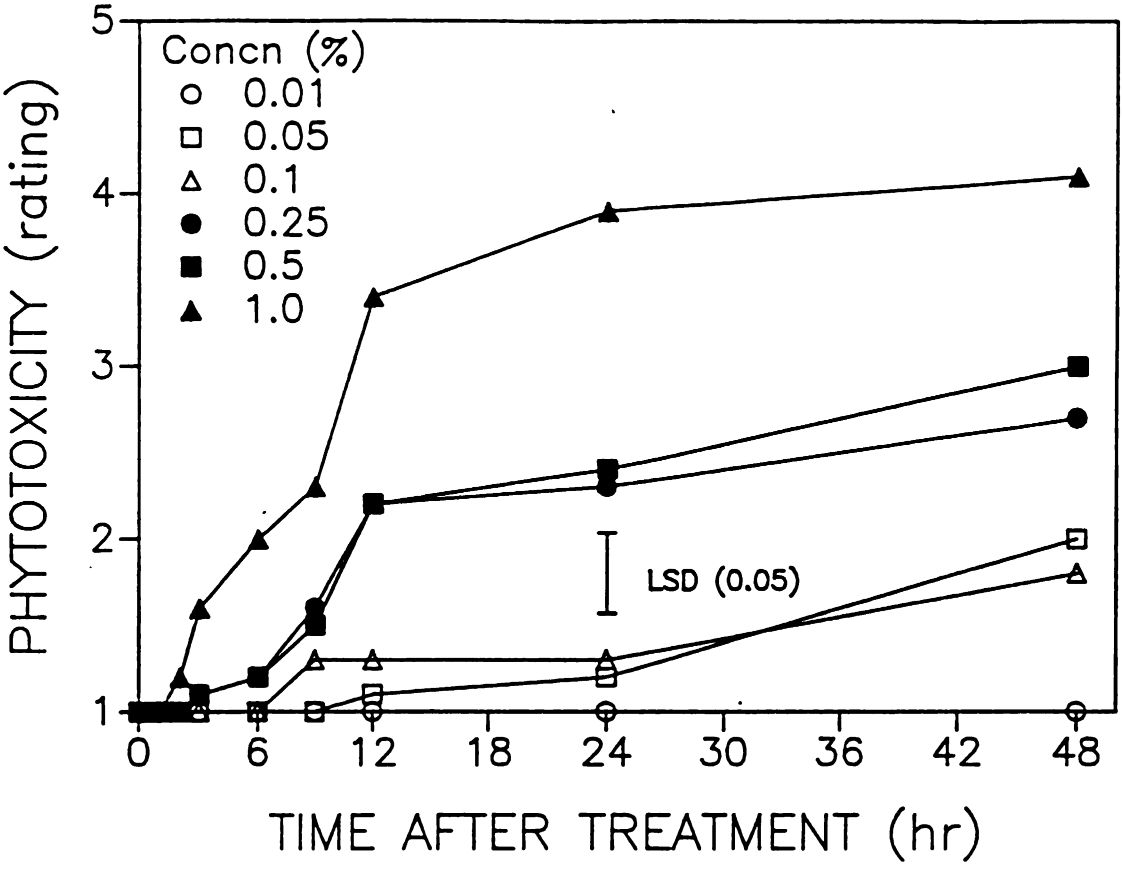

A
B
C
200 µm

Figure 5. Effect of concentration on surfactant-induced phytotoxicity. Triton X-100 was applied as 1 μl droplets to the adaxial surface of cowpea leaves. Ratings based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic). LSD is for 24 hr.

Figure 6. Effect of droplet volume on surfactant-induced phytotoxicity. Triton X-100 (0.5%) was applied to the adaxial surface of cowpea leaves. Ratings based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic). LSD is for 24 hr.


PHYTOTOXICITY (rating)
TIME AFTER TREATMENT (hr)
Concn (%)
○ 0.01
□ 0.05
△ 0.1
● 0.25
■ 0.5
▲ 1.0
LSD (0.05)
1
2
3
4
5
0
6
12
18
24
30
36
42
48

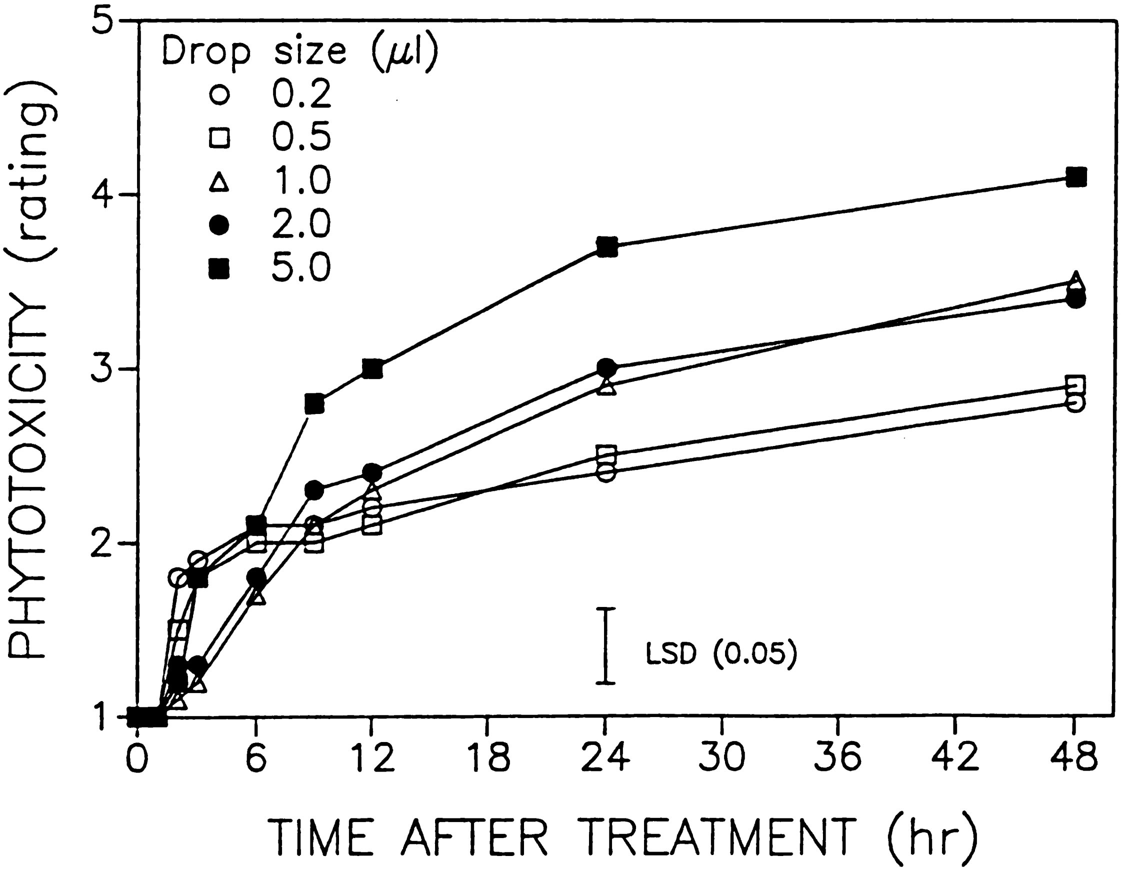
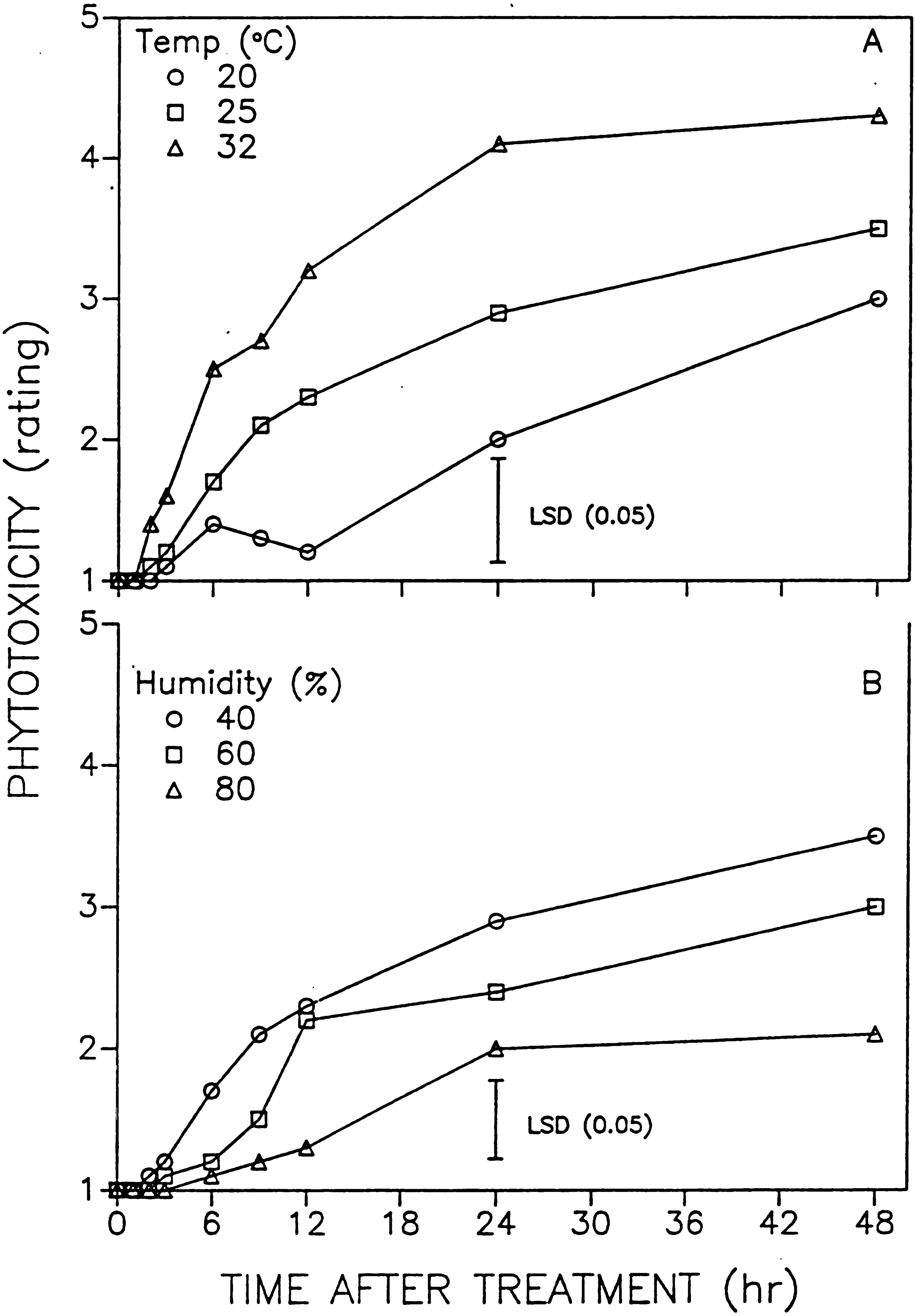

PHYTOTOXICITY (rating)
TIME AFTER TREATMENT (hr)
Drop size (μl)
○ 0.2
□ 0.5
△ 1.0
● 2.0
■ 5.0
LSD (0.05)
1
2
3
4
5
0
6
12
18
24
30
36
42
48

Figure 7. Effect of temperature (A) and humidity (B) on surfactant-induced phytotoxicity. Triton X-100 (0.5%) was applied as 1 μl droplets to the adaxial surface of cowpea leaves. Ratings based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic). LSD is for 24 hr.


PHYTOTOXICITY (rating)
TIME AFTER TREATMENT (hr)
A
Temp (°C)
○ 20
□ 25
△ 32
LSD (0.05)
B
Humidity (%)
○ 40
□ 60
△ 80
LSD (0.05)
1
2
3
4
5
0
6
12
18
24
30
36
42
48

humidity (Fig. 7B). Initial symptoms developed slowly and were apparent after 2, 3 and 6 hr for 40, 60 and 80% relative humidity, respectively. Greatest leaf damage was consistently observed at 40% relative humidity.

Effect of moles EO. For the OP surfactants, phytotoxicity generally decreased with increasing EO chain length (Table 1). This relationship was linearly related to log(EO chain length). The LA+7EO, LA+9EO and LA+12EO surfactants induced the greatest phytotoxicity while less leaf injury was observed with LA+30EO. Phytotoxicity was related to log(EO chain length) in a quadratic manner.

Discussion

We have shown that surfactants can induce phytotoxicity in cowpea leaves and cause localized tissue necrosis. The first appearance of phytotoxicity at the periphery of the droplet:leaf interface was not associated with specific leaf surface features. Injury remained localized within the droplet:leaf interface area and was probably related to the nature of droplet drying, the distribution of the surfactant deposit on the leaf surface (2) and penetration into the leaf (23). According to Hartley and Graham-Bryce (11) evaporation from a drop on a surface (leaf) would occur most rapidly at the liquid:air:leaf interface resulting in preferential chemical deposition in the form of an annulus. Thus, those areas first showing symptoms of phytotoxicity probably represent areas of initial and greatest surfactant deposition and penetration. With continuing evaporation, surfactant deposition would occur toward the center of the treatment site and, depending on surfactant and concentration, tissue damage

Table 1. Effect of ethylene oxide (EO) chain length on surfactant-induced phytotoxicity.[z]

EO chain	Surfactant hydrophobe	
length	Octylphenol	Linear alcohol
3	2.0[y]	2.0[x]
5	2.5	-
7	-	3.0
9	-	2.9
9.5	1.3	-
12	-	3.0
30	1.0	1.0
40	1.0	-

[z] Rating based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic).

[y] Linear component significant (P=0.001) for log(EO chain length).

[x] Quadratic component significant (P=0.001) for log(EO chain length).

followed.

As phytotoxicity became more severe, larger areas within the treatment site became discolored as underlying epidermal, palisade and then mesophyll cells lost membrane integrity and collapsed (Figs. 2-4). The rate of symptom development was related to surfactant chemistry and was concentration dependent. The LA surfactant was found to be slightly more toxic.

The relationship between phytotoxicity and EO chain length (Table 1) was similar to that observed in other systems (1,6,8,10,22) and generally followed the relationship between EO content and foliar penetration of OP surfactants where greater penetration occured with decreasing EO chain length (increasing surfactant lipophilicity; 23). The severity of phytotoxicity would be expected to be a function of surfactant penetration to the underlying cells, where surfactants can induce membrane disruption. Thus, the relationship between EO and phytotoxicity is most likely related primarily to differences in penetration and to affinity for lipoidal membranes.

The primary effect of the surfactant on cell membranes is consistent with data that have shown surfactant interactions with lipids in membranes leading to increased permeability (24,26) and solubilization of membrane associated proteins (5). Walls of surfactant damaged cells increased in affinity for Safranin O (Fig. 4) indicating changes in cell wall chemistry or association of cytoplasmic constituents with the cell wall following membrane damage.

Localization of injury to the cells immediately beneath the treatment site probably reflects accumulation of the absorbed surfactant and may indicate a compartmentalization of damage (10).

This view is supported by data showing rapid absorption of foliar applied surfactants but no significant transport of absorbed surfactants away from the treatment site in leaves of Vicia faba (23).

Factors associated with spray application (e.g. spray distribution on the plant surface, concentration, droplet volume, temperature, humidity) altered the magnitude of phytotoxicity induced by a given surfactant dose. Concentration (Fig. 5) may be of particular practical significance since surfactants are routinely applied as components of formulated pesticides in concentration ranges of 0.01 to 0.5% (20,21). In low-volume spraying and/or where additional surfactant may be tank-mixed with formulated materials, concentrations may approach 1.0%. Such concentrations have been shown to induce significant lesions that may alter physiological processes in leaves (16; Noga, unpublished data) and russeting of apple fruit (19).

Similarly, droplet size, temperature and relative humidity at time of application impact on surfactant activity (Figs. 6 and 7). For a given increase in droplet volume, dose per droplet increased more than droplet:leaf interface area. Therefore, droplet volume effects may result from increased dose per unit area (similar to increased concentration at constant droplet volume). Although the droplet volumes (sizes) used in this study were larger than those usually formed by conventional spray nozzels, droplet over-strikes and coalescence would produce droplets comparable to those used in our study. A component not addressed but which could be important is droplet density of the spray deposit. Since surfactant-induced phytotoxicity was confined to the treatment site, the number of lesions would be related to number of droplets retained containing a biologically active dose. In addition, droplet over-strikes and coalescence would increase the quantity of surfactant deposited per unit area which could increase phytotoxicity.

Increasing temperature and/or decreasing humidity increased phytotoxicity (Fig. 7). Both conditions increased droplet drying and would be expected to produce more uniform deposits (dose) over the treatment area (11). Thus, a greater portion of the treated area would be exposed to a dose adequate to induce phytotoxicity.

Although not measured directly, physiological tissue function was most likely lost as cells became stressed and damaged (1,24,26). The absence of tissue recovery suggests long term importance. The potential for surfactant-induced phytotoxicity is real, especially with current low-volume application practices. The impact of phytotoxic responses on the plant and on the performance of foliar applied agrochemicals must be assessed in terms of the extent of the damage and the physiological process involved. These interactions must be considered in the development and use of foliar applied chemicals.

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SECTION II

SURFACTANT-INDUCED ETHYLENE PRODUCTION BY LEAF TISSUE

SURFACTANT-INDUCED ETHYLENE PRODUCTION BY LEAF TISSUE

Abstract

Ethylene evolution induced by nonionic (Triton X-100, Triton X-405, Tween 20, Ortho X-77 and Regulaid), anionic (Aerosol OT and Dupanol ME) and cationic (Arquad C-50 and Arquad 2C-75) surfactants was characterized using cowpea (Vigna unguiculata (L.) Walp. supsb. unguiculata 'Dixielee') seedlings. Surfactants (0.1%) of each ionic class induced ethylene evolution. The rate decreased markedly as incubation time was extended beyond 2 hr. The rate of ethylene evolution increased significantly the first 6 to 12 hr after treatment and then decreased slowly for the next 12 to 36 hr, returning to control levels within 48 hr. Ethylene production was related to surfactant chemistry and concentration and was significantly greater following treatment of the abaxial than adaxial surface of sour cherry (Prunus cerasus 'Montmorency') leaves. Surfactants that induced ethylene evolution also induced phytotoxicity characterized by discoloration and localized disruption of epidermal cells. Similar surfactant-induced responses were observed with corn (Zea mays 'B73 X MO17'), wheat (Triticum aestivum 'Hillsdale'), soybean (Glycine max 'McCall') and apple (Malus spp. 'Golden Delicious'). The horticultural implications of our findings are discussed in relation to spray application since surfactants are frequently used in the formulation of agrochemicals and incorporated as tank additives.

Introduction

Surfactant-induced ethylene evolution has recently been reported for a number of nonionic surfactants (13). Concomitant with ethylene evolution, varying degrees of phytotoxicity were observed that may be associated with the ethylene response (13). The nature and physiological significance of surfactant-induced biological responses are not well understood, but could be important in the performance of foliar-applied agrochemicals.

Surfactants are integral components of most foliar sprays and thus are repeatedly applied to horticultural crops during pesticide applications. While generally present in high volume (HV) sprays at less than 0.2% (4,15,18), the concentration in low volume (LV) sprays may approach 1.0%. The surfactant component of a spray may affect the performance of foliar-applied compounds not only by modifying the physical and chemical characteristics of the spray solution (5,6), but also by inducing biological responses (13) which could alter plant response to an active ingredient. For herbicide application, ethylene evolution as a stress response may enhance herbicidal activity (1). Alternatively, surfactant-induced ethylene production may reduce the effectiveness of a growth regulator spray intended to reduce pre-harvest drop. Such antagonistic activities have been documented for surfactant promotion and daminozide inhibition of internode elongation in bean (17).

In this paper we show that surfactants important in agrochemical formulation induce ethylene production in crop plants and characterize the nature of this response. These findings are discussed in relation to surfactant properties, phytotoxicity and their potential importance to spray applications.

Materials and Methods

Plant material and growing conditions. Cowpea seeds were pre-germinated for 24 hr in the dark at 30°C on moist paper towels. Healthy seeds of uniform size and radicle length were selected and planted into disposable AC-4-8 "Cell Paks" (Geo. J. Ball Co., W. Chicago, IL) using PROMIX BX (Premier Brands, Inc., New Rochelle, NY) as a growing medium. Seedlings were grown in a growth chamber under day/night temperatures of 25/20°C and relative humidities of 45-55/65-75%, respectively. Light was provided for 16 hr daily at 220 μmol m^{-2} s^{-1} (400-700 nm; cool-white fluorescent supplemented with 15% incandescent).

Surfactant chemistry and properties. The surfactants selected (Table 1) represented nonionic; Tween 20 (ICI Americas, Inc., Wilmington, DE), Triton X-100 and X-405 (Rohm and Haas, Philadelphia, PA), Ortho X-77 (Chevron Chemical Co., San Francisco, CA), and Regulaid (Kalo Laboratories, Inc., Kansas City, MO), anionic; Dupanol ME (E.I. Dupont deNemours, & Co., Wilmington, DE) and Aerosol OT (American Cyanamid, Wayne, NJ) and cationic; Arquad C-50 and 2C-75 (Armac Co., Chicago, IL), classes of commercially important chemistries or blends. The two nonionic Triton surfactants differed in the average ethylene oxide (EO) chain length, 9.5 and 40 for X-100 and X-405, respectively. Ortho X-77 and Regulaid are mixtures of various components and thus molecular weights were not available. Critical micelle concentrations (CMC) were obtained from the literature (16) or determined experimentally (Ortho X-77, Regulaid, Arquad C-50 and 2C-75). Briefly, solutions of varying surface tension were prepared by adding increasing concentrations of surfactant to deionized distilled water having a surface tension of 72.5 mN m^{-1}. Surface tension was measured with a surface tensiometer (Fisher Model 20, Fisher Scientific Co., Pittsburg, PA) and plotted against log concentration. The point of inflection of these curves concides with the CMC (9).

Wetting was indexed by contact angles (θ) calculated from measurements made from projected silhouettes of drops (1 μl) approximately 5 sec after application to the adaxial surface of 10-day-old cowpea leaves (10 replicates) using the formula (14):

$$\theta = 2 \arctan ({}^{\text{height}}/_{0.5 \text{ base}})$$

All surfactant solutions were prepared on a weight/volume (w/v) basis with distilled deionized water.

<u>Treatment procedure, phytotoxicity rating and ethylene measurement</u>. Primary leaves of 10-day-old cowpea, selected for uniformity and freedom from defects, were treated with nonionic (Tween 20, Triton X-405, Triton X-100 and Ortho X-77), anionic (Dupanol ME and Aerosol OT) and cationic (Arquad 2C-75 and Arquad C-50) surfactants at 0.1%. Each treatment was applied 2.5 to 3.5 hr after the beginning of the light period as 20 drops (5 μl each) to the adaxial or abaxial surface (avoiding major veins) using a microsyringe fitted with an automatic dispenser. Leaf orientation was horizontal and when necessary individual leaves were supported in a horizontal position to prevent drop coalescence and excessive spreading. Surfactant penetration was allowed to proceed under conditions previously described for 12 or 24 hr after which treated leaves were rated for phytotoxicity and sampled for ethylene determination.

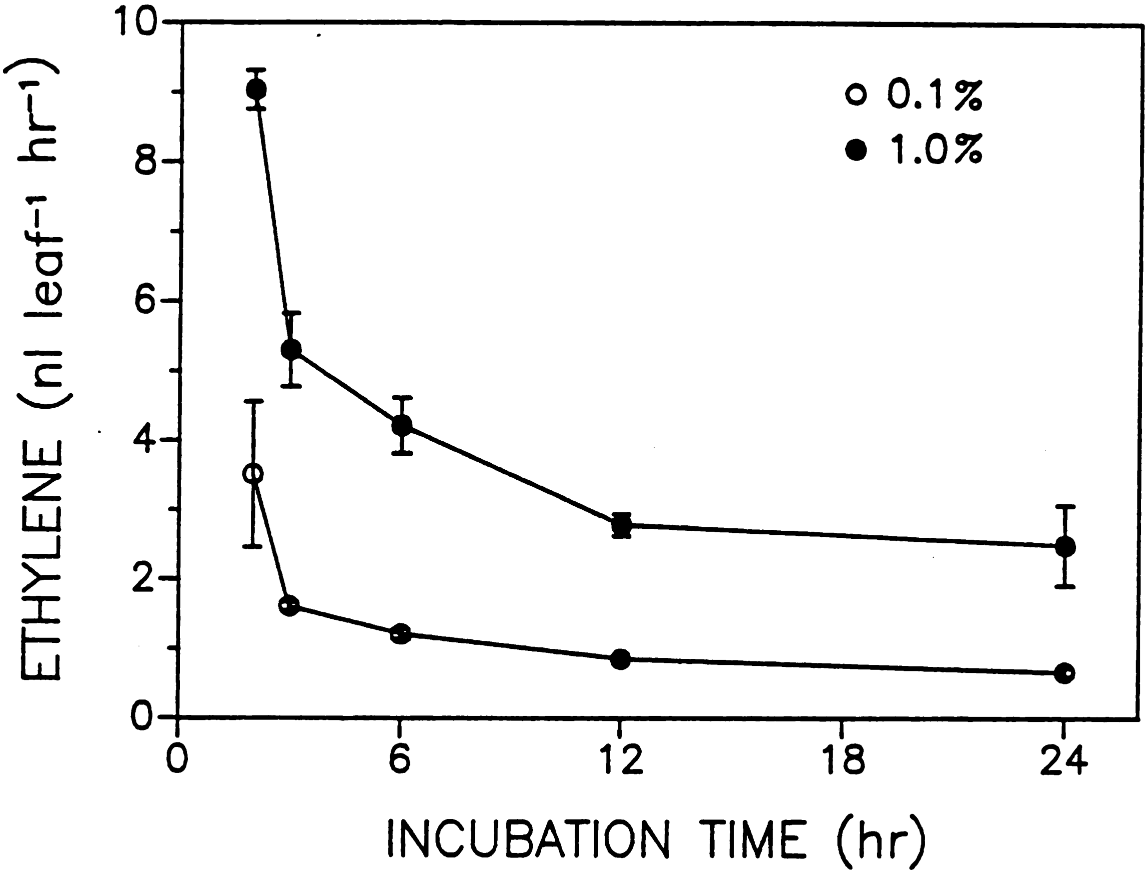
Leaves were visually inspected and assigned a phytotoxicity rating of 1 to 5 based on degree of tissue discoloration and/or necrosis as previously reported (12).

For ethylene determination leaves were excised and positioned abaxial side outward with minimum overlap in 25 x 200 mm test tubes containing 2 ml of distilled water. The tubes were allowed to equilibrate (uncapped) for 1 hr in a constant temperature (30°C) water bath, flushed with air for 30 sec, sealed with rubber serum stoppers and incubated for 2 hr (3).

Ethylene was determined on a 1 ml headspace sample by gas chromatography (Varian 1440, Varian Associates, Inc., Palo Alto, CA) using a 1.2 m steel column packed with activated alumina and a flame ionization detector. The injection port, column and detector temperatures were 130, 100 and 150°C, respectively. N_2 flow was maintained at 15 ml min^{-1}. Data were expressed as rate of ethylene evolution, nl $leaf^{-1}$ hr^{-1}.

Incubation conditions. Effect of duration of incubation and light and dark were evaluated with leaves treated with Triton X-100 (0.1 and 1.0%). After 24 hr leaves were incubated at 30°C, optimum for ethylene production (20), in the light (115 μmol m^{-2} s^{-1}) for up to 24 hr and the headspace sampled at 2, 3, 6, 12, and 24 hr. In a parallel study ethylene was measured on leaves incubated in the dark for 2 or 12 hr. The rate of surfactant-induced ethylene evolution was greatest with a 2-hr incubation period and decresed markedly with longer incubation times (Fig. 1). There were no differences between incubation in light and dark after 2 hr. After a 12-hr incubation period ethylene production was reduced in light and dark with greater reduction in the

Figure 1. Effect of incubation time on the rate of ethylene evolution by primary cowpea leaves 24 hr after treatment with Triton X-100 (0.1, 1.0%). Vertical bars represent standard error. Where standard error bars are not shown they were smaller than the data symbol.


ETHYLENE (nl leaf^{-1} hr^{-1})
10
8
6
4
2
0
0
6
12
18
24
INCUBATION TIME (hr)
○ 0.1%
● 1.0%

light (data not presented). For maximum ethylene production and convenience, incubation conditions for subsequent experiments were standardized at 2 hr in the light.

Non-biological degradation of surfactants. Cowpea leaves were freeze-dried, coarsely macerated, and the dry weight equivalent of 2 leaves (0.3 g) weighed into test tubes. A duplicate set of empty tubes was used for comparison. 200 μl of treatment solution, water or polyoxyethylene surfactants (Triton X-405 or Ortho X-77; 1.0%), was added to each of 10 replicate tubes from each set (with and without lyophilized tissue). The tubes were capped and incubated for 24 hr at 30°C in an illuminated water bath before ethylene measurement.

Time-course. Primary cowpea leaves were treated as previously described with surfactants selected from each ionic class; Triton X-100 and Tween 20 (nonionic), Aerosol OT (anionic) and Arquad 2C-75 (cationic) at 1.0%. Treated leaves were sampled for ethylene determination at designated times over a 48-hr period.

Concentration response. Primary cowpea leaves were treated with Triton X-100 and Tween 20 at concentrations ranging from 0.001 to 1.0%. Ethylene was measured after 12 hr.

Effect of leaf surface. The effect of leaf surface on surfactant-induced ethylene production was examined using sour cherry leaves. Recently fully expanded leaves (4-6 nodes from apex) on potted trees growing in the greenhouse (12-hr photoperiod, 25-27/20-22°C day/night, respectively) were treated by applying 20 drops (5 μl each) of Tween 20 (0.1 to 10%) to the astomatous adaxial or stomatous abaxial surface as described for cowpea. Phytotoxicity and ethylene evolution were determined after 24 hrs.

Plant species response. A number of diverse plant species, differing in leaf cuticle morphology and permeability, were selected to establish relative sensitivity to surfactant-induced ethylene formation. Wheat and corn were selected as annuals rich in epicuticular wax, soybean for the high degree of pubescence and apple and sour cherry as representative perennial tree fruits with astomatous adaxial surfaces.

Corn, wheat and soybean were grown under conditions described for cowpea. Twenty drops (1 μl each) of Triton X-100 (1.0%) were applied to each of 10 wheat (1st leaf, adaxial surface, 8 days post-emergence), corn (1st leaf, adaxial surface, 8 days post-emergence) and soybean (1st trifoliate leaf, adaxial surface, 10 days post-emergence) leaves. Treated wheat and soybean leaves were sampled 18 hr and corn leaves 24 hr after treatment for ethylene determination. Leaves were rated for phytotoxicity at sampling.

Fully expanded apple and sour cherry leaves (field grown) were sprayed to runoff (both surfaces) using a hand held sprayer. The surfactants used were; Triton X-100, Ortho X-77, Tween 20 and Regulaid at 0.1 and 1.0%. Phytotoxicity and ethylene evolution were determined 24 hr after treatment.

Experimental design and statistics. All experiments were conducted using a randomized complete block design with 8 to 10 replications. The mean, standard deviation, standard error and coefficient of variation were calculated for the ethylene data for each treatment. Where appropriate, data were further analyzed using mean separation and trend analysis.

Results

Wetting. All surfactants markedly decreased surface tension and increased wetting (lower contact angles) by 16 to 56% relative to the water control (Table 1). The degree varied both between and within ionic classes with no clear trends apparent.

Surfactant-induced ethylene and phytotoxicity. Surfactants from all 3 ionic classes induced ethylene evolution by cowpea leaves (Table 2). Of the nonionics examined Triton X-100 and Ortho X-77, induced ethylene evolution while Tween 20 and Triton X-405 did not. Similarly, the anionic surfactant, Aerosol OT, induced ethylene evolution while Dupanol ME was without effect. Both cationic surfactants induced ethylene evolution, Arquad C-50 showing greater activity. Increased penetration time (12 to 24 hr) resulted in decreased or no significant change in ethylene evolution with the single exception of Arquad C-50 (Table 2).

No ethylene was detected in the headspace atmosphere of any treatment (± surfactant, ± leaf tissue) of freeze dried tissue (data not presented).

Surfactants that induced ethylene evolution also induced phytotoxicity (Table 2). The degree of phytotoxicity increased with increasing penetration time (12 to 24 hr) and in no case was visual phytotoxicity observed without a measurable increase in ethylene.

Time-course. The rate of surfactant-induced ethylene evolution increased during the first 6 to 12 hr after treatment then decreased slowly for the next 12 to 36 hr and returned to control rates within 48 hr (Fig. 2). The time required to reach the maximum rate of ethylene evolution varied with ionic class. Cationic, Arquad 2C-75,

Table 1. Physical and chemical properties of selected surfactants.

Ionic class and Common name	Principle chemistry	Molecular Weight	Critical micelle concn^{z}	Surface tension (mN m^{-1})^{y}	Wetting (θ)^{x}
Nonionic					
Tween 20	Sorbitan monolaurate	1244	0.006	45.4	86
Triton X-405	Octylphenoxypoly(ethoxy)ethanol	1966	0.16	48.0	92
Triton X-100	Octylphenoxypoly(ethoxy)ethanol	628	0.019	31.0	48
Ortho X-77	Alkylaryl polyoxyethylene glycols free fatty acids / isopropanol	-	0.01	31.4	50
Regulaid	Polyoxyethylenepolypropoxypropanol	-	0.008	42.3	76
	Alkyl 2-ethoxyethanol / dihydroxypropane				
Anionic					
Dupanol ME	Sodium lauryl sulfate	288	0.24	43.6	70
Aerosol OT-B	Dioctyl sodium sulfosuccinate	444	0.03	28.7	49
Cationic					
Arquad 2C-75	Dicoco dimethyl ammonium chloride	447	0.009	30.0	62
Arquad C-50	Monococo trimethyl ammonium chloride	278	0.01	41.0	83

^{z}% (w/v).

^{y}0.1% surfactant solutions, 25^{o}C. Control (water) = 72.5 mN m^{-1}.

^{x}Contact angles formed by 1 μl drops of surfactant solution (0.1%) on the adaxial surface of cowpea leaves. Control (water) = 110^{o}.

Table 2. Effects of selected surfactants (0.1% w/v) on ethylene evolution by and phytotoxicity on primary leaves of cowpea.

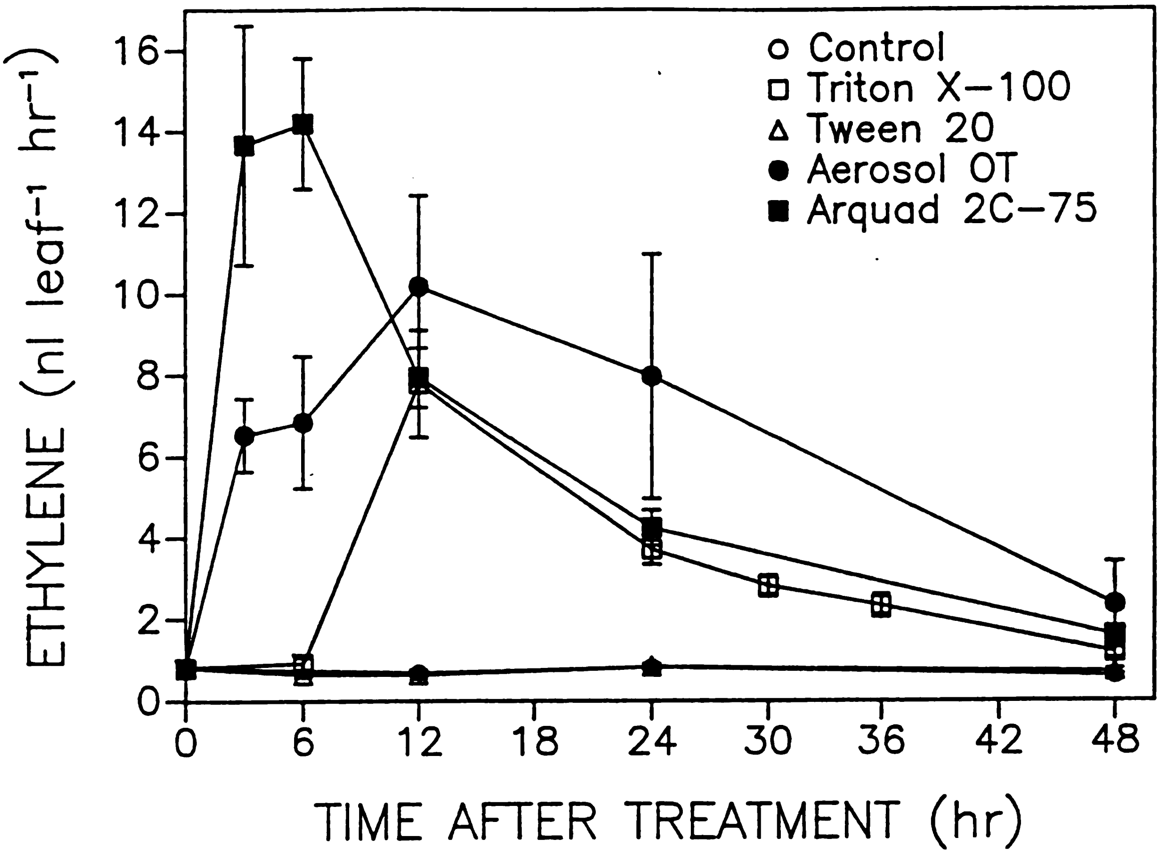
	Penetration-12hr		Penetration-24hr	
Surfactant	Ethylene (nl $leaf^{-1}$ hr^{-1})	Phyto-toxicity[z]	Ethylene (nl $leaf^{-1}$ hr^{-1})	Phyto-toxicity[z]
Control (water)	0.99 ± 0.13[y]	1	1.19 ± 0.07	1
<u>Nonionic</u>				
Tween 20	0.83 ± 0.06	1	0.72 ± 0.16	1
Triton X-405	0.95 ± 0.15	1	0.78 ± 0.05	1
Triton X-100	2.57 ± 0.69	2	3.51 ± 1.05	4
Ortho X-77	5.88 ± 2.26	3	2.33 ± 0.53	4
<u>Anionic</u>				
Dupanol ME	0.72 ± 0.06	1	0.70 ± 0.05	2
Aerosol OT	6.47 ± 1.03	4	4.16 ± 0.26	5
<u>Cationic</u>				
Arquad 2C-75	1.62 ± 0.22	4	1.23 ± 0.07	5
Arquad C-50	2.64 ± 0.24	3	3.51 ± 0.40	5

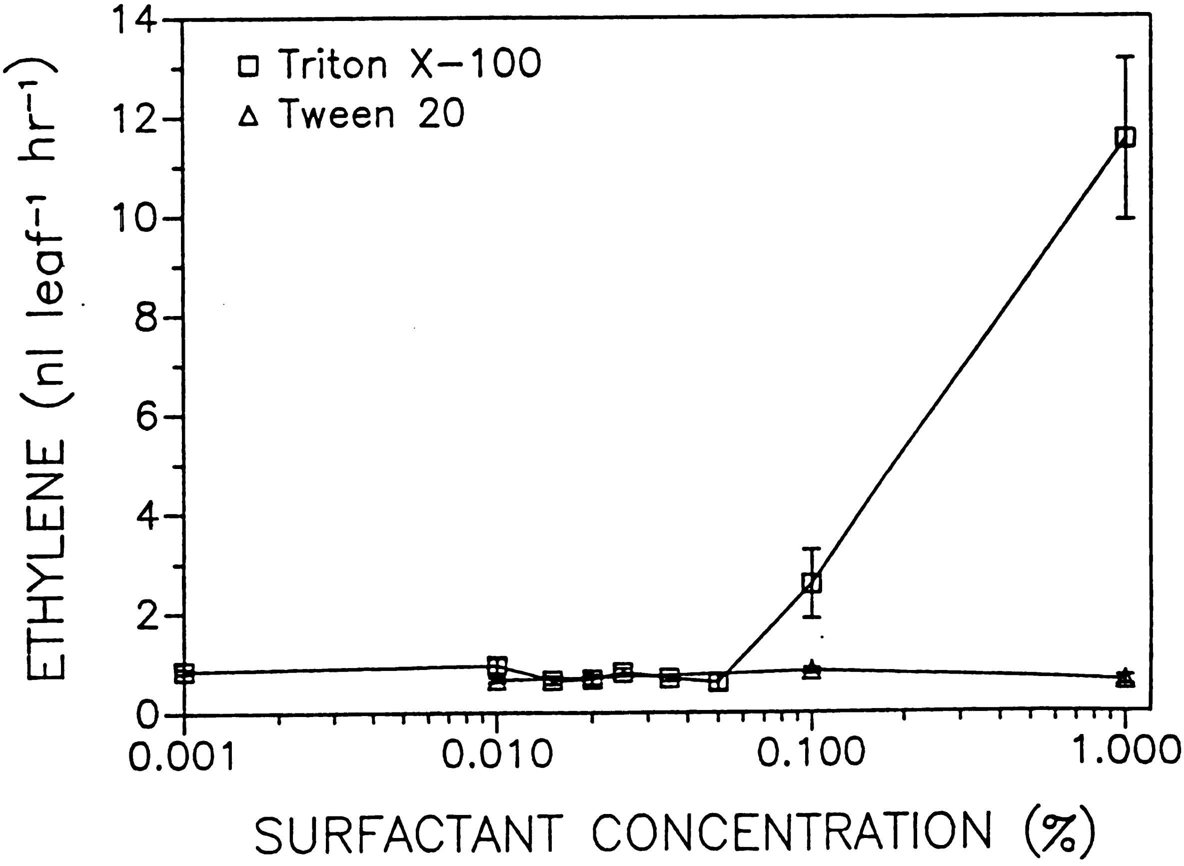
[z] Rating based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic).

[y] Mean ± SE

Figure 2. Time-course of ethylene evolution from cowpea leaves following treatment with water (control) or 20 drops (5 μl each) of nonionic (Triton X-100, Tween 20), anionic (Aerosol OT) or cationic (Arquad 2C-75) surfactants (1.0%). Vertical bars represent standard error. Where standard error bars are not shown they were smaller than the data symbol (<0.2).

Figure 3. Effect of concentration of Triton X-100 and Tween 20 on rate of ethylene evolution by primary leaves of cowpea 12 hr after treatment. Vertical bars represent standard error. Where standard errors are not shown they were smaller than the data symbol (<0.2).


ETHYLENE (nl leaf⁻¹ hr⁻¹)
TIME AFTER TREATMENT (hr)
0
2
4
6
8
10
12
14
16
0
6
12
18
24
30
36
42
48
○ Control
□ Triton X-100
△ Tween 20
● Aerosol OT
■ Arquad 2C-75


ETHYLENE (nl leaf⁻¹ hr⁻¹)
SURFACTANT CONCENTRATION (%)
0
2
4
6
8
10
12
14
0.001
0.010
0.100
1.000
□ Triton X-100
△ Tween 20

induced maximum evolution in 3 to 6 hr, anionic, Aerosol OT, in 6 to 12 hr, and nonionic, Triton X-100, in 12 to 18 hr. Maximum response was also related to surfactant concentration, but the trend between ionic classes remained unchanged. No significant ethylene evolution was induced by Tween 20.

Concentration response. The concentration response curves were markedly different for Triton X-100 and Tween 20 (Fig. 3). Triton X-100 induced significant ethylene evolution at concentrations of 0.1% and greater. This concentration response curve is representative of that observed for other ethylene-inducing surfactants namely, Triton X-35 and Ortho X-77. In contrast, ethylene evolution was not induced by Tween 20 at any concentration tested.

Effect of leaf surface. Tween 20 (0.1 to 10%) applied to the astomatous adaxial surface of sour cherry leaves did not induce ethylene or phytotoxicity (Table 3). In contrast, when applied to the abaxial surface Tween 20 induced ethylene evolution that increased linearly with increasing concentration ($r=0.99^{***}$) concomitant with the induction of phytotoxicity (Table 3).

Plant species response. Triton X-100 (1.0%) induced ethylene evolution and phytotoxicity in wheat, corn and soybean with soybean showing the greatest sensitivity (Table 4). When applied as a foliar spray, all surfactants examined induced ethylene production in apple leaves with markedly higher levels obtained with 1.0 than 0.1%. Phytotoxicity was induced with 1.0% but was confined primarily to the abaxial leaf surface. Similar results were obtained with sour cherry. Low levels of ethylene production were induced at 0.1% and significantly more at 1.0% (Table 4). Phytotoxicity was induced only

Table 3. Effect of leaf surface on surfactant-induced ethylene evolution and phytotoxicity with increasing concentrations of Tween 20.

	Adaxial		Abaxial	
Concn[z]	Ethylene ($nl\ leaf^{-1}\ hr^{-1}$)	Phyto-toxicity[y]	Ethylene ($nl\ leaf^{-1}\ hr^{-1}$)	Phyto-toxicity[y]
0.0	0.39 ± 0.04[x]	1	0.71 ± 0.08	1
0.1	0.41 ± 0.03	1	1.43 ± 0.33	2
1.0	0.40 ± 0.03	1	2.06 ± 0.18	3
5.0	0.39 ± 0.08	1	4.13 ± 0.18	4
10.0	0.60 ± 0.14	1	7.12 ± 0.15	4

[z] % (w/v)

[y] Rating based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic)

[x] Mean ± SE

Table 4. Effects of selected surfactants on inducing ethylene evolution and phytotoxicity in diverse plant species.

Common name	Cultivar	Surface[z]	Surfactant	Concn[y]	Ethylene (nl leaf^{-1} hr^{-1})	Phyto-toxicity[x]
Wheat[w]	'Hillsdale'	U	Control		0.06 ± 0.01[v]	1
		U	Triton X-100	1.0	0.12 ± 0.02	2
Corn[u]	'B73 X MO17'	U	Control		0.01 ± 0.01	1
		U	Triton X-100	1.0	0.27 ± 0.08	2
Soybean[w]	'McCall'	U	Control		0.05 ± 0.02	1
		U	Triton X-100	1.0	18.62 ± 2.72	5
Apple[t]	'Golden Delicious'	B	Control		1.69 ± 0.51	1
		B	Ortho X-77	0.1	3.49 ± 0.16	1
		B	Regulaid	0.1	3.90 ± 1.11	1
		B	Tween 20	0.1	5.60 ± 1.12	1
		B	Triton X-100	0.1	5.99 ± 1.58	1
		B	Regulaid	1.0	26.74 ± 6.91	1
		B	Triton X-100	1.0	30.98 ± 6.75	4
		B	Ortho X-77	1.0	33.00 ± 3.36	4
		B	Tween 20	1.0	50.02 ±12.07	2
Cherry[t]	'Montmorency'	B	Control		2.25 ± 0.16	1
		B	Ortho X-77	0.1	3.24 ± 1.75	1
		B	Regulaid	0.1	6.21 ± 1.73	1
		B	Tween 20	0.1	8.65 ± 3.27	1
		B	Triton X-100	0.1	9.06 ± 1.78	1
		B	Tween 20	1.0	9.24 ± 2.78	1
		B	Regulaid	1.0	11.66 ± 3.46	1
		B	Triton X-100	1.0	43.91 ± 8.83	2
		B	Ortho X-77	1.0	100.29 ±27.27	2

[z] Legend: U = upper, B = both.

[y] % (w/v).

[x] Rating based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic).

[w] 20, 1 µl drops per leaf, 18 hr penetration.

[v] Mean + SE

[u] 20, 1 µl drops per leaf, 24 hr penetration.

[t] Sprayed to runoff, 24 hr penetration, phytotoxicity localized on abaxial surface.

with Triton X-100 and Ortho X-77 and was confined to the abaxial surface. Relative ethylene production rates at 1.0% in increasing order were Tween 20, Regulaid, Triton X-100 and Ortho X-77.

Discussion

Our data demonstrate that surfactants can induce ethylene evolution in leaf tissue from a diverse group of plants. Such a response is of interest for two primary reasons. First, surfactants are commonly incorporated into foliar sprays (2,4,15,18) and are repeatedly applied to plants. Second, ethylene can affect numerous physiological processes (11,19), and thus surfactant-induced ethylene could affect plant responses to foliar applied chemicals.

Surfactants of all 3 ionic classes (nonionic, anionic and cationic) and a range of chemistries (Table 1) induced ethylene formation in cowpea leaves (Table 2). Ethylene produced was derived from plant tissue and was not from non-biological degradation of the surfactant.

The effect of incubation time on the rate of ethylene production (Fig. 1) and the time-course of surfactant-induced ethylene evolution (Fig. 2) were similar for all surfactants suggesting a common mode of action for the different ionic classes. However, there were differences in time to reach maximum ethylene evolution and duration of the response (Fig. 2). These differences, including the lack of induction of ethylene by Tween 20, may be related to differences in inherent surfactant activity or to differences in penetration into the leaf.

Phytotoxicity was generally associated with surfactant-induced ethylene evolution (Table 2), but the response was influenced by ionic class. There was a close linear relationship between ethylene production and phytotoxicity for the anionic and nonionic types, but cationic surfactants induced extensive phytotoxicity while there were low levels of ethylene production. It was not clear if these events were cause and effect, but frequently severe phytotoxicity was coupled with low ethylene production.

The transient nature of surfactant-induced ethylene evolution and the high correlation between ethylene production and phytotoxicity for the nonionic and anionic surfactants suggests that this may be a stress response. If so, ethylene evolution should increase rapidly but return to base levels in a short time (11,19). Such a stress response could explain the decrease in ethylene evolution observed with increasing penetration time (Fig. 2). However, if phytotoxicity is severe and results in membrane disruption and cell necrosis, ethylene formation would be inhibited (11,19) and the evolution rate would rapidly decline as observed for cationic Arquad 2C-75 (Table 2, Fig. 2). Similar examples of marked phytotoxicity by cationic surfactants have been observed elsewhere (7,8,10).

The concentration of surfactant in solution, especially near the CMC, may have marked effects on solution properties that may be associated with biological activity (5,16). Triton X-100-induced ethylene evolution, however, does not appear to be directly related to the CMC. Ethylene evolution was induced only at concentrations of 0.1% and greater (Fig. 3), well above the CMC (0.019%). The surfactant in each drop, regardless of initial concentration, would be expected to pass through and exceed the CMC as water was lost during drop drying.

Based on this criteria, drops containing biologically active surfactants at concentrations below the CMC would have the potential to induce ethylene evolution but this did not occur. The significance of 0.1% may be because the dose per droplet (5 μg) was adequate to induce ethylene evolution. In terms of spray application, surfactant concentrations of 0.1% are commonplace (2,15,18) and thus biological effects must be considered.

The marked effect of leaf surface on surfactant-induced ethylene evolution and phytotoxicity is most likely related to differences in penetration (Table 3). Stomata and other specialized structures on the abaxial surface may serve as preferred sites of entry.

It is significant that surfactants induced ethylene evolution and phytotoxicity not only in cowpea but in a diverse group of plant species varying in cuticular properties (Table 4). Differences in response to a given surfactant dose by different species may reflect innate physiological differences and/or differences in permeability of the cuticular membranes. The marked ethylene production induced by Tween 20 (1.0%) in apple and cherry leaves was unexpected since Tween 20 had low activity on cowpea. Further studies are needed to establish the basis of this response.

Performance of foliar-applied agrochemicals may depend on surfactant chemistry, interactions between the surfactant and active ingredient, interactions between the spray solution and plant surface (5) and may be modified by surfactant-induced biochemical responses in the plant. The role and potential impact of surfactant-induced ethylene evolution and phytotoxicity are not yet clear. However, identification of surfactants that induce biological responses and characterization of the responses would provide a rational means for selecting surfactants for formulation of agrochemicals and for use in tank mixes. For example, an ethylene inducing, phytotoxic surfactant may be acceptable (desirable) for herbicide applications while undesirable with a growth regulator used to control a process sensitive to ethylene.

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SECTION III

RELATIONSHIPS BETWEEN SURFACTANT-INDUCED ETHYLENE PRODUCTION AND ETHYLENE OXIDE CHAIN LENGTH FOR OCTYLPHENOL AND LINEAR ALCOHOL SURFACTANTS

RELATIONSHIPS BETWEEN SURFACTANT-INDUCED ETHYLENE PRODUCTION AND ETHYLENE OXIDE CHAIN LENGTH FOR OCTYLPHENOL AND LINEAR ALCOHOL SURFACTANTS

Abstract

The effects of the ethylene oxide (EO) chain on surfactant-induced ethylene production for selected octylphenol (OP) and linear alcohol (C_{12-15}, C_{9-11}, C_9) (LA) surfactants were established with cowpea (Vigna unguiculata (L.) Walp. subsp. unguiculata 'Dixielee'). OP-induced ethylene production was concentration dependent and decreased log linearly with increasing EO. C_{12-15} LA-induced ethylene production (0.1%) also decreased log linearly with increasing EO, however, at 1.0% the relationship was curvilinear. Relationships for C_{9-11} and C_9 LA series were nonlinear with greatest biological activity at intermediate (8-12) EO content. A similar relationship was found for surfactant-induced phytotoxicity and EO chain length as between ethylene formation and EO content. Similar relationships between EO chain length and ethylene production were found in germinating mung bean (Vigna radiata) seedlings as in cowpea. Radicle growth was markedly inhibited and in some cases radicles were irreversibly damaged by ethylene producing surfactants. A mixture of a short chain (OP+1EO) non-water soluble surfactant with a long chain (OP+40EO) non ethylene producing surfactant significantly increased biological activity on cowpea.

Introduction

Recent studies have shown that surfactants of widely different chemistries may induce ethylene production in plants (Lownds, 1987). However, specific relationships between surfactant structure and ethylene production have not been described.

Numerous physico-chemical properties of surfactants change markedly with the chemistry of the hydrophobe and hydrophile (Seaman, 1982). In addition, surfactant-plant interactions indexed by sorption by cuticles (Shaffer and Bukovac, 1986), root elongation (Buchanan, 1965), phytotoxicity to soybean cells (Davis and Stolzenberg, 1986) and apple leaves (Furmidge, 1959) may be related to the ethylene oxide (EO) content for a given surfactant hydrophobe. The mechanisms are not clear but may be related to degree of penetration (Silcox and Holloway, 1986).

Surfactant chemistry is also important in enhancement of pesticide activity (Seaman, 1982). This may be related to physical and/or chemical interactions between surfactant + pesticide, surfactant + plant and/or surfactant + pesticide + plant. Several mechanisms have been proposed (Currier and Dybing, 1959), and recent reports suggest that humectancy (Stevens and Bukovac, 1987) and degree of surfactant penetration (Silcox and Holloway, 1986; Stevens and Bukovac, 1987) may also be important.

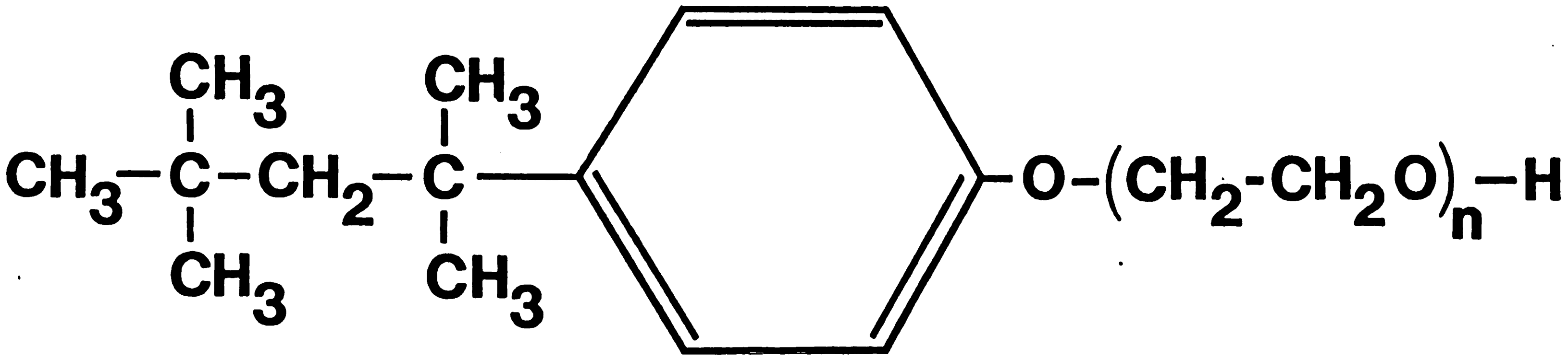
In this paper we report on the relationships between surfactant-induced ethylene production, a plant physiological response, and degree of ethoxylation of octylphenol and selected linear alcohols.

Materials and Methods

Plant material and growing conditions. Cowpea seeds were pregerminated for 24 h in the dark at 30° C on moist paper towels. Healthy seeds of uniform size and radicle length were selected and planted into disposable AC-4-8 "Cell Paks" (Geo. J. Ball Co., W. Chicago, IL) using PROMIX BX (Premier Brands, Inc., New Rochelle, NY) as a growing medium. Seedlings were grown in a growth chamber under day/night temperatures of 25/20 °C and relative humidities of 45-55/65-75%, respectively. Light was provided for 16 h daily at 220 μmol m^{-2} s^{-1} (400-700 nm; cool-white fluorescent supplemented with 15% incandescent).

Surfactants. 4-(1,1,3,3-tetramethyl)butylphenol (OP) condensed with 3 (OP+3EO), 5 (OP+5EO), 9.5 (OP+9.5EO), 30 (OP+30EO) and 40 (OP+40EO) ethylene oxide (EO) units were selected (Triton X surfactants; Rohm and Haas Co., Philadelphia, PA). A similar OP surfactant series (Igepal CA; GAF Corporation, Wayne, NJ) condensed with 5 (OP+5EO), 7 (OP+7EO), 9 (OP+9EO), 13 (OP+13EO) and 40 (OP+40EO) EO units was also used (Fig. 1). In addition, 3 linear alcohol ethylene oxide (LAEO) surfactants (Neodol; Shell Chemical Co., Houston, TX) were selected namely, C_{12-15} (Neodol 25) condensed with 3 (C_{12-15} +3EO), 7 (C_{12-15}+7EO), 9 (C_{12-15}+9EO), 13 (C_{12-15}+13EO) and 30 (C_{12-15}+30EO) EO units, C_{9-11} (Neodol 91) condensed with 6 (C_{9-11}+6EO), 8 (C_{9-11}+8EO), 10 (C_{9-11}+10EO), 12 (C_{9-11}+12EO), and 20 (C_{9-11}+20EO) EO units and a purified nonanol (C_9) condensed with 2 (C_9+2EO), 3 (C_9+3EO), 4 (C_9+4EO), 6 (C_9+6EO), 9 (C_9+9EO) and 12 (C_9+12EO) EO units (Fig.1). All surfactants were mixtures of oligomers where the EO number represents an average value and the ethoxymer mole ratio distribution follows a Poisson distribution (Anon., 1982). The hydrophobe of the C_{9-11} and C_{12-15} linear alcohols are mixtures of C_9, C_{10}, C_{11} or C_{12}, C_{13}, C_{14}, C_{15} with approximate ratios of 1:1:1 and 2:3:3:2, respectively (McCoy and Bullock, 1969).

Figure 1. **Generalized structure of octylphenol (Triton X) and linear alcohol (Neodol) surfactants.**



OP+nEO

$CH_3-(CH_2)_m-O-(CH_2-CH_2O)_n-H$

LA+nEO

All surfactant solutions were prepared on a weight/volume (w/v) basis with distilled deionized water.

Treatment procedure, phytotoxicity rating and ethylene measurement. Primary leaves of 10-day-old cowpea, selected for uniformity and freedom from defects, were treated by applying the appropriate surfactant solution as 20 discrete droplets (5 μl each) on the adaxial surface (avoiding the veins) using a microsyringe fitted with an automatic dispenser. All treatments were made 2.5 to 3.5 h after the beginning of the light cycle. Leaf orientation was horizontal and when necessary individual leaves were supported in a horizontal position to prevent droplet coalescence and excessive spreading. Surfactant penetration was allowed to proceed under environmental conditions previously described for 12 or 24 h after which treated leaves were rated for phytotoxicity and sampled for ethylene determination.

Leaves were visually inspected and assigned a phytotoxicity rating of 1 to 5 based on degree of tissue discoloration and/or necrosis as previously reported (Lownds, 1987). Briefly, ratings were as follows;

Rating 1 - no discoloration or necrosis
2 - isolated areas of discolored, sunken and/or necrotic tissue
3 - discolored, sunken and/or necrotic tissue at the entire periphery of the droplet:leaf interface
4 - entire droplet:leaf interface area discolored and/or sunken, less than 50% necrotic

5 - entire droplet:leaf interface area discolored
and/or sunken, greater than 50% necrotic

To measure the rate of ethylene evolution, leaves were excised and positioned abaxial side outward with minimum overlap in 25 x 200 mL test tubes containing 2 mL of distilled water. The tubes were allowed to equilibrate (uncapped) for 1 h in a constant temperature (30 °C) water bath, flushed with air for 30 sec, sealed with rubber serum stoppers and incubated for 2 h (Lownds, 1987).

Ethylene was determined on a 1 mL headspace sample by gas chromatography (Varian 1440, Varian Associates, Inc., Palo Alto, CA) using an activated alumina column and flame ionization detector. The injection port, column and detector temperatures were 130, 100 and 150 °C, respectively. N_2 flow was maintained at 15 ml^{-1} min^{-1}. Data were expressed as rate of ethylene evolution, nl $leaf^{-1}$ hr^{-1}.

Relationship between EO chain length and ethylene. Primary cowpea leaves were treated as previously described with each ethoxylate of the five surfactant hydrophobes at 0.1 and 1.0%. Ethylene was measured and phytotoxicity rated after 12 and 24 h.

Mung bean studies. Mung bean seeds were selected for uniform size and color and freedom from defects. Twenty seeds, found to provide adequate response and the maximum number convenient for a 25 mL Erlemeyer flask (data not presented), were pregerminated with 3 mL of distilled water and fitted with a plug of cotton. Following the 24-h pregermination period, 3 mL of the designated treatment solution was pipetted into each flask and held in the dark at 30 °C. After 4 h, the flasks were removed , flushed with air for 30 s, capped with rubber serum stoppers and returned to the dark for incubation. After 2 h of incubation, 1 mL of the headspace was removed and ethylene determined by gas chromatography as described for the cowpea assay. The flasks were then uncapped, flushed with air and returned to the dark for further penetration. A similar procedure of flushing, capping and ethylene determination was employed 24 h after treatment. Upon completion of the second ethylene determination, the seeds were removed and the radicles excised and weighed (fresh weight).

Surfactant mixtures. OP+1EO was insoluble in water and therefore could not be compared to the other ethoxymers used in these studies. Thus, it was mixed with OP+40EO (non-ethylene producer) at equal concentrations (0.1 or 1.0% w/v) and the mixture applied to cowpea leaves as previously described. Leaves were rated for phytotoxicity and ethylene determined 12 and 24 h after treatment.

Experimental design and statistics. All experiments were conducted using a randomized complete block design with 8 or 10 replications. Relationships between EO content and ethylene were determined by curve fitting with PlotIT (Eisensmith, 1985), a program designed for the fitting of linear and nonlinear regression models, as well as the graphic display of data. Models were chosen on the basis of residual sums of squares, coefficient of determination (r^2) and visual fit of the regression lines in relation to the observed data. Relationships between phytotoxicity and EO chain length were determined using linear regression analysis.

Results and Discussion

Relationship between ethylene oxide chain length and ethylene production. Selected ethoxymers of all hydrophobes were biologically

Table I. Effects of octylphenol (OP) surfactants, applied alone or in combination, on ethylene production by cowpea leaves 12 h after treatment.

surfactant	ethylene (nl leaf^{-1} hr^{-1})		phytotoxicity (rating)[a]	
	0.1%	1.0%	0.1%	1.0%
control	1.05	1.10	1.0	1.0
OP+1EO	1.22	7.57	2.1	3.1
OP+40EO	1.26	1.22	1.0	1.0
OP+1EO + OP+40EO	2.70	29.56	2.9	4.7
LSD (P=0.05)	0.70	4.91	0.5	0.5

[a] Rating based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic).

active in inducing ethylene formation by cowpea leaves (Figs. 2-4, Table I). Because ethylene is an active plant growth regulator at low concentrations, this response may have important implications in the selection and use of surfactants in formulation, application and performance of agrochemicals.

Surfactants are selected as spray additives primarily based on their ability to modify the physical characteristics of the spray solution and, hence, the performance of the active ingredient (Seaman, 1982). Physical:chemical properties (e.g. hydrophile:lipophile balance, critical micelle concentration, surface tension, solubilization) depend on surfactant chemistry, and for a given hydrophobe, may be related to EO content. Resultant surfactant:plant interactions and plant responses to surfactants, especially phytotoxicity, are also related to degree of ethoxylation (Lownds, 1987).

Ethylene production induced by OP surfactants (Triton X) decreased log linearly with increasing EO content (Fig. 2). The magnitude of the response was concentration dependent and the relationship to EO content was highly correlated ($r^2 = 0.94-0.97$) at both concentrations. A similar relationship was observed for a structurally related OP hydrophobe (Igepal CA). At 0.1% the equation describing the response was not significantly different than that presented in Fig. 2, however, at 1.0% the response was significantly greater being, $\hat{Y} = -74.24(X) + 111.4$. The basis for these differences are not clear and will require further study into the relative purities, ethoxymer distribution and branching of the two OP hydrophobes.

The relationship between EO content and ethylene production for

Figure 2. Relationship between surfactant-induced ethylene production by primary leaves of cowpea and log (ethylene oxide (EO) content) for octylphenol (Triton X) surfactants at 0.1 and 1.0% 12 h after treatment.

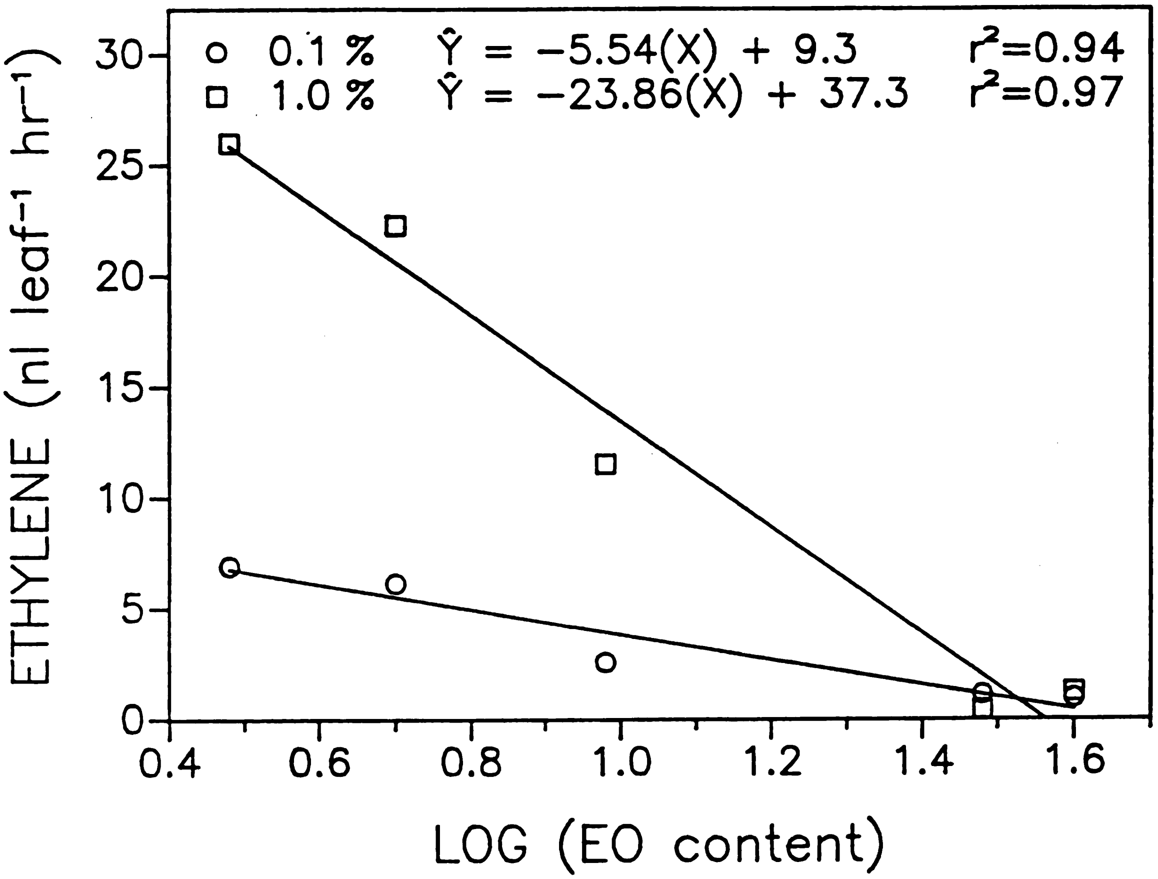
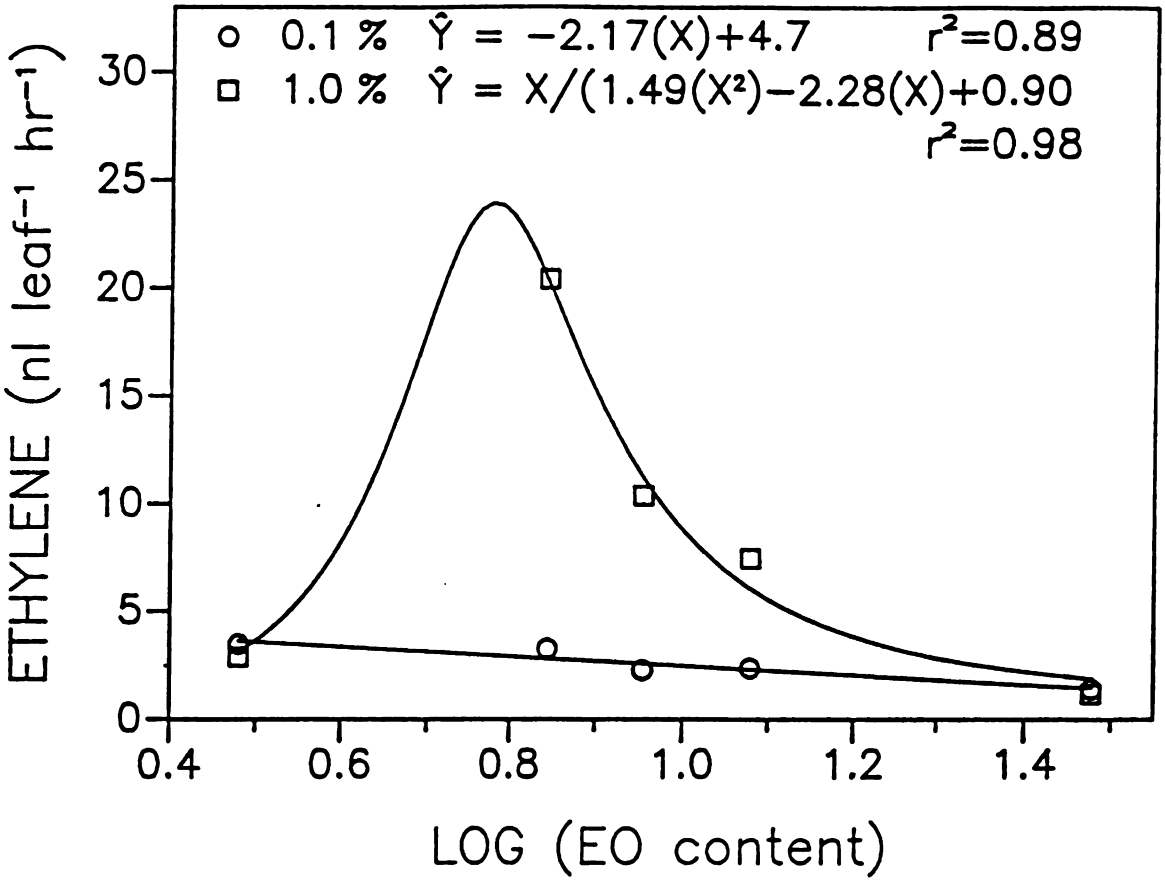
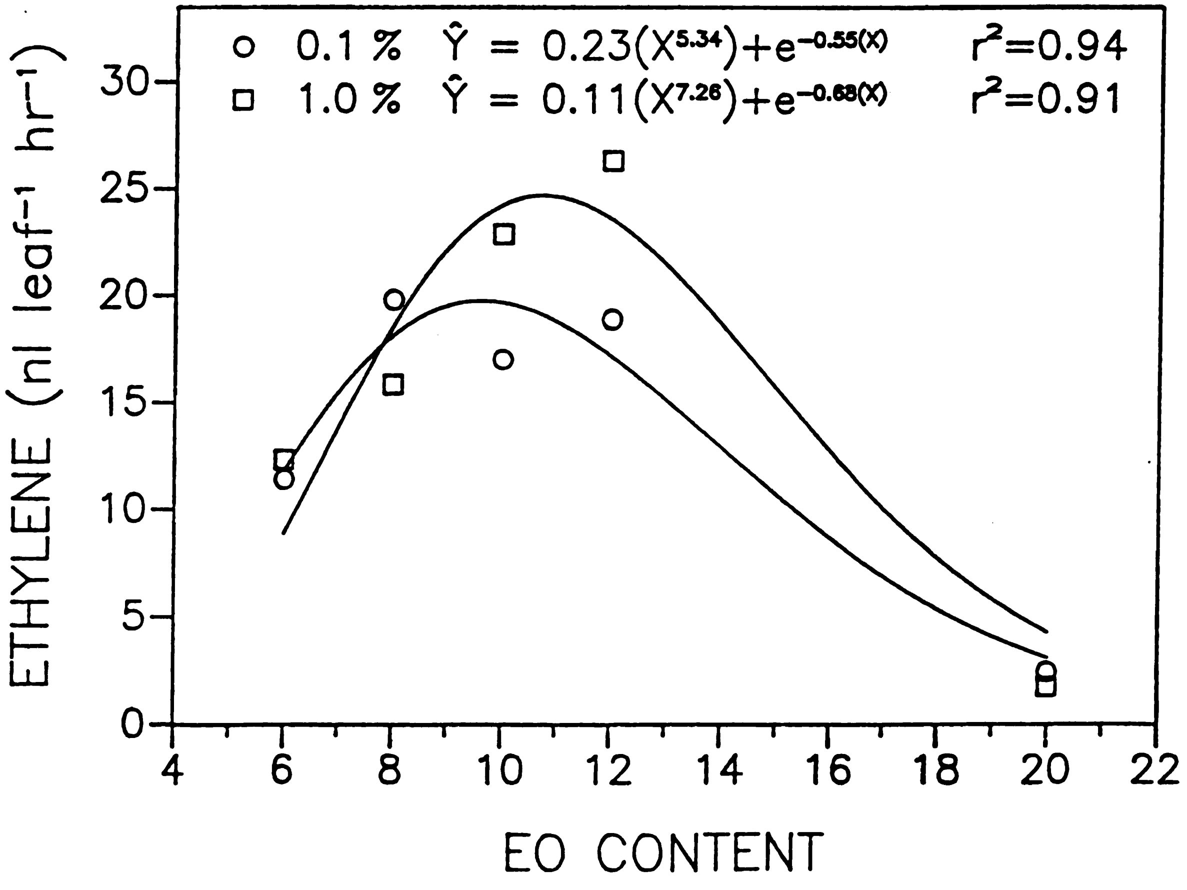

○ 0.1 % $\hat{Y} = -5.54(X) + 9.3$ $r^2=0.94$
□ 1.0 % $\hat{Y} = -23.86(X) + 37.3$ $r^2=0.97$
ETHYLENE (nl leaf⁻¹ hr⁻¹)
0
5
10
15
20
25
30
LOG (EO content)
0.4
0.6
0.8
1.0
1.2
1.4
1.6

Figure 3. Relationship between surfactant-induced ethylene production by primary leaves of cowpea and log(ethylene oxide (EO) content) for C_{12-15} linear alcohol surfactants at 0.1 and 1.0% 12 h after treatment.

Figure 4. Relationship between surfactant-induced ethylene production by primary leaves of cowpea and ethylene oxide (EO) content for C_{9-11} linear alcohol surfactants at 0.1 and 1.0% 12 h after treatment.


○ 0.1 % Ŷ = −2.17(X)+4.7 r²=0.89
□ 1.0 % Ŷ = X/(1.49(X²)−2.28(X)+0.90 r²=0.98
ETHYLENE (nl leaf⁻¹ hr⁻¹)
0
5
10
15
20
25
30
0.4
0.6
0.8
1.0
1.2
1.4
LOG (EO content)


○ 0.1 % Ŷ = 0.23(X^5.34)+e^−0.55(X) r²=0.94
□ 1.0 % Ŷ = 0.11(X^7.26)+e^−0.68(X) r²=0.91
ETHYLENE (nl leaf⁻¹ hr⁻¹)
0
5
10
15
20
25
30
4
6
8
10
12
14
16
18
20
22
EO CONTENT

the C_{12-15} linear alcohol hydrophobe was concentration dependent (Fig. 3). At 0.1%, ethylene evolution decreased log linearly with increasing EO content similar to the OP surfactants. In contrast, at 1.0% maximum ethylene production occurred at 7EO and decreased markedly with shorter (3) EO and longer (9,12,30) EO chains (Fig. 3). This relationship was nonlinear and could best be simulated using a hoop model (r^2=0.98). A nonlinear relationship was also observed for the C_{9-11} linear alcohol hydrophobe with the best fit against EO content (Fig. 4). Greatest ethylene production was induced with an EO content between 8 and 12, and the relationship was best simulated using a multiplicative exponential model, r^2=0.94 and 0.91 for 0.1 and 1.0%, respectively. The fit against log(EO content) was r^2 = 0.84 and 0.77 for 0.1 and 1.0%, respectively. The relationship for the C_9 linear alcohol hydrophobe was also nonlinear and followed the pattern of the C_{9-11} hydrophobe (data not presented).

Relationships between EO content and ethylene production may reflect differences in innate biological activity, penetration and/or the ability of cowpea to metabolize the ethoxymers. Foliar uptake of OP (Stevens and Bukovac, 1986) and nonlyphenol (Silcox and Holloway, 1986) surfactants was inversely related to EO content. The linear decrease in ethylene production with increasing EO content for the OP surfactants (Fig. 2) suggests a direct relationship to surfactant penetration. Ethylene production induced by LA surfactants (Figs. 3,4) is probably also a result of surfactant penetration. The relatively low rates induced by shorter EO chains may be related to their high lipophilicity. Thus, they would be expected to penetrate into the cuticle, where they may remain failing to reach the site(s) of ethylene production. Further uptake and localization studies are needed to clarify these relationships. Metabolism of surfactants has been documented (Stolzenburg et.al., 1982; Silcox & Holloway, 1986) but there are insufficient data on rates of metabolism of different ethoxymers to determine its relative significance.

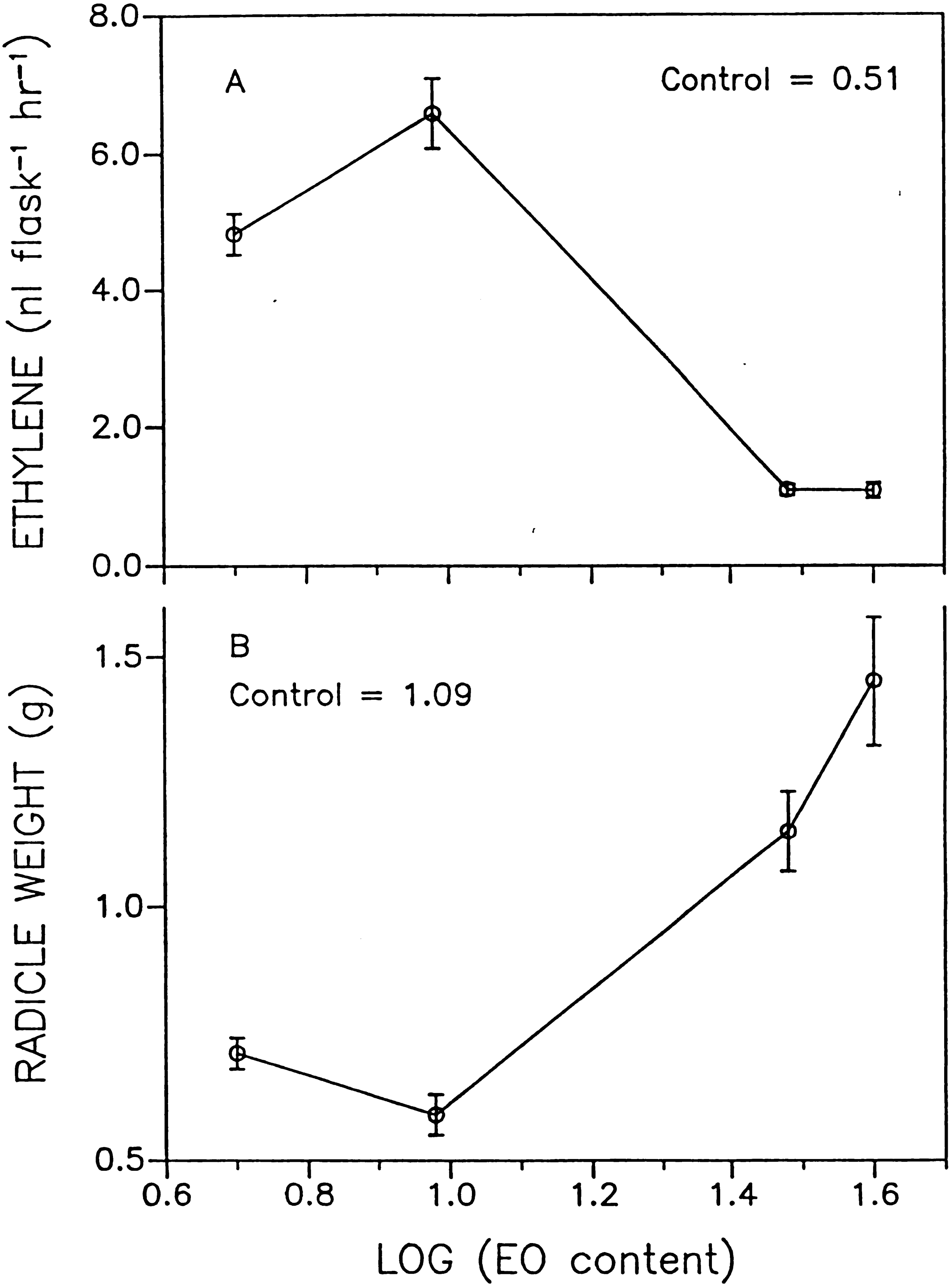
Relationships between ethylene oxide chain length and phytotoxicity. Surfactant-induced phytotoxicity in cowpea leaves decreased with increasing EO (Lownds, 1987). Further, phytotoxicity was consistently directly related to ethylene production (Lownds, 1987) and has been related to surfactant uptake (Silcox and Holloway, 1986). Therefore, the relationship between phytotoxicity and EO chain length would be expected to parallel that of ethylene production. Such relationships were found for OP-induced (Triton X, Igepal CA) phytotoxicity being linearly related to EO chain length (Table II) while LA-induced (Neodol 25, Neodol 91, nonanol) phytotoxicity was not (Table II), but generally followed the nonlinear relationships observed for ethylene production (Figs. 3,4). The importance of surfactant-induced phytotoxicity remains to be determined but its effects on leaves (Furmidge, 1959) and contribution to russeting of apple fruit (Noga and Bukovac, 1986) suggest that it may be important both physiologically and economically.

Mung bean studies. Germinating mung bean seeds provided a plant system where the EO effect on ethylene production and radicle growth could be assessed simultaneously with the same tissue. Maximum ethylene production rates for OP surfactants were induced by 10 EO, less by 5 and no significant ethylene production by 30 and 40 EO (Fig. 5A). With the 5 EO, some surfactant separated out of solution during

Table II. Relationship between log(ethylene oxide (EO) chain length) and phytotoxicity for selected surfactant hydrophobes (0.1%) 12 h after treatment.

surfactant hydrophobe	EO range	coefficient of determination (r^2)
octylphenol (Triton X)	5-40	0.958
octylphenol (Igepal CA)	5-40	0.922
C_{12-15} linear alcohol	3-30	0.614
C_{9-11} linear alcohol	6-20	0.401
C_9 linear alcohol	2-12	0.014

Figure 5. Relationship between surfactant-induced ethylene production by mung bean seedlings (A) and radicle fresh weight (B) and ethylene oxide (EO) content for octylphenol (Triton X) surfactants at 1.0%. Ethylene production rates were determined 4 h and radicle weight 24 h after treatment. Vertical bars represent standard error. Where standard errors are not shown they were smaller than the data symbol.


ETHYLENE (nl flask^{-1} hr^{-1})
8.0
6.0
4.0
2.0
0.0
A
Control = 0.51
RADICLE WEIGHT (g)
1.5
1.0
0.5
B
Control = 1.09
0.6
0.8
1.0
1.2
1.4
1.6
LOG (EO content)

the treatment period, which may have resulted in decreased absorption and may account for the decrease in ethylene production observed (Fig. 5).

The root growth (fresh weight) curve was a mirror image of ethylene production (Fig. 5B). Those ethoxymers that induced ethylene production (5 and 10 EO) significantly decreased root weight while those with 30 and 40 EO stimulated root growth. Inhibition of root growth may be a direct surfactant effect or the result of ethylene inhibition of root elongation. Although not examined experimentally, we suggest this is a direct surfactant effect. Roots that were inhibited also displayed marked visual changes. Root tips (1-2 cm) were affected most severly, becoming flaccid and taking on a 'water soaked' appearance suggesting membrane damage. In parallel experiments we observed significant reductions in root growth without increased ethylene production (data not presented). In some studies there was also evidence of root thickening, a typical ethylene effect, but this was small relative to surfactant-induced damage.

Surfactant mixtures. Surfactants containing short EO chains are very lipophilic with low water solubility. We could not, therefore, directly compare them to freely water soluble ethoxymers. Because lipophilic surfactants may be important in formulations and formulated materials often contain more than one surfactant (or mixture of ethoxymers), we combined the slightly soluble OP+1EO with the non-ethylene producing, non-phytotoxic OP+40EO ethoxymer. At both 0.1 and 1.0% ethylene production by the combination of OP+1EO and OP+40EO was significantly greater than that of either ethoxymer alone (Table I). Surfactant-induced phytotoxicity followed a similar pattern. We view this increase in response from the mixture as the result of increased solubility of the OP+1EO in the presence of OP+40EO. Based on degree of phytotoxicity, greater penetration of the OP+1EO occured from the surfactant mixture than from OP+1EO applied alone. The interactions of surfactant mixtures with the plant surface and on plant responses offer opportunities for further study.

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SECTION IV

EFFECT OF TRITON X-100 ON ACC AND ETHYLENE FORMATION AND LACK OF MOVEMENT IN COWPEA LEAVES

EFFECT OF TRITON X-100 ON ACC AND ETHYLENE FORMATION AND LACK OF ACC MOVEMENT IN COWPEA LEAVES

Abstract

ACC and ethylene production were induced in cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Dixielee') leaves by foliar applied Triton X-100, 1-naphthaleneacetic acid (NAA) and 1-aminocyclopropane-1-carboxylic acid (ACC). The addition of Triton X-100 (1.0%) to NAA (1mM) increased both ACC and ethylene production approximately 3-fold. Similarly, addition of Triton X-100 to ACC (1 mM) resulted in incresed ethylene production. ACC and ethylene formation increased markedly the first 12 hr after treatment and then decreased but at a much slower rate. The apparent rate constant for ACC to ethylene conversion increased between 0 and 6 hr following treatment with Triton X-100, NAA and the combination of Triton X-100 and NAA and then remained almost constant over the next 30 hr at 0.10, 0.24 and 0.08, respectively. There was no significant translocation of ACC from the treatment site as evidenced directly by ACC measurements and indirectly by ethylene production rates in treated and non-treated tissue.

Introduction

Surfactants have been shown to induce ethylene production by plant leaves (17). Ethylene production is also promoted by a variety of environmental and physiological factors (14,22) all of which appear to involve a single biosynthetic pathway: methionine -> S-adenosyl-methionine (SAM) -> ACC -> ethylene (1,12,22,26). Stress factors such as wounding, drought, waterlogging, chilling, virus infection and noxious chemicals can enhance ethylene production by increasing the conversion of SAM to ACC (5,7,9,21,22). This is the same step at which auxin induces ethylene production (13,23,25) and has generally been found to be the rate limiting step in ethylene biosynthesis (13,23,26). In contrast, surfactants, and stress factors that modify membrane structure can reduce the rate of ethylene production by inhibiting conversion of ACC to ethylene (4,6,21,24).

Ethylene biosynthesis and associated physiological responses are not necessarily confined to stressed tissues. For example, ACC is produced in tomato roots under anaerobic stress, but its conversion to ethylene is inhibited by the lack of oxygen (8,9,10,11). The ACC produced is translocated through the xylem to the above ground organs where it is converted to ethylene and may induce physiological responses (8,9,10,11). Further, ACC transport is not confined to the xylem but may also occur in the phloem even under conditions where ACC to ethylene conversion is not limiting (2).

Surfactants are extensively used as components of spray formulations and are, therefore, almost ubiquitous in spray applications. They can induce phytotoxicity and this response appears to be associated with ethylene production (17). To better understand the mechanism of surfactant-induced ethylene production and its potential impact on the performance of foliar-applied agrochemicals, our study focused on the effects of a foliar-applied surfactant, Triton X-100, on ACC formation and movement in relation to ethylene production.

Materials and Methods

Plant material and growing conditions. Cowpea seeds were pregerminated (24 hr) in the dark at 30°C on moist paper towels. Healthy seeds of uniform size and radicle length were selected, seed coats removed to facilitate uniform epicotyl emergence and planted into disposable AC-4-8 "Cell Paks" (Geo. J. Ball Co., W. Chicago, IL) using PROMIX BX (Premier Brands Inc., New Rochelle, NY) as a growing medium. Germination was completed and seedlings grown in a growth chamber under day/night temperatures of 25/20°C. Light was provided for 16 hr daily at 180-230 μmol m^{-2} s^{-1} (400-700 nm; cool-white fluorescent, GE F48T12 CW-1500 supplemented with 15% incandescent). Relative humidity during the light period varied from 45-55% and 65-75% during the dark.

Chemicals. Triton X-100 (Rohm and Haas Co., Philadelphia, PA), an oligomeric mixture containing an average 9.5 ethylene oxide units (EO;20), was used at 1.0% (w/v), a concentration above the critical micelle concentration (CMC; 20). Two chemicals, NAA and ACC (Sigma Chemical Co., St. Louis, MO), whose effects on ethylene production are well established (14,22) were used at 1 mM for comparative purposes. All solutions were prepared with distilled deionized water.

Treatment proceedure, ethylene and ACC determination. Primary leaves of uniform 10-day-old cowpea seedlings were treated by applying Triton X-100 (1.0%), NAA (1mM), ACC (1mM), a combination of Triton X-100 and NAA (1.0% and 1mM, respectively) and a combination of Triton X-100 and ACC (1.0% and 1mM, respectively) as 10 drops (5 μl each) to the apical or basal half of the adaxial surface using a microsyringe fitted with an automatic dispenser. All treatments were applied 2.5 to 3.5 hr after the beginning of the light period and penetration was allowed to proceed for 24 hr in the growth chamber under the specified conditions. After the designated penetration period both primary leaves were removed and separated into treated and non-treated halves and each set (2 leaf halves) was carefully positioned abaxial side outward, into 25 x 200 mm test tubes containing 2 ml of distilled water. The leaf tissue was allowed to equilibrate, uncapped, for 1 hr in a constant temperature (30°C) water bath after which each tube was flushed with air for 30 sec, sealed with a rubber serum stopper and incubated for an additional 2 hr before sampling the gas phase for ethylene and assaying the tissue for ACC.

Gas samples (1 ml) were removed and assayed for ethylene by gas chromatography (Varian 1440, Varian Associates, Inc., Palo Alto, CA) using an activated alumina column and flame ionization detector. The injection port, column and detector temperatures were 130, 100 and 150°C, respectively. N_2 flow was maintained at 15 ml min^{-1}. Data were calculated (using void volume, incubation time and fresh tissue weight) as rates of ethylene production, nmol g fresh $weight^{-1}$ hr^{-1}.

Leaves (or leaf tissues) were removed immediately after ethylene measurements, frozen on dry ice and stored at -30°C. Frozen leaf tissue was homogenized in 80% ethanol and extracted for 16 hr by continuously shaking at 4°C. The extract was centrifuged for 35 min at 1800 *g*, the pellet resuspended in ethanol, centrifuged and the 2 supernatants combined and evaporated to dryness under reduced pressure at 40°C (Evapo-mix, Buchler Instruments, Fort Lee, NJ). The residue was then taken up in 0.1% L-77 (silicone based surfactant; Union Carbide Co., New York, NY) and clarified by passing through a glass-paper filter. A 0.2 ml aliquot was assayed for ACC according to the method of Lizada and Yang (15). The efficiency of ACC to ethylene conversion in the presence of the extract was determined by adding 1 nmol of ACC as an internal standard. Conversion efficiency was between 60 and 77%. ACC content was calculated based on ethylene liberated and the conversion efficiency.

Time-course study. Triton X-100 (1.0%), NAA (1mM) and a combination of Triton X-100 and NAA (1.0% and 1mM, respectively) were applied as 20 drops (5 μl each) to the adaxial surface of primary cowpea leaves. Following penetration periods of 6, 12, 24, 30, and 36 hr, treated leaves were sampled for ethylene and ACC determinations. Other procedures were as previously described.

ACC to ethylene conversion. Using data from the time-course experiments, the apparent rate constants (k_a) for the conversion of ACC to ethylene were calculated by dividing the rate of ethylene production (nmol g^{-1} hr^{-1}) by the ACC content (nmol g^{-1}) of the tissue.

Localization study. Primary leaves of 10-day-old cowpea seedlings were treated by applying Triton X-100 (1.0%), NAA (1mM) and a combination of Triton X-100 and NAA (1.0% and 1mM, respectively) as 5 drops (5 μl each). Each drop was placed on the adaxial surface avoiding veins at least 2.5 cm apart. Following a 12-hr penetration period, the primary leaves were detached and discs (16.3 mm diam.) containing the treated areas and surrounding tissue were excised from each leaf with a cork borer. A second disc (8.6 mm diam.), containing only the treated area, was then excised from the center of each 16.3 mm disc. The discs containing the treatment site (5 per replication) were placed in 15 x 85 mm test tubes fitted with filter paper moistened with 0.5 ml of distilled water. The 16.3 mm diam. rings of surrounding tissue (non-treated) were placed abaxial side outward into 25 x 200 mm test tubes containing 2 ml distilled water. All tubes were placed in a 30°C water bath, equilibrated, flushed, capped, and incubated as previously described for ethylene measurement. ACC was determined in leaf tissue immediately after ethylene assay.

Experimental design. Experiments were conducted using a randomized complete block design with 8 replications, except the time-course study (4 replications), and analyzed as factorials. The mean, standard deviation, standard error and coefficient of variation were also calculated for the ACC and ethylene data. All experiments were repeated at least 2 times.

Results

ACC and ethylene production. Both Triton X-100 and NAA induced a greater than 3-fold increase in the ACC level in treated cowpea leaves (Table 1). The combination of Triton X-100 and NAA induced the greatest increase in ACC level, approximately 3-fold greater than either compound alone (interaction significant at $P=0.05$).

ACC content following treatment with Triton X-100 or the combination of Triton X-100 and NAA increased markedly during the first 12 hr then decreased but at a much lower rate (Fig. 1A). ACC production was greatest for leaves treated with the combination of Triton X-100 and NAA, intermediate for Triton X-100 but consistently lower for NAA, probably due to poor NAA penetration.

Ethylene production rates following all treatments increased rapidly the first 12 hr followed by slowly declining rates (Fig. 1B). The highest ethylene production rates were observed for Triton X-100

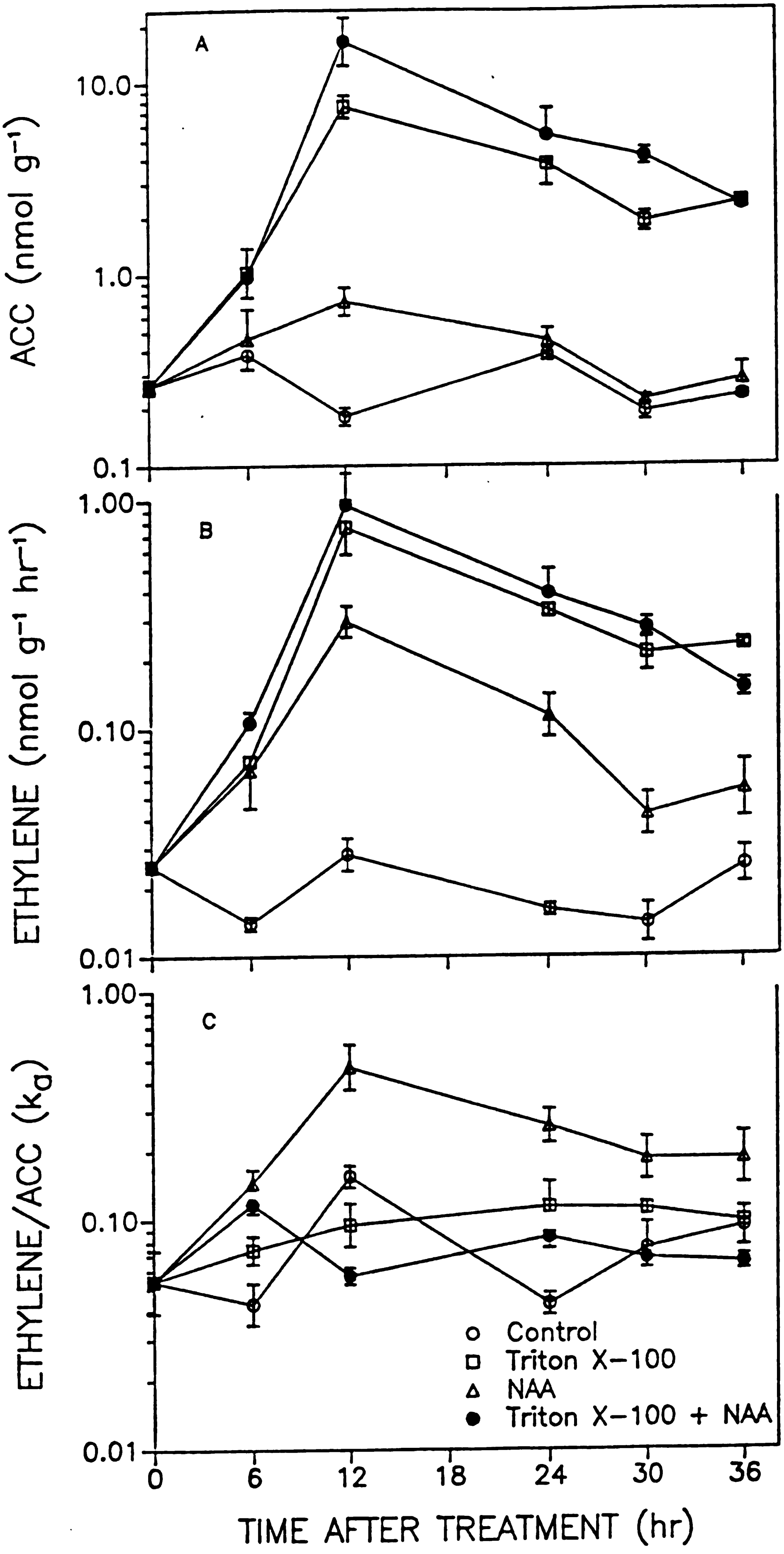
Table 1. Surfactant- and auxin-induced ACC production by cowpea leaves.

Treatment	ACC (nmol g^{-1})[z]
Control	0.78 ± 0.29[y]
Triton X-100 (1.0%)	2.55 ± 0.46
NAA (1mM)	2.54 ± 0.09
Triton X-100 + NAA	7.56 ± 0.89

[z] ACC concentration determined 30 hr after treatment

[y] Mean ± SE

Figure 1. Time-course of ACC (A) and ethylene (B) production and apparent rate constant for ACC to ethylene conversion (C) in cowpea leaves following treatment with Triton X-100 (1.0%), NAA (1mM) or the combination of Triton X-100 and NAA (1.0% and 1mM, respectively). Vertical bars represent standard error.


A
B
C
ACC (nmol g⁻¹)
10.0
1.0
0.1
ETHYLENE (nmol g⁻¹ hr⁻¹)
1.00
0.10
0.01
ETHYLENE/ACC (ka)
1.00
0.10
0.01
0
6
12
18
24
30
36
TIME AFTER TREATMENT (hr)
○ Control
□ Triton X−100
△ NAA
● Triton X−100 + NAA

and the combination of Triton X-100 and NAA and the lowest for NAA alone. The relative differences were much less than those observed for ACC content (Fig. 1A).

The k_a for the conversion of endogenous ACC to ethylene increased between 0 and 6 hr for all treatments (Fig. 1C). The k_a for Triton X-100 and the combination of Triton X-100 and NAA remained almost constant over the next 30 hr at approximately 0.10 and 0.08, respectively. For NAA, k_a was greatest (0.46) at 12 hr then decreased and remained almost constant between 24 and 36 hr at approximately 0.21.

Exogenously applied ACC increased the ethylene production rate 14-fold (Table 2). The combination of Triton X-100 and ACC induced rates of ethylene production greater than either compound alone (interaction significant at $P=0.05$).

Localization study. There was no increase in ACC in the surrounding leaf tissue (16.3 mm disc with treated area removed) following treatment with Triton X-100, NAA or the combination of Triton X-100 and NAA, while there was a 100-fold increase in the ACC concentration within the treated area (8.6 mm disc; Table 3). Triton X-100 and the combination of Triton X-100 and NAA induced a small but significant increase in ethylene production by the surrounding tissue. We have also examined ACC and ethylene distribution between treated and non-treated halves of leaves (apical vs. basal and right vs. left) and between treated primary leaves and adjacent trifoliate leaves. In these experiments both ACC and ethylene production were limited to the treated portions of leaves (data not presented).

Table 2. Effect of surfactant on ACC-induced ethylene production in cowpea leaves.

Treatment	Ethylene (nmol g^{-1} hr^{-1})[z]
Control	0.10 ± 0.01[y]
Triton X-100 (1.0%)	0.44 ± 0.03
ACC (1mM)	1.42 ± 0.13
Triton X-100 + ACC	4.39 ± 0.59

[z] Ethylene determinations made 27 hr after treatment

[y] Mean ± SE

Table 3. Localization of surfactant- and NAA-induced ACC and ethylene production to the treated portion of cowpea leaves.

	ACC (nmol g^{-1})^{z}		Ethylene (nmol g^{-1} hr^{-1})^{y}	
Treatment	Treated	Surrounding	Treated	Surrounding
Control	0.65 ± 0.25^{x}	1.22 ± 0.20	0.27 ± 0.01	0.26 ± 0.02
Triton X-100 (1.0%)	25.91 ± 8.28	1.30 ± 0.42	1.40 ± 0.09	0.42 ± 0.02
NAA (1 mM)	2.24 ± 0.69	0.61 ± 0.14	0.38 ± 0.03	0.26 ± 0.01
Triton X-100 + NAA	99.68 ± 11.93	1.04 ± 0.23	2.33 ± 0.09	0.38 ± 0.02

^{z}ACC determinations made 15 hr after treatment

^{y}Ethylene determinations made 12 hr after treatment

^{x}Mean ± SE

Discussion

The production of ethylene by vegetative tissues can be induced by a variety of environmental and physiological factors (14,22) through the pathway: methionine -> SAM -> ACC -> ethylene (1,12,22,26). Many of these factors affect ethylene production by regulating conversion of SAM to ACC through ACC synthase activity (5,7,9,21,22) although some can regulate conversion of ACC to ethylene (4,6,18,21,24). Our data demonstrate that ACC and ethylene production can also be enhanced by the surfactant Triton X-100.

Increases in tissue levels of ACC following treatment with Triton X-100, NAA and the combination of Triton X-100 and NAA (Tables 1 and 3, Fig. 1A) suggest increased ACC synthase activity. Differences in response, including the Triton X-100 by NAA interaction may result from differences in penetration, and therefore the quantity of active compound available at the reaction site, and/or the inherent ability of these compounds to stimulate the enzyme(s). Differences in NAA penetration may account for the differences in ACC content in the separate NAA experiments (Table 1, Fig. 1A). NAA-induced ethylene production is directly related to NAA penetration (16). Comparison of the ethylene production rates for the two sets of data (0.487 vs. 0.042) indicates an 11.6-fold decrease in NAA penetration which agrees with the 11.2-fold decrease in ACC content (2.54 vs. 0.225).

Surfactants are known to increase the penetration of foliar applied NAA (16). Therefore, the increases in ACC content of tissues treated with the combination of Triton X-100 and NAA (Table 1, Fig. 1A) probably involve increased NAA penetration. Alternatively, Triton X-100 can induce phytotoxicity in cowpea leaves (17) and did so in these studies (data not presented). Therefore, it is possible that increases in ACC content are, at least in part, stress responses associated with surfactant-induced phytotoxicity.

Surfactants could also affect ethylene production by acting on the conversion of ACC to ethylene. Previous work established that surfactants, particularly Triton X-100, can inhibit conversion of ACC to ethylene in mung bean hypocotyl segments (19), apple protoplasts (3) and apple tissue discs (4) preincubated with ACC. This inhibition has been related to surfactant-induced changes in membrane structure and integrity (3,4,19).

The conversion of exogenously applied ACC to ethylene was not inhibited by Triton X-100 in cowpea (Table 2). Instead there was enhanced ethylene production from ACC when applied with Triton X-100 (Table 2). Similarily, NAA-induced ethylene production increased in the presence of Triton X-100 (Fig. 1B, Table 3). These increases may have resulted from increased penetration (16) and/or movement to the conversion site. Preliminary data (not presented) indicate that Triton X-100 increased the penetration of ACC into cowpea leaves. Further support comes from recent work with isolated microsomal membrane fractions, where the addition of Triton X-100 increased ethylene production from ACC-treated fractions, at least partially, because of increased ACC availability at the active site (18). This suggests that Triton X-100 increased penetration of ACC and NAA but did not inhibit ACC to ethylene conversion. It is probable that under conditions of more extensive phytotoxicity and membrane damage that ACC to ethylene conversion may also be affected.

The increase in k_a for each treatment (Fig. 1C) indicates an increase in the conversion efficiency of ACC to ethylene. This could result from higher levels of ethylene forming enzyme (EFE), greater EFE activity and/or increased transport of ACC to the EFE site. The mean k_a between 6 and 36 hr of 0.21, 0.10 and 0.08 for NAA, Triton X-100 and the combination of Triton X-100 and NAA, respectively, may indicate different modes of action. However, further study will be required to separate these possibilities.

Our studies established that ACC produced following surfactant or NAA treatment remained localized at the treated site (Table 3). In addition, there was no evidence of ACC movement to non-treated leaves or to adjacent tissue within the same leaf. It is possible, however, that ACC may have moved to adjacent non-treated tissues, been rapidly converted to ethylene and gone undetected. A comparison of ethylene production by treated and non-treated adjacent tissue showed that most of the ethylene production was from the treated areas. Slight but significant increases in ethylene production were found in the surrounding tissue (within 2-3 mm of the treatment site) in the presence of Triton X-100 (Table 3) indicating that at most only some short distance movement occurred. This is in contrast to the response of anaerobic tomato roots where ACC was produced in the roots and transported through the xylem and induced physiological responses in above ground plant parts (8,9,10,11).

While Triton X-100 increased both ACC and ethylene production, the localization at the treatment site suggests that associated physiological responses should also remain localized. The degree to which these responses may affect the performance of foliar applied agrochemicals has yet to be determined, but should be considered in the formulation and application of foliar sprays.

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SUMMARY AND CONCLUSIONS

These studies demonstrate that selected surfactants, applied to plant leaves, penetrate the leaf surface, disrupt membranes, induce phytotoxicity, ACC and ethylene production, and these effects are related to surfactant chemistry. The characterization of these responses provides information not previously available and establishes a data base of surfactant-plant interactions on which rational decisions concerning surfactant usage can be made.

The practical significance of surfactant-induced phytotoxicity and ethylene production, as responses to the surfactant component of a foliar spray, is difficult to predict. It will depend on a variety of factors including, the tissue affected (i.e. leaves, flowers, fruit), the stage of tissue development, the nature of the active ingredient and both short and long term effects on other physiological processes. In addition, there may be marked effects on penetration and translocation of the active ingredient in the foliar spray. Thus, the overall effects and their importance will result from complex interactions of these factors and could vary greatly. These studies, therefore, focused on describing and characterizing surfactant-induced phytotoxicity and ethylene production as a step toward understanding their importance.

Surfactant-induced phytotoxicity remained localized at the treatment site appearing as discolored and sunken areas. The amount of tissue damage was related to spray coverage, surfactant concentration and rate of droplet drying and could therefore be influenced by a variety of factors. At the cellular level, there was deformation and collapse of epidermal, palisade and mesophyll cells and cell walls. Histological staining suggested a marked change in the membranes and proteins within damaged tissues. Phytotoxicity increased with increasing time after treatment and when maximum, the entire treated area was necrotic. Although phytotoxicity was localized, there was no evidence of tissue recovery.

Ethylene production was induced by surfactants of each ionic class (nonionic, anionic, cationic) at concentrations of 0.1% and greater. The response was transient (similar to 'wound' induced ethylene production), increasing rapidly until reaching a maximum between 6 and 18 hr after treatment (dependent on surfactant chemistry) and then declining to control rates by 48 hr. Similar surfactant-induced ethylene production was observed in a range of plant species with various nonionic surfactants. In most studies, there was a strong correlation between ethylene production and phytotoxicity suggesting that the two may be closely related.

Surfactant-induced ethylene production was related to surfactant chemistry. For octylphenol surfactants there was an inverse linear relationship between ethylene oxide (EO) content and ethylene production. For linear alcohol surfactants this relationship was non-linear, with greatest activity at intermediate EO and decreased activity at shorter and longer EO. These relationships appear to be related to surfactant penetration (30,31) but may also result, to some extent, from differences in intrinsic surfactant bioactivity. A similar relationship was found between EO and phytotoxicity, again suggesting that surfactant-induced phytotoxicity may be related to ethylene production.

Two important steps in the ethylene biosynthesis pathway were affected by surfactants. First, surfactants increased the production of 1-aminocyclopropane-1-carboxylic acid (ACC) leading to increased ethylene production. Second, surfactants increased the apparent rate constant for the conversion of ACC to ethylene. The mechanism(s) for these effects were not studied, but may involve enzyme levels, activity and/or substrate transport. Production of ACC and ethylene remained localized within the treated area.

Based on this information we can propose a sequence of events that may occur. Initially, during droplet drying, the surfactant molecues in solution will tend to orient themselves at the interface (28). Some of these molecules will penetrate into the cuticle, the number and degree of penetration dependent on their lipophilicity and size. In addition, there will be a deposit formed on the surface, its distribution largely determined by the rate of droplet drying (16), from which further penetration may occur. On penetration through the cuticle surfactants will interact with cell membranes, the hydrophobic portion of the surfactant becoming associated with the lipophilic domain of the membranes. These interactions may induce a localized stress response resulting in the production of ACC and subsequently increased ethylene production. The specific mode of action, however, remains unclear. With increasing time after treatment, surfactant penetration would increase resulting in a higher concentration at the membrane, and further surfactant-membrane interactions. At some point membrane disruption and cell wall deformation and collapse could occur.

This damage would then be visually evident as discolored and sunken tissues. Thus, a series of surfactant-plant interactions would lead to overall surfactant effects on phytotoxicity and ethylene production.

Phytotoxicity and ethylene production were observed in a range of plant species indicating that these are general surfactant effects. In addition, the relationships between EO and ethylene production in cowpea and germinating mung bean seeds suggest that these effects might be predicted based on surfactant chemistry and could have important applications in the formulation of agrochemicals for foliar application. Surfactant effects on plants (ethylene production) can now be taken into consideration in the selection of surfactants. As more specific and active agrochemicals are developed, selection of surfactants, based on surfactant-plant interactions, will become even more important.

In conclusion, these studies provide a starting point in the understanding of surfactant-plant interactions. The overall effects and importance of these interactions will also depend on a number of other factors. Therefore, additional study on the effects of surfactants on other plants processes (i.e. photosynthesis, transpiration) and their relationships to surfactant chemistry and spray solution properties will be needed. Greater understanding of the effects of spray application parameters (coverage, droplet overlap, runoff) will also be required as this information is applied to field situations. In time these and additional studies should help to maximize chemical efficiency while minimizing plant stress.

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APPENDICES

APPENDIX A

GENERAL STRUCTURES OF SELECTED SURFACTANTS

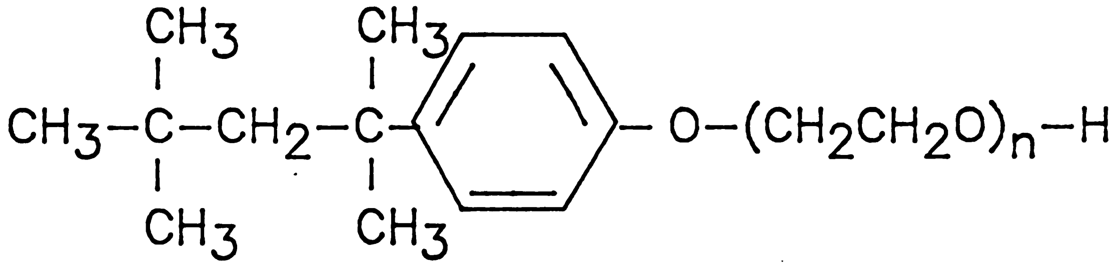


Figure A1. Structure of Triton X surfactants. (1)

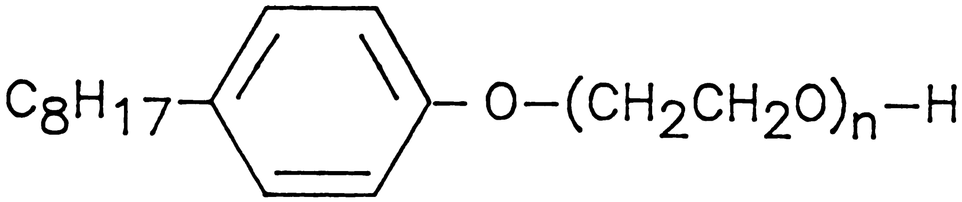


Figure A2. Structure of Igepal CA surfactants. (2)

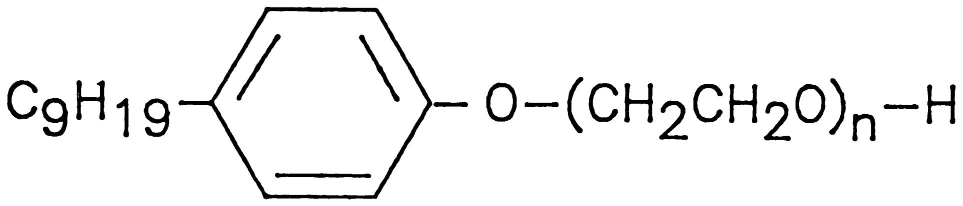


Figure A3. Structure of Igepal CO surfactants. (3)

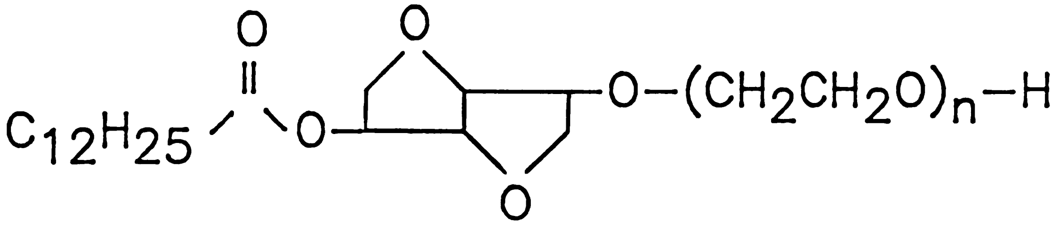


Figure A4. Structure of Tween 20[z]. (4)

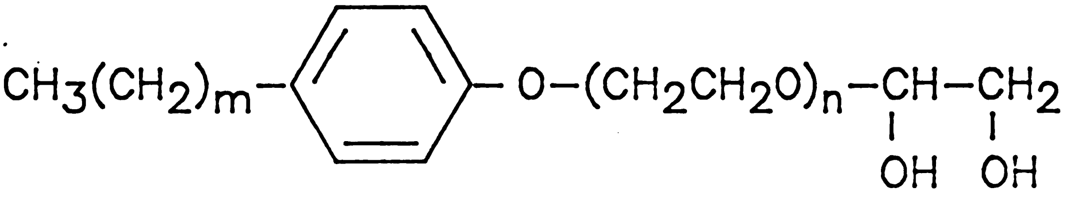


Figure A5. Possible structure of Ortho X-77[y]. (5)

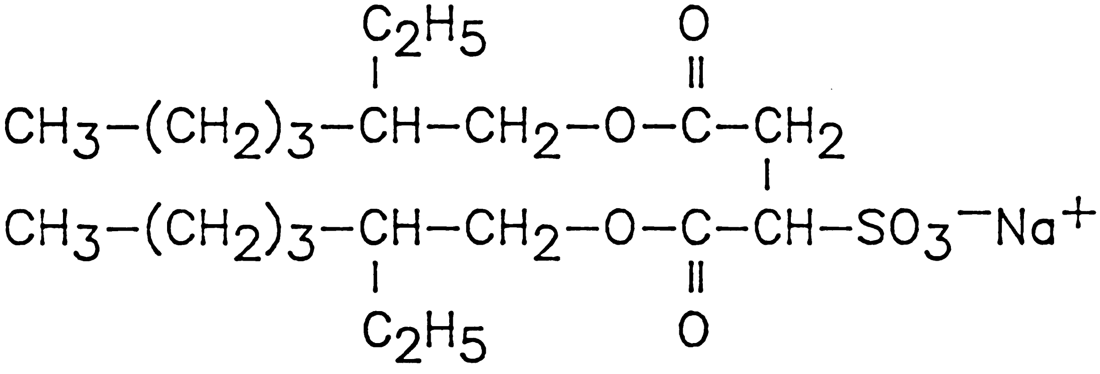
$$CH_3(CH_2CH_2O)_n{-}(CH_2CH_2CH_2O)_o{-}CH_2CH_2CH_2OH$$

$$CH_3{-}(CH_2)_m{-}CH_2CH_2O{-}CH_2CH_2OH$$

Figure A6. Possible structure of Regulaid[y]. (6)

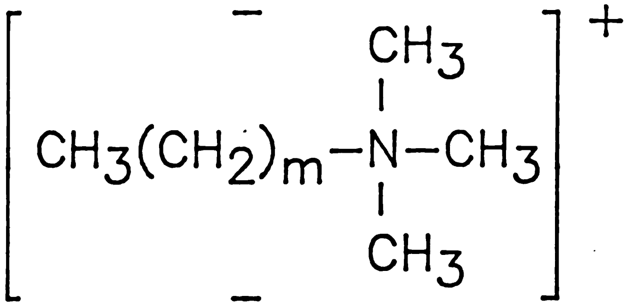
$CH_3-(CH_2)_m-CH_2-O-SO_3^-Na^+$

Figure A7. Structure of Dupanol. (7)



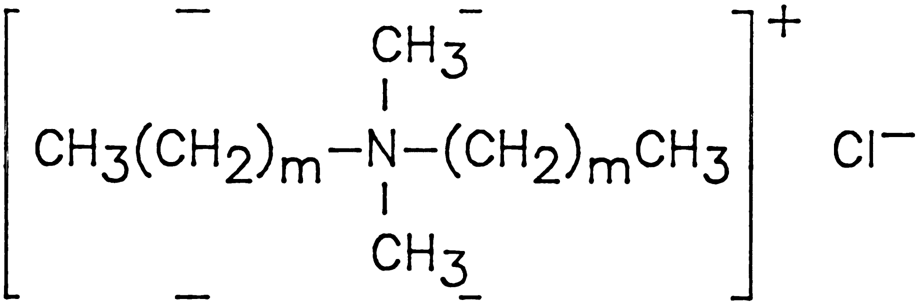
$$\begin{aligned}
&CH_3-(CH_2)_3-\overset{C_2H_5}{\overset{|}{C}}H-CH_2-O-\overset{O}{\overset{\|}{C}}-\underset{|}{CH_2}\\
&CH_3-(CH_2)_3-\underset{C_2H_5}{\underset{|}{C}}H-CH_2-O-\underset{O}{\underset{\|}{C}}-CH-SO_3^-Na^+
\end{aligned}$$

Figure A8. Structure of Aerosol OT. (8)



$$\left[\mathrm{CH_3(CH_2)_m{-}\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{N}}{-}CH_3}\right]^{+}\ \mathrm{Cl^-}$$

Figure A9. Structure of Arquad C-50^{x}. (9)



$$\left[\mathrm{CH_3(CH_2)_m{-}\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{N}}{-}(CH_2)_mCH_3}\right]^{+}\ \mathrm{Cl^-}$$

Figure A10. Structure of Arquad 2C-75^{x}. (10)

[z] $n=20$

[y] Proprietary surfactant blend, therefore this is a possible structure only

[x] $m=7\text{-}17$

<u>Surfactant chemistries and sources</u>:

1. Octylphenoxypoly(ethoxy)ethanol; Rohm and Haas Co., Philadelphia, PA.
2. Octylphenoxypoly(ethoxy)ethanol; GAF Corp., Wayne, NJ.
3. Nonylphenoxypoly(ethoxy)ethanol; GAF Corp., Wayne, NJ.
4. Polyoxyethylene (20) sorbitan monolaurate; ICI Americas, Inc. Wilmington, DE.
5. Alkylaryl polyoxyethylene glycol; Chevron Chemical Co., San Francisco, CA.
6. Polyoxyethylenepolypropoxypropanol and alkyl 2-ethoxyethanol; Kalo Laboratories, Inc., Kansas City, MO.
7. Sodium lauryl sulfate; E.I. Dupont deNemours, & Co., Wilmington, DE.
8. Dioctyl sodium sulfosuccinate; American Cyanamid, Wayne, NJ.
9. Monococo trimethyl ammonium chloride; Armac Co., Chicago, IL.
10. Dicoco dimethyl ammonium chloride; Armac Co., Chicago, IL.

APPENDIX B

SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES OF SELECTED SURFACTANTS AND SURFACTANT INTERACTIONS WITH COWPEA LEAVES

Table B1. Physical and chemical properties of selected surfactants.

Surfactant	Moles EO	MW	HLB^z	CMC (% w/v)	Partition coefficient^y	% EO^x
Triton						
X-15	1.0	250	3.6	0.001	5.3	18
X-35	3.0	338	7.8	0.004	4.0	39
X-45	5.0	426	10.4	0.005	2.7	52
X-100	9.5	628	13.5	0.019	-0.3	67
X-305	30.0	1526	17.3	0.110	-13.8	87
X-405	40.0	1966	17.9	0.170	-20.4	90
Igepal						
CA 520	5.0	420	10.0	0.005	2.7	50
CA 620	7.0	514	12.0	0.010	1.4	60
CA 630	9.0	596	13.0	0.015	0.1	65
CA 730	13.0	778	14.8	0.031	-2.6	74
CA 897	40.0	1966	18.0	0.055	-20.4	90
Neodol						
25-3	3.0	336	7.9	0.0001	6.1	39
25-7	7.0	522	12.2	0.0009	4.4	60
25-9	9.0	610	13.3	0.0018	2.0	66
25-12	12.0	729	14.4	0.0027	0.3	72
25-30	30.0	1548	17.1	0.016	-11.7	85
Neodol						
91-6	6.0	425	12.4	0.025	2.0	62
91-8	8.0	529	14.0	0.027	0.5	70
91-10	10.0	600	14.7	0.029	-0.6	71
91-12	12.0	680	15.3	0.031	-1.9	72
91-20	20.0	1040	16.9	0.039	-7.2	76
Nonanol						
9-2	2.0	232	7.9	0.038	4.1	37
9-3	3.0	276	9.6	0.058	3.4	47
9-4	4.0	320	11.0	0.071	2.8	55
9-6	6.0	408	12.9	0.12	1.4	64
9-9	9.0	540	14.7	0.25	-0.5	73
9-12	12.0	672	15.7	0.47	-2.5	78

Continued . . . .

Table B1. (continued)

Surfactant	Moles EO	MW	HLB[z]	CMC (% w/v)	Partition Coefficient[y]	% EO[x]
C_9						
9-3	3.0	276	9.6	0.06	3.4	48
9-5	5.0	364	12.0	0.095	2.1	60
9-8	8.0	496	14.2	0.19	0.1	71
C_{12}						
12-3	3.0	318	8.3	0.0021	5.2	41
12-5	5.0	406	10.8	0.0032	3.9	54
12-8	8.0	538	13.1	0.0059	1.9	65
C_{15}						
15-3	3.0	360	7.3	0.0007	7.0	37
15-5	5.0	448	9.8	0.001	5.7	49
15-8	8.0	580	12.2	0.002	3.7	61
Misc. nonionic						
Tween 20	20.0	1244	16.7	0.006	-	71
Ortho X-77[w]	-	-	-	0.01	-	-
Regulaid[w]	-	-	-	0.008	-	-
Anionic[v]						
Aerosol	-	444	-	0.030	-	-
Dupanol ME	-	288	-	0.240	-	-
Cationic[v]						
Arquad C-50	-	278	-	0.009	-	-
Arquad 2C-75	-	447	-	0.01	-	-

[z] HLB = (weight % EO)/5

[y] Calculated as follows: Octylphenol; 6 - (#EO)0.66
Linear alcohol; (#C)0.6 - (#EO)0.66

[x] % EO = [(weight of EO)/MW] x 100

[w] Surfactant blends, therefore, specific properties are not available

[v] Moles EO, HLB and % EO (as calculated) are inappropriate parameters for ionic surfactants. Partition coefficients were not calculated.

Table B2. Selected surfactant properties and interactions with cowpea leaves at 0.1%.

Surfactant	% monomer^z	Surface tension (mN m^{-1})	Contact angle (^{o})^y	Relative interface area^x	Phyto-toxicity (rating)^w	Ethylene^v (nl leaf^{-1} hr^{-1})
Triton						
X-15	1	insol.	62	3.0	2.0	1.29 ± 0.19^u
X-35	4	29.0	40	4.4	2.0	6.88 ± 0.60
X-45	5	28.5	35	4.9	3.0	6.11 ± 0.91
X-100	19	31.0	52	3.6	2.0	2.57 ± 0.69
X-305	100	38.0	71	3.6	1.0	1.11 ± 0.16
X-405	100	48.0	91	1.9	1.0	0.95 ± 0.15
Igepal						
CA 520	5	28.0	42	4.3	2.5	6.10 ± 1.19
CA 620	10	29.0	45	4.0	2.0	1.98 ± 0.56
CA 630	15	30.0	52	3.6	2.0	1.35 ± 0.37
CA 730	31	32.0	66	2.8	2.0	0.96 ± 0.23
CA 897	55	49.0	96	1.7	1.0	0.20 ± 0.03
Neodol						
25-3	0	29.3	44	4.1	3.0	3.47 ± 0.29
25-7	1	27.8	43	4.2	3.0	3.27 ± 0.43
25-9	2	30.3	49	3.8	3.0	2.28 ± 0.47
25-12	3	33.6	60	3.1	3.0	2.37 ± 0.18
25-30	16	46.8	89	1.9	1.0	1.40 ± 0.24
Neodol						
91-6	25	28.0	43	4.2	3.0	11.40 ± 1.74
91-8	27	30.0	56	3.3	3.0	19.78 ± 2.14
91-10	29	32.8	63	3.0	3.5	17.02 ± 0.84
91-12	31	35.5	69	2.7	3.5	18.89 ± 1.24
91-20	39	43.1	90	1.9	1.5	2.43 ± 0.61
Nonanol						
9-2	38	28.5	58	3.2	1.1	0.62 ± 0.08
9-3	58	27.8	54	3.4	1.4	2.45 ± 0.38
9-4	71	29.6	52	3.6	1.5	5.70 ± 0.80
9-6	100	34.0	63	3.0	2.3	4.52 ± 0.32
9-9	100	41.4	78	2.3	1.6	5.32 ± 0.93
9-12	100	43.5	91	1.9	1.0	0.35 ± 0.05
C_9						
9-3	60		35	4.9	2.5	0.73 ± 0.16
9-5	95		55	3.4	3.0	0.69 ± 0.12
9-8	100		83	2.2	4.0	2.60 ± 0.66

Continued . . . .

Table B2. (continued)

Surfactant	% monomer[z]	Surface tension ($mN\ m^{-1}$)	Contact angle (^{o})[y]	Relative interface area[x]	Phyto- toxicity (rating)[w]	Ethylene[v] (nl leaf-1 hr-1)
C_{12}						
12-3	2		40	4.4	3.5	7.95 ± 1.49[u]
12-5	3		47	3.9	3.5	3.36 ± 0.46
12-8	6		60	3.1	3.5	2.27 ± 0.36
C_{15}						
15-3	1		61	3.1	4.0	4.46 ± 0.80
15-5	1		51	3.6	4.0	8.67 ± 1.50
15-8	2		65	2.9	3.5	1.60 ± 0.37
Misc. nonionic						
Tween 20	6	45.4	86	2.1	1.0	0.83 ± 0.06
Ortho X-77	10	31.0	50	3.7	3.0	5.88 ± 2.26
Regulaid	8	42.3	76	2.2	1.0	0.48 ± 0.07
Anionic						
Aerosol	30	28.7	49	3.8	4.0	6.47 ± 1.03
Dupanol ME	100	43.6	70	2.7	1.0	0.72 ± 0.06
Cationic						
Arquad C-50	9	41.0	83	2.2	3.0	2.64 ± 0.24
Arquad 2C-75	10	30.0	62	3.0	4.0	1.62 ± 0.22

[z] (CMC/concn) x 100

[y] 1 μl drops, adaxial surface of 10-day-old cowpea leaves. Water = 110^{o}.

[x] Relative area =
$$\pi \left[\frac{6V}{\pi(3 \tan \frac{\theta}{2} + \tan^{3} \frac{\theta}{2})} \right]^{2/3}$$

[w] Based on scale of 1 (no visual damage, e.g. water control) to 5 (greater than 50% of treated area necrotic)

[v] Determined following 12 hr penetration, 2 hr incubation

[u] Mean ± SE

Table B3. Selected surfactant properties and interactions with cowpea leaves at 1.0%.

Surfactant	% monomer^{z}	Surface tension (mN m^{-1})	Contact angle (^{o})^{y}	Relative interface area^{x}	Phyto-toxicity (rating)^{w}	Ethylene^{v} (nl leaf^{-1} hr^{-1})
Triton						
X-15	0.1		63	3.0	2	7.57 ± 1.32^{u}
X-35	0.4		37	4.7	2	25.97 ± 4.73
X-45	0.5	28.0	34	5.0	4	22.27 ± 4.39
X-100	1.9	30.0	43	4.2	3	11.51 ± 1.63
X-305	11.0	41.0	73	2.5	2	0.43 ± 0.04
X-405	17.0	44.0	86	2.1	1	1.22 ± 0.11
Igepal						
CA 520	0.7	28.0	42	4.3	4	68.65 ± 5.76
CA 620	0.8	29.0	45	4.0	4	54.14 ± 4.99
CA 630	0.9	30.0	51	3.6	3	32.26 ± 2.94
CA 730	1.1	32.0	66	2.8	2	17.47 ± 1.46
CA 897	1.6	43.0	96	1.7	1	0.63 ± 0.07
Neodol						
25-3	0.0	28.7	42	4.3	3	2.89 ± 0.53
25-7	0.1	28.0	49	3.8	5	20.41 ± 2.12
25-9	0.2	30.0	54	3.4	5	10.38 ± 0.70
25-12	0.3	34.0	65	2.9	4	7.45 ± 0.63
25-30	1.6	46.6	94	1.8	1	1.19 ± 0.16
Neodol						
91-6	2.5	29.0	40	4.4	5	12.32 ± 1.30
91-8	2.7	31.0	54	3.4	5	15.84 ± 2.00
91-10	2.9	35.2	58	3.2	4	22.87 ± 1.40
91-12	3.1	36.8	63	3.0	4	26.29 ± 1.50
91-20	3.9	38.2	69	2.7	2	1.75 ± 0.40
Nonanol						
9-2	3.8	27.4	26	6.1	5	5.12 ± 0.32
9-3	5.8	27.6	31	5.4	5	13.25 ± 0.98
9-4	7.1	28.1	32	5.3	5	11.28 ± 1.16
9-6	12.0	29.2	39	4.5	5	12.95 ± 0.64
9-9	25.0	33.2	60	3.1	4	27.39 ± 5.26
9-12	47.0	35.6	69	2.7	4	19.27 ± 2.27
C_9						
9-3	6.0		32	5.3	5	3.82 ± 0.68
9-5	9.5		51	3.6	5	5.15 ± 0.81
9-8	19.0		67	2.8	5	15.25 ± 1.82

Continued . . . .

Table B3. (continued)

Surfactant	% monomer^z	Surface tension (mN m^{-1})	Contact angle (^{o})^y	Relative interface area^x	Phyto-toxicity (rating)^w	Ethylene^v (nl leaf^{-1} hr^{-1})
C_{12}						
12-3	0.2		36	4.8	5	8.88 ± 1.82^u
12-5	0.3		45	4.0	5	3.69 ± 0.56
12-8	0.6		55	3.4	5	10.18 ± 1.50
C_{15}						
15-3	0.1		64	2.9	4	5.66 ± 1.40
15-5	0.1		51	3.6	5	9.68 ± 1.30
15-8	0.2		63	3.0	5	4.32 ± 0.81
<u>Misc. nonionic</u>						
Tween 20	0.6	44.4	81	2.2	1	0.63 ± 0.09
Ortho X-77	1.0	30.9	46	3.9	3	9.04 ± 2.10
Regulaid	0.8	41.3	66	2.8	1	0.52 ± 0.08
<u>Anionic</u>						
Aerosol	3.0	25.6	23	6.6	4	15.26 ± 4.14
Dupanol ME	10.0	35.1	36	4.8	1	0.97 ± 0.06
<u>Cationic</u>						
Arquad C-50	0.9	39.9	52	3.6	4	4.07 ± 0.56
Arquad 2C-75	1.0	28.8	39	4.5	4	4.90 ± 0.12

[z] (CMC/concn) x 100

[y] 1 μl drops, adaxial surface of 10-day-old cowpea leaves. Water = 110^o.

[x] Relative area = $\pi \left[\dfrac{6V}{\pi(3 \tan \frac{\theta}{2} + \tan^3 \frac{\theta}{2})} \right]^{2/3}$

[w] Based on sacle of 1 (no visual damage, e.g. water control) to 5 (greater than 50 % of treated area necrotic)

[v] Determined following 12 hr penetration, 2 hr incubation

[u] Mean ± SE

APPENDIX C

RELATIONSHIPS BETWEEN SELECTED SURFACTANT PROPERTIES FOR OCTYLPHENOL AND LINEAR ALCOHOL SURFACTANTS

Table C1. Correlations between properties of octylphenol (OP) and linear alcohol (LA) surfactants.

Regression equation	Correlation coefficient (r)	Probability level (P)
1) HLB^{z} = 9.18(log EO) + 4.26	0.979	0.001
2) Surface tension (mN m^{-1})		
a) At 0.1%		
OP^{y}: surface tension = 0.56(EO) + 25.0	0.985	0.001
LA^{x}: surface tension = 0.75(EO) + 26.6	0.808	0.001
b) At 1.0%		
OP^{y}: surface tension = 0.45(EO) + 25.9	0.995	0.001
LA^{x}: surface tension = 0.72(EO) + 25.5	0.955	0.001
3) Wetting^{z} (Cos Θ)^{w}		
= -0.04(surface tension) + 1.83	-0.973	0.001

[z] All surfactants

[y] Excluding OP+1EO and OP+3EO because of low solubility

[x] Excluding C_{12-15}+3EO and C_9+2EO because of low solubility

[w] Contact angles formed by 1 µl drops of surfactant solution (0.1%) on the adaxial surface of 10-day-old cowpea leaves. Control (water) = 110°. Excluding OP+1EO, OP+3EO, C_{12-15}+3EO and C_9+2EO because of low solubility

APPENDIX D

ETHYLENE PRODUCTION INDUCED BY C_9, C_{12} AND C_{15} LINEAR ALCOHOL SURFACTANTS

The relative effect of the hydrophobe chain length and ethylene oxide (EO) chain length on induction of ethylene production from cowpea leaves was examined. Purified C_9, C_{12} and C_{15} linear alcohol surfactants with 3, 5 or 8 EO were provided by Shell Development Co. These compounds were assayed at 0.1 and 1.0% v/v for induction of ethylene production using the cowpea assay over a 12- and 24-hr penetration period.

No consistent trend for the effect of hydrophobe chain length or EO chain length was apparent (Tables D1 and D2). Comparing the means for both penetration (12- and 24-hr) periods at the 0.1% rate revealed no differences between the 3 and 5 ethoxymers, but the 8 EO compound was considerably less active (Table D1). In contrast, at 1.0% the 8 EO compound was most active (Table D2). The C_{15} hydrophobe, taken over all EO chain lengths was more active than the C_9 or C_{12} hydrophobe.

The general mean of the ethylene production rate (over both penetration periods and concentrations) for 3, 5 and 8 EO was 6.5, 6.7 and 6.9 nl leaf^{-1} hr^{-1}, respectively, while for the C_9, C_{12} and C_{15} hydrophobes the mean rate was 5.6, 5.2 amd 9.2 nl leaf^{-1} hr^{-1}, respectively. Thus only the C_{15}+8EO compound was consistently more active.

It is not clear why a general relationship between EO chain length and ethylene production was not apparent. There are several possibilities; a) all are quite active compounds, b) chemical purity may not be sufficient and c) the cowpea assay may not be sufficiently sensitive to discriminate among ethoxymers differing by only 5 EO or hydophobe chain lengths differing by only 6 carbon atoms.

Table D1. Effect of C_9, C_{12} and C_{15} linear alcohol hydrophobes with 3, 5 and 8 ethylene oxides (0.1% v/v) on ethylene production (nl leaf^{-1} hr^{-1}) by primary leaves of cowpea seedlings.

Linear alcohol	Ethylene oxide content 3	5	8	mean
	12-hr penetration, 2-hr incubation			
C_9	0.73 ± 0.16[z]	0.69 ± 0.12	2.60 ± 0.66	1.13
C_{12}	7.95 ± 1.49	3.36 ± 0.46	2.27 ± 0.36	4.53
C_{15}	4.46 ± 0.80	8.67 ± 1.50	1.60 ± 0.37	4.91
mean	4.38	4.24	2.16	
	24-hr penetration, 2-hr incubation			
C_9	0.63 ± 0.12	0.76 ± 0.16	4.24 ± 1.23	1.88
C_{12}	0.51 ± 0.77	2.35 ± 0.41	2.12 ± 0.37	1.66
C_{15}	14.26 ± 1.70	19.28 ± 1.10	1.00 ± 0.19	11.18
mean	5.13	7.46	2.45	
overall mean	4.76	5.85	2.30	

[z] Mean ± SE

Table D2. Effect of C_9, C_{12} and C_{15} linear alcohol hydrophobes with 3, 5 and 8 ethylene oxides (1.0% v/v) on ethylene production (nl leaf^{-1} hr^{-1}) by primary leaves of cowpea seedlings.

Linear alcohol	Ethylene oxide content 3	5	8	mean
	12-hr penetration, 2-hr incubation			
C_9	3.82 ± 0.68[z]	5.15 ± 0.81	15.25 ± 1.82	8.07
C_{12}	8.88 ± 1.82	3.69 ± 0.56	10.18 ± 1.50	7.58
C_{15}	5.66 ± 1.40	9.68 ± 1.30	4.32 ± 0.81	6.55
mean	6.12	6.17	9.92	
	24-hr penetration, 2-hr incubation			
C_9	3.37 ± 0.58	7.01 ± 1.50	23.11 ± 3.03	11.16
C_{12}	2.96 ± 0.44	5.42 ± 0.65	12.21 ± 1.58	6.86
C_{15}	24.52 ± 3.44	13.99 ± 1.50	3.96 ± 0.66	14.16
mean	10.28	8.81	13.09	
overall mean	8.20	7.49	11.50	

[z] Mean ± SE

APPENDIX E

ADAXIAL SURFACE OF COWPEA LEAVES

In all studies treatments were applied to the adaxial surface of cowpea leaves. Therefore the nature of this surface is important to penetration of these compounds. Herein we provide documentation as to the nature of this surface on 10-day-old plants and changes in the surface with increasing age.

Scanning electron microscopy. Fresh tissue sections were mounted on aluminum stubs with O.C.T. compound (Miles Laboratories, Inc., Naperville, IL), quick frozen in slush nitrogen, etched at -80°C, coated with gold (100A) and observed on a cold stage using a JSM-35C SEM (JEOL) operating at 15 KV. Scanning electron micrographs were taken on positive/negative 665 Polaroid film.

Epicuticular wax appeared as groups of vertically oriented platelets, some folded over, randomly distributed over the surface (Fig. E1). Epicuticular wax density increased with increasing leaf age (Fig. E2).

Figure E1. Scanning electron micrographs of a primary leaf of 10-day-old cowpea seedling illustrating the nature of the surface.

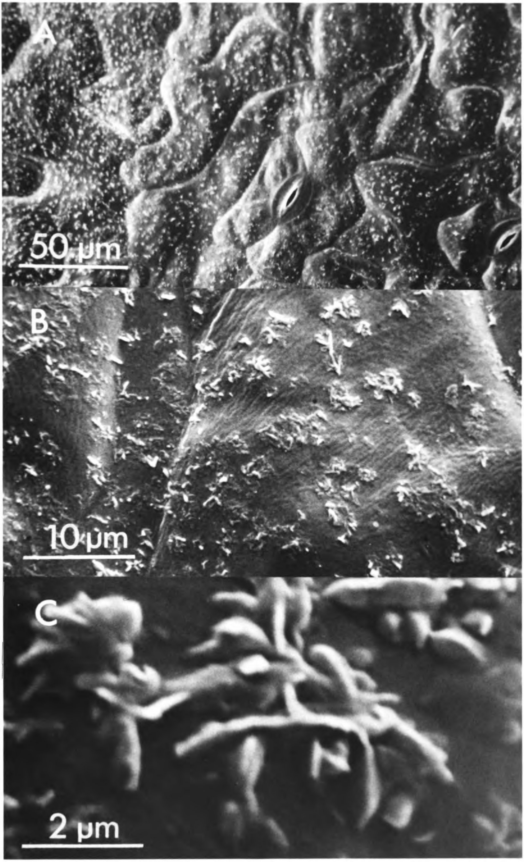
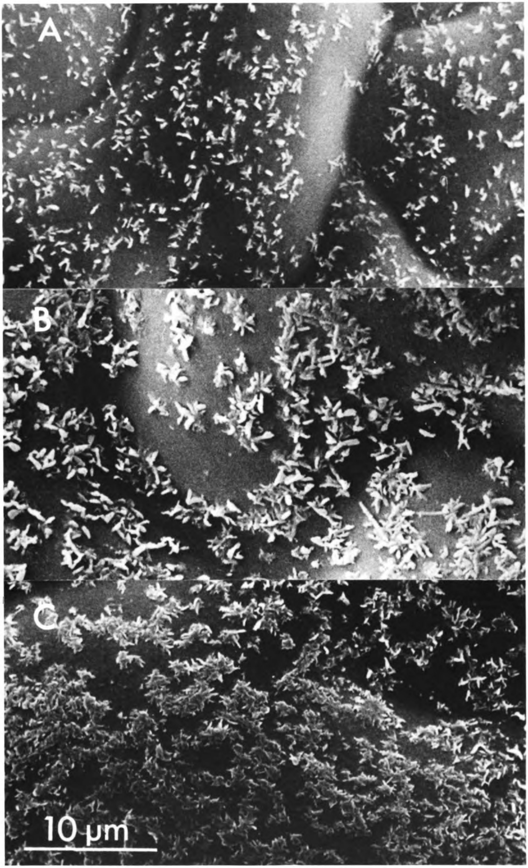

A
50 μm
B
10 μm
C
2 μm

Figure E2. Scanning electron micrographs of a primary leaf of 4-day-old (A), 10-day-old (B) and 13-day-old (C) cowpea seedlings illustrating the nature and distribution of epicuticular wax.


A
B
C
10 μm