Evidence for Surfactant Solubilization of Plant Epicuticular Wax

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The solubilization of isolated, reconstituted tomato (*Lycopersicon esculentum* Mill.) fruit and broccoli (*Brassica oleracaea* var. *botrytis* L.) leaf epicuticular waxes (ECW) by nonionic octylphenoxypolyethoxy ethanol surfactant (Triton X-100) was demonstrated in a model system by TLC and fluorescence analysis using pyrene as a fluorescent probe. ECW was solubilized at or above the surfactant critical micelle concentration; solubilization increased with an increase in micelle concentration. As shown by the fluorescence quenching of pyrene, surfactant solubilization of the ECW increased rapidly for the first 12 h, then approached a plateau, increased linearly with an increase in temperature (22−32 °C), and decreased linearly with the log of the polyoxyethylene chain length (range 5−40 oxyethylenes). These data are discussed in relation to surfactant effects on phytotoxicity and performance of foliar spray application of agrochemicals.

Keywords: *Cuticular penetration; micelles; fluorescence quenching; tomato; Triton X-100; Lycopersicon esculentum; Brassica oleracea; broccoli*

INTRODUCTION

Surfactants are commonly used in the foliar application of agrochemicals to improve performance. One mechanism by which surfactants may enhance performance is by solubilizing or modifying the epicuticular wax (ECW) barrier covering the aerial plant surface. We are not aware of any direct evidence that nonionic surfactants solubilize ECW. To the contrary, Riederer and Schönherr (*1*) did not observe solubilization of ECW by surfactant residues deposited on orange and pear leaves over a 2−4-day period.

There is considerable evidence that surfactants modify the fine-structure of ECW (*2*−*5*). This response has been observed most frequently with plants from *Brassica* sp. (*5*), in which the ECW is characterized by a high degree of delicate fine-structure and low wettability. Furthermore, surfactants have been shown to increase the mobility (the diffusion coefficient) of NAA and 2,4-D in isolated plant cuticles (*6*, *7*), and this effect was greatest when the surfactant was presented to the ECW-rich outer cuticular surface (*8*).

Because the cuticle and the ECW, in particular, play determinant roles (e.g., retention, spreading, distribution, and penetration) in the performance of foliarly applied chemicals (*9*−*15*), we investigated if surfactants solubilize ECW in a model system.

EXPERIMENTAL PROCEDURES

SEM−Surfactant Deformation of ECW. The effect of TX-100 (0.1%) on ECW fine-structure was evaluated by application of simulated spray droplets (1 μL) to the surface of mature tomato fruit and abaxial surfaces of newly expanded broccoli leaves. For tomato, a series of droplets were carefully applied with a microsyringe in a defined area on the equator of the fruit. The location of each droplet was marked by silver conducting paint. After 24 h, the treated area was positioned in contact with distilled water and, after four 30-min desorptions, the surface was dried under a stream of air. One-centimeter-diameter disks were removed from the fruit, each containing a treatment site, and an epidermal segment was carefully excised, blotted dry, and attached to an aluminum stub with silver conducting adhesive. The treated ECW surface was viewed immediately (without coating) with a model JEOL JSM-35C SEM at 12 kV. Photomicrographs were prepared with Polaroid PN 55 film. For control purposes, disks were excised from nontreated areas adjacent to the treatment site and processed as described above.

A similar experiment was performed using the abaxial surfaces of newly expanded broccoli leaves where droplets of TX-100 (0.1%) were applied by microsyringe, avoiding veinal tissue. After 24 h, leaf disks were excised containing droplet sites and from adjacent nontreated areas and placed treated (abaxial) surface in contact with distilled water (disks supported by a plastic grid) in a Petri dish to desorb the surfactant residue. After desorption, the tissue was processed and viewed as described above.

Chemicals. The surfactants used were of the Triton X (TX) octylphenol hydrophobe series, α-[4-(1,1,3,3-tetramethylbutyl) phenyl-ω-hydroxypolyoxy-1,2-ethanediyl (*16*), with 5, 7.5, 9.5, 16, 30, and 40 oxyethylene (OE, or POE) groups (Table 1). All surfactants were used as received and were mixtures of oligomers; the OE number represents the average value, and the mole ratio distribution follows a Poisson distribution (*16*). These surfactants were selected because they represent an important surfactant chemistry used in pesticide formulation and in spray application of agrochemicals.

Pyrene, benzo[*def*]phenanthrene (Aldrich Chemical Co., Milwaukee, WI), was recrystallized once from redistilled chloroform. Authentic octadecane (99% CP, Sigma, St. Louis, MO) octadecanoic acid, methyl octadecanoate, *n*-tricosane, octadecylpalmitate, 2-octadecanone, and 1-eicosanol (98+% CP, Analabs Inc., New Haven, CT) were used as received.

ECW Isolation. Tomatoes (*Lycopersicon esculentum* Mill. cv. Pik-Red) were field-grown pesticide-free in an isolated area of our research facility. The ECW was removed by dipping (five times for ∼2 s each) the apical two-thirds of mature fruit, free of blemishes, into redistilled chloroform at room temperature.

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Table 1. Physical Properties of Triton X Surfactants Used[a]

Triton X-	OE no.[b]	av MW	cmc[c] (wt %)	HLB[d] (calcd)	γ[e] ($mN \cdot m^{-1}$)
45	5	426	0.005	10.4	29
114	7−8	536	0.009	12.4	30
100	9−10	628	0.019	13.5	31
165	16	910	0.04	15.8	33
305	30	1526	0.11	17.3	38
405	40	1966	0.17	17.9	48

[a] *Rohm and Haas, Surfactants and Dispersants, Handbook of Physical Properties*; 1988; 16 pp, and ref *47* for Triton X-45. [b] Mean number of oxyethylene units. [c] Critical micelle concentration. [d] Calculated hydrophile−lipophile balance. [e] Surface tension, 0.01% w/v solution.

The chloroform extract was filtered, dried over anhydrous sodium sulfate, concentrated under vacuum, and held as a source of ECW for solubilization studies. ECW was also isolated from fully expanded broccoli (*Brassica oleracea* var. *botrytis* L. cv. Green Comet) leaves of plants grown without the use of pesticides as described above. The leaves were slightly wilted to ensure stomatal closure before extraction.

ECW Preparations. Five milligrams of tomato fruit or broccoli leaf ECW was reconstituted as a film on the bottom of glass scintillation vials (20 mL) by transferring 3 mL of the chloroform/wax solution (1.67 mg mL^{-1}) and evaporating to dryness overnight at 50 °C. The deposited wax was dried further under vacuum at room temperature for 6 h. Additional vials were similarly prepared with 5 mg of selected reference compounds (see Table 2). All vials and Teflon-lined caps were rinsed three times with redistilled chloroform before use.

Solubilization Procedure. Ten milliliters of the designated surfactant at appropriate concentration (w/v) made up in deionized, distilled water was added to each vial. The vials were capped, and wax was extracted by shaking slowly horizontally in a water bath at 25 °C. The supernatant was decanted from each sample and filtered through Anotop 25 disposable syringe filters (pore size = 0.1 μm, Alltec Associates, Inc., Deerfield, IL). This filtrate represented our ECW extract sample (or referred to as wax sample). Blank vials without wax were subjected to the same procedure and served as controls. A chloroform extract was also prepared of tomato and broccoli ECW for comparison to the surfactant extract.

Effect of TX-100 Concentration. ECW samples from tomato fruit and broccoli leaves were extracted for 7 days with 0, 0.001, 0.01, 0.03, 0.1, 0.25, 0.5, and 1.0% w/v TX-100 and processed as described above. This concentration range provided TX-100 extraction solutions either with only the monomeric form (0.001 and 0.01%), the critical micelle concentration (cmc) being 0.019% w/v, or with the monomeric plus increasing concentrations of the micellar form (0.03−1%). The extracts were partitioned against chloroform, concentrated, and subjected to TLC.

Time Course. Tomato fruit ECW samples were extracted with TX-100 (0.03%) for 0, 1, 3, 6, 12, and 24 h in the short-term study and for 1, 2, 4, 8, and 10 days in the long-term study. Extracts were processed as described above, and wax solubilization was determined by fluorescence spectroscopy.

Effect of Temperature. The effect of temperature on TX-100 (0.03%) solubilization of tomato fruit ECW was determined by extraction at 22, 25, 27, and 32 °C for 8 days. Solubilization was determined by fluorescence spectroscopy.

Effect of Surfactant POE Chain Length. The relative solubilizing effectiveness of a series of Triton surfactants differing in the length of the POE chain was determined at concentrations relative to their cmc so that the micelle concentration was comparable. The surfactants selected and physical characteristics are listed in Table 1. Solutions were prepared at concentrations ~1.6 times their respective cmc. The concentrations in w/v were 0.008% (TX-45), 0.014% (TX-114), 0.030% (TX-100), 0.063% (TX-165), 0.174% (TX-305), and 0.268% (TX-405). Extracts were processed after extraction for 8 days and assayed by fluorescence spectroscopy.

Micelle Terminology. Micelles formed by surfactant monomers of two chemistries are often referred to as mixed micelles. In our study, the micelles present in our surfactant solutions were composed of mixtures of oligomers differing only in the POE chain length. However, for clarity in our presentation, we will refer to such micelles as pure (surfactant only) micelles and to those that contain a solubilizate (e.g., solubilized ECW) as mixed micelles.

TLC. ECW extracts were spotted on tetrachloroethane prewashed silica gel G (250 μm) plates. Extracts of blank vials without wax served as controls, and chloroform extracts of ECW and authentic samples of *n*-tricosane, octadecylpalmitate, 2-octadecanone, and 1-eicosanol were included for reference purposes. Plates were developed with redistilled chloroform. Chromatograms were visualized by spraying with 50% H_2SO_4 and charring at 125 °C.

Fluorescence Quenching as a Measure of Micelle Solubilization. Pyrene has been used extensively as a fluorescence probe to characterize the solubilization of nonpolar substances by surfactant micelles in aqueous systems (*17*−*19*). In theory, when pyrene is added to an aqueous surfactant solution containing micelles, the nonpolar pyrene partitions into the micelle. When excited, the emission spectrum produced exhibits at least five vibronic peaks in the region from λ_{350nm} to λ_{500nm} (see Figure 4 for the emission spectrum of the surfactant probe solution of TX-100/pyrene). When an additional solubilizate is introduced into the micelle (we will refer to it as a mixed micelle), for example, in our case the solubilized ECW, fluorescence intensity is depressed (quenched). The first (I) and third (III) fluorescence intensity peaks are particularly sensitive to changes in solvent polarity (*20*, *21*). These peaks may respond differently due to induced changes in the microenvironment of the micelle (*22*). The degree of quenching of peak I, that is, the ratio of intensity after to that before (I′/I) wax solubilization, is a useful measure of micelle solubilization of a solubilizate. The ratios of peaks III/I for micellar solutions in the absence of a solubilizate and peaks III′/I′ in the presence of a solubilizate may provide an index of the micelle microenvironment, often referred to as the polarity parameter (*20*, *22*, *23*).

TX-100 can serve as a fluorophore on its own, because the surfactant has an aromatic moiety (phenol) that produces a useful emission spectrum with one vibronic peak below the cmc at λ_{306nm} and one (λ_{336nm}) above the cmc (see Figure 5). The λ_{336nm} peak appears to be associated with micelle formation. Changes in the ratio of these two peaks can provide information on the microenvironment near the phenyl ring in the micelle. We adapted this approach to provide further evidence that the solubilized epicuticular wax was localized in the surfactant micelles.

Fluorescence Measurements and Emission Spectra. For pyrene, 10 μL of a methanolic solution of pyrene (0.2 μg μL^{-1}) was added as a fluorescence probe to each of the reference samples, ECW extract, or blank. The samples were excited at λ_{340nm}, and the intensity of the fluorescence emission spectra was scanned from λ_{350nm} to λ_{500nm} at 120 nm min^{-1} with an Aminco Bowman spectrofluorometer at 25 °C. The slit widths for excitation and emission were 0.55 and 0.20 mm, respectively. Samples were not degassed. The fluorescence of control blanks (surfactant or surfactant plus pyrene) was determined prior to measurement of the fluorescence intensity of samples.

The fluorescence intensity of TX-100 before and after solubilization of tomato fruit ECW was scanned from λ_{270nm} to λ_{500nm} following excitation at λ_{286nm}. Other experimental conditions were as above for pyrene.

Optimizing TX-100 and Pyrene Concentrations. The fluorescence assay was optimized for TX-100 and pyrene concentrations by determining the effect of varying the TX-100 concentration from 0.01 to 0.5% (w/v) on fluorescence emission from pyrene at a probe concentration of 1 μM; then pyrene concentration was varied from 0.05 to 1 μM in the presence of TX-100 at 0.1% w/v. Changes in fluorescence intensity, measured at λ_{370nm} (peak I) and λ_{380nm} (peak III), were used to identify optimum concentrations (Figure 1).

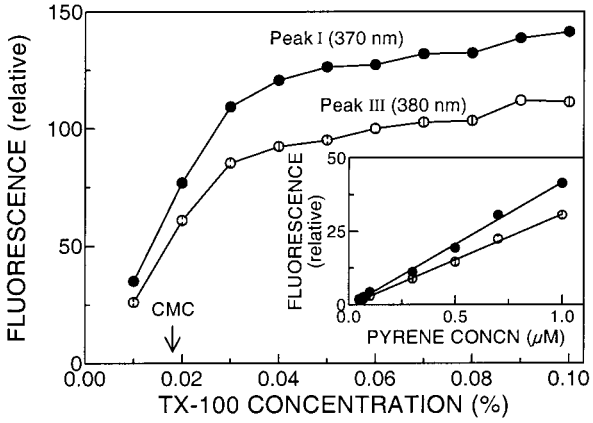

Figure 1. Effect of TX-100 concentration on fluorescence intensity of pyrene (1 μM) and pyrene concentration on fluorescence intensity in the presence of 0.1% TX-100 (inset): (●) peak I; (○) peak III. The peak III/I ratio remained constant.

The fluorescence intensity of pyrene (1 μM) increased rapidly initially with an increase in TX-100 concentration to ~0.03% and then increased at a decreasing rate with higher concentrations (Figure 1). Fluorescence intensity increased linearly with increasing concentrations of pyrene in the range of 0.05−1 μM in the presence of 0.1% TX-100 (Figure 1, inset). The ratio of peaks III/I remained constant, averaging 0.78 (SE = 0.004, n = 36) and 0.75 (SE = 0.011, n = 21) for the TX-100 and pyrene concentration responses, respectively. Unless otherwise stated, 1 μM pyrene and 0.03% TX-100 were used in subsequent experiments.

Statistics. Complete randomized designs were used with three replications. When appropriate, data were subjected to analysis of variance. Regression analysis was performed using treatment means. Mean comparisons were made using Duncan's multiple-range test. Standard errors are presented in the figures with data points except when smaller than the data symbol. Statistical significance for coefficients of determination at the P = 0.05 and 0.01 levels is indicated by * and **, respectively.

RESULTS

SEM—Surfactant Deformation of ECW. The ECW on tomato fruit CM was smooth and featureless (*24*), and there was no consistent visual evidence that the wax was significantly altered by the surfactant treatment (data not presented). The absence of fine-structure made it difficult to visually observe wax solubilization.

The fine-structure of the ECW on broccoli leaves appeared as a dense covering of upright, tapered rodlets with extensive secondary and tertiary branching near the apex (Figure 2A). The primary rodlets, square to polygonal, measured ~0.5−1.0 μm in cross section, and the secondary and teritary branches were 100−~300 nm in diameter.

Application of small droplets (1 μL) of aqueous TX-100 (0.1%) caused a deformation (softening) of the secondary and tertiary branchlets that then collapsed and draped over the primary branch structure of the rodlets (Figure 2B). The ECW fine-structure was deformed throughout the droplet deposit site but most extensively at the perimeter of the droplet deposit.

TLC. Tomato fruit ECW was solubilized by TX-100 at concentrations above the cmc (0.03−0.5%). Although the higher concentrations (0.25−0.5%) of TX-100 appeared to solubilize greater quantities of the wax, the presence of the additional mass of surfactant in the extract made separation of the more polar constituents difficult (data not presented). In contrast, there was no evidence of TX-100 solubilization at concentrations below the cmc, that is, at 0.001 or 0.01%. Thus, for subsequent studies, unless noted otherwise, concentrations of 0.03 and 0.1% TX-100 were utilized, which effectively solubilized the wax with minimum or no effect on detection of the wax in the extract (data not presented).

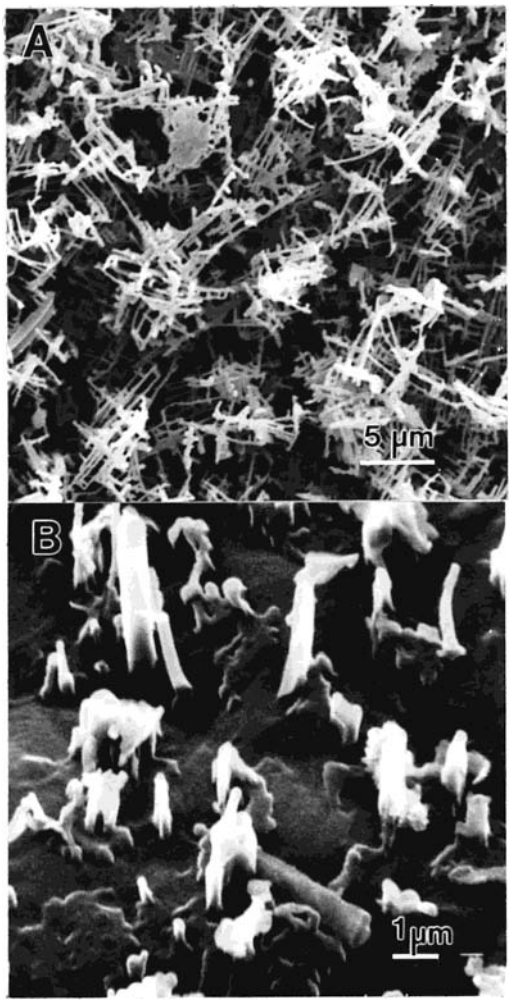

Figure 2. Secondary electron micrographs of epicuticular wax fine-structure on the abaxial surface of broccoli leaves: (A) nontreated control; (B) deformed wax rodlets near the periphery of a deposit from an aqueous droplet of TX-100 (1 μL, 0.1%) after 24 h and desorption of the residue.

A TLC comparison of solubilization of tomato fruit and broccoli leaf ECW by TX-100 at 0.03 and 0.1% is illustrated in Figure 3, along with chloroform extracts of the respective epicuticular waxes. TX-100 solubilized constituents of at least two principal wax classes (Figure 3). Other components were probably extracted but not adequately separated from the surfactant near the baseline.

Effects of ECW Solubilization on Pyrene and TX-100 Emission Spectra. The fluorescence emission spectra for pyrene in a TX-100 micellar solution (0.03% w/v) and excitation at λ_{340nm} are given in Figure 4. Five vibronic peak intensities are apparent in the region of λ_{350nm}−λ_{460nm}. All spectra after solubilization of authentic octadecane or ECW of tomato fruit or broccoli leaves revealed marked quenching of pyrene fluorescence, particularly at the shorter wavelengths. The greatest degree of quenching (I′/I) occurred with tomato ECW (−84%) and the least with octadecanol, octadecanone,

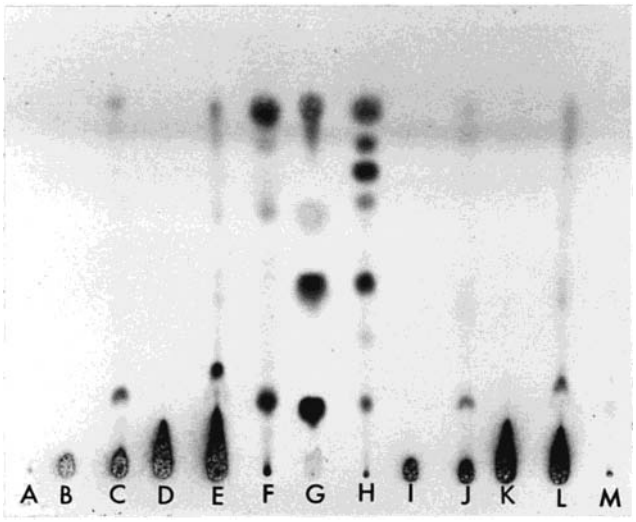

Figure 3. Thin-layer chromatogram of isolated tomato fruit and broccoli leaf ECW: tomato fruit ECW extract, water (A), TX-100, 0.03% (C), TX-100, 0.1% (E), chloroform (F); broccoli leaf ECW extract, chloroform (H), TX-100, 0.03% (J), TX-100, 0.1% (L), water (M); TX-100 blanks (no ECW), 0.03% (B, I), 0.1% (D, K). Authentic standards (G) from solvent front to baseline were *n*-tricosane, octadecylpalmitate, 2-octadecone, and eicosanol.

octadecanoic acid, and methyl octadecanoate, whereas broccoli ECW and octadecane were intermediate (Table 2).

The III′/I′ ratio of vibronic peak intensities of pyrene in TX-100 micellar solution was ~0.74 and increased to 0.83 and 0.81 following solubilization of tomato fruit and broccoli leaf ECW, respectively (Table 2; Figure 4). Comparative (III′/I′) ratios for the reference compounds ranged from 0.73 to 0.91 (Table 2).

The fluorescence emission spectrum (excitation at λ_{286nm}) of TX-100 (0.03%) before and after solubilization of tomato fruit ECW exhibited two broad vibronic peak intensities at about (A) λ_{306nm} and (B) λ_{336nm} (Figure 5). Both peaks were markedly quenched by the solubilized ECW, with the B peak being more sensitive than the A peak. The fluorescence intensity ratio (B/A) increased linearly (B/A = 0.24 + 38.71 concn, for the concentration range of 0.005−0.025%) with an increase in TX-100 concentration up to the cmc and then plateaued at ~1.22 (unpublished data). However, after solubilization of ECW, the B/A ratio decreased from ~1.22 for TX-100 micelles to ~0.88 for the TX-100/ECW mixed micelles (Figure 5), suggesting a change in polarity of the mixed micelle microenvironment after solubilization of the ECW.

Time Course. Surfactant solubilization of tomato fruit ECW was time dependent (Figure 6). The rate of solubilization was rapid initially and linear (Figure 6, inset) during the first 12 h and then decreased, reaching a plateau after 6 days.

Temperature. Fluorescence intensity decreased linearly (I′/I = 1.40−0.04 (temp), r^2 = 0.985**) with increasing temperature over the range from 22 to 32 °C (Figure 7), indicating greater quenching and thus greater wax solubilization with an increase in temperature.

Surfactant OE Effect. Surfactant solubilization (greater quenching) of tomato fruit ECW was inversely related to OE content (Figure 8). Fluorescence quenching (I′/I) was linearly related to the log of OE content I′/I = −0.49 + 0.67 log OE, r^2 = 0.927**), whereas the

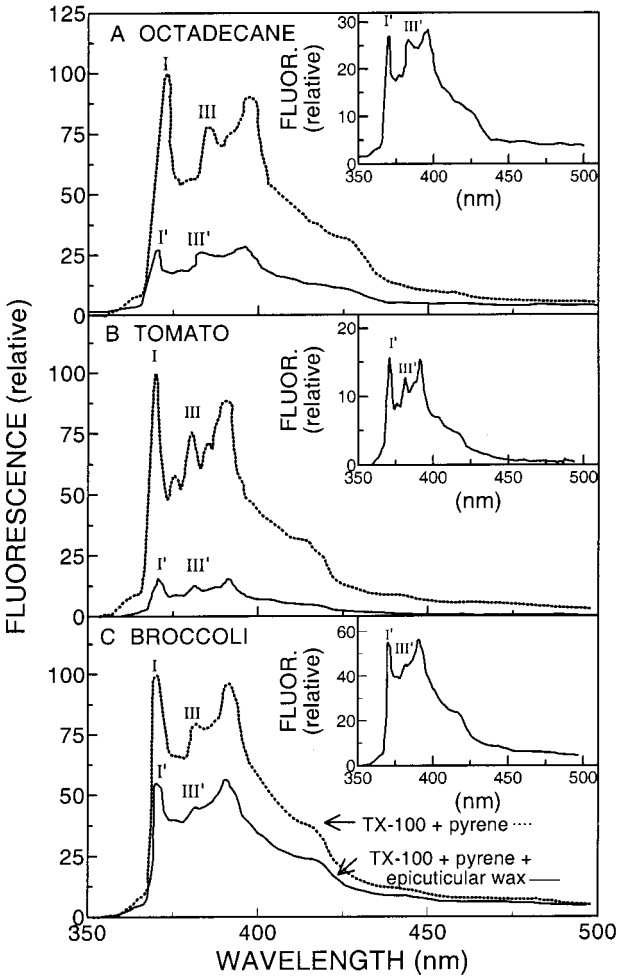

Figure 4. Fluorescence emission spectra of pyrene/TX-100 (0.03%) probe solutions before and after solubilizing octadecane (A), tomato fruit (B), and broccoli leaf (C) ECW. (Insets) Expanded ordinate scale for fluorescent solution after solubilization of ECW.

Table 2. Effect of Solubilizing Tomato Fruit and Broccoli Leaf Epicuticular Waxes and the Selected Reference Compounds Octadecane, Octadecanol, Octadecanone, Octadecanoic Acid, and Methyl Octadecanoate with Triton X-100 (0.03% w/v, Extracted for 8 Days) on Quenching of Pyrene Fluorescence As Indexed by the Ratio of Peaks I′/I and III′/I′ (See Figure 4 for Description of Ratios)

epicuticular wax and reference compounds	peak ratio ± SE	
	peak I′/I	peak III′/I′
control[a]	1.00 ± 0.008	0.74 ± 0.004
tomato	0.16 ± 0.019	0.83 ± 0.005
broccoli	0.54 ± 0.001	0.81 ± 0.007
octadecane	0.29 ± 0.012	0.91 ± 0.014
octadecanol	0.97 ± 0.008	0.76 ± 0.001
octadecanone	0.84 ± 0.011	0.76 ± 0.009
octadecanoic acid	0.96 ± 0.007	0.78 ± 0.007
methyl octadecanoate	0.95 ± 0.007	0.73 ± 0.004

[a] Triton X-100 at 0.03% w/v, no wax.

relationship with surfactant HLB was best described by the quadratic equation I′/I = 1.20 − 0.22 (HLB) + 0.10 $(HLB)^2$, r^2 = 0.938* (Figure 8, inset).

DISCUSSION

Solubilization of wax has been suggested as one mode of surfactant action to enhance foliar penetration (*4*, *15*,

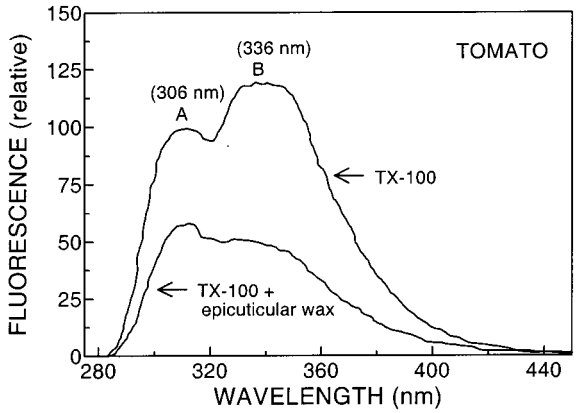

Figure 5. Fluorescence emission spectra of TX-100 before and after solubilizing tomato fruit ECW.

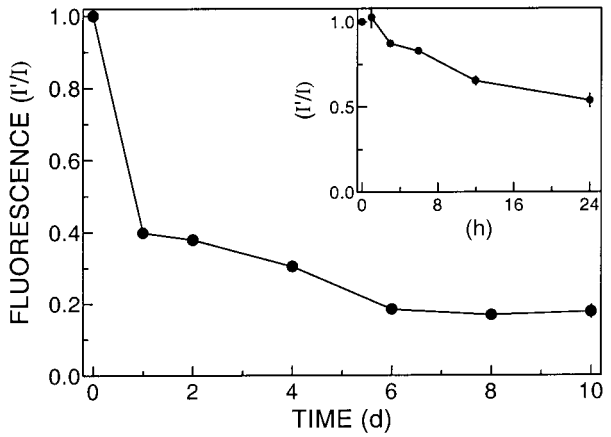

Figure 6. Time course of TX-100 (0.03%) solubilization of tomato fruit ECW as indexed by quenching (I′/I) of pyrene fluorescence. (Inset) Short-term time course. Regression equation for first 12 h of time course is I′/I = 1.01−0.03 (h).

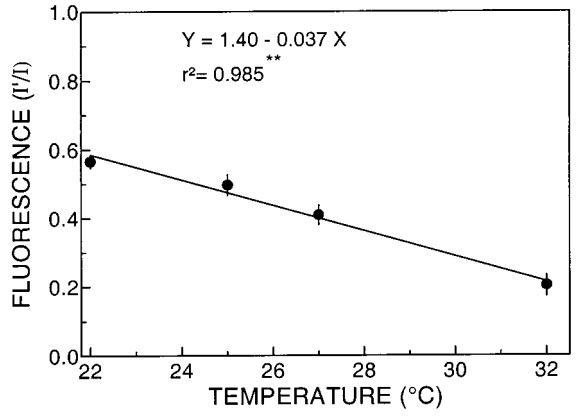

Figure 7. Effect of temperature on TX-100 solubilization of tomato fruit ECW as measured by fluorescence quenching (I′/I) of pyrene.

25), but no direct evidence is available. Early conclusions were based on observations of enhanced pesticide performance and surfactant-induced phytotoxicity (*26*, *27*), whereas more recent SEM observations have shown that surfactants often altered the micromorphology of ECW fine-structure (Figure 2; *2*−*4*). We now provide evidence, using a model system, that surfactants commonly used in spray application solubilize epicuticular wax.

TLC of TX-100 extracts of ECW of tomato fruit and broccoli leaves provided conclusive evidence for surfactant solubilization of components of two major constitu-

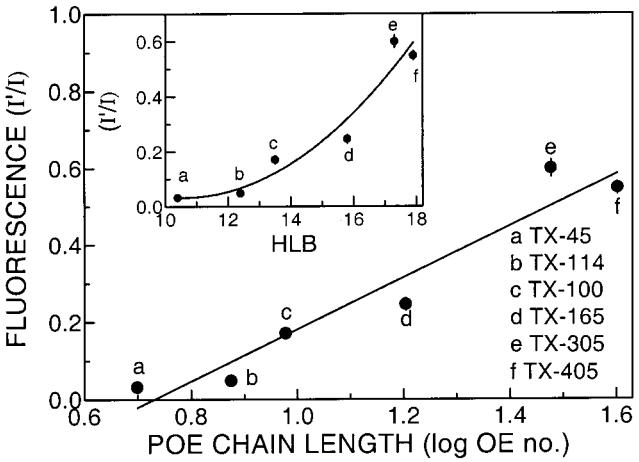

Figure 8. Effect of POE chain length on nonionic octylphenoxy (Triton X) surfactant solubilization of tomato fruit ECW measured by fluorescence quenching (I′/I) of pyrene. (Inset) Quenching in relation to surfactant HLB. Regression equation for fluorescence quenching on log OE content is I′/I = −0.49 + 0.67 log OE and for fluorescence on HLB is I′/I = 1.20−0.22 (HLB) + 0.10 (HLB)^2.

ent classes, namely, unsaturated hydrocarbons and triterpenols (*28*). ECW solubilization was detected only at TX-100 concentrations near or above the cmc, indicating the critical role of micelles. Our observation that micellar surfactant solutions were effective in solubilizing ECW is consistent with classical studies on the role and mechanisms of micelle solubilization of nonpolar substances in aqueous systems (*17*, *29*).

Here it is important to describe the nature and solubilizing characteristics of the nonionic octylphenol POE surfactant micelles to adequately explain our findings. Micelles are formed in aqueous systems at and above the cmc as thermodynamically stable aggregates. The octylphenol hydrophobic groups make up the micelle core and the hydrophilic POE chains, spiraling from the core outward into the aqueous solution, form the palisade mantle (*30*). The core is very nonpolar and viscous. The palisade layer varies in polarity; it is essentially nonpolar at the core/palisade interface and becomes more hydrophilic (hydrated), acquiring characteristics of the aqueous solution, with increasing distance from the core (*30*). TX-100 micelles, in particular, have been shown to have a high capacity to solubilize nonpolar components because of the fluidity of the core and structural flexibility, that is, change in morphology on solubilization to reconstruct a thermodynamically stable structure (*18*).

The degree of solubilization may be determined by a number of factors; most important for our discussion are the chemistry of the solubilizate and structure and microenvironment of the micelle [for an extensive review see Myers (*30*), Chapters 3 and 4). On solubilization, the nonpolar solubilizates (e.g., hydrocarbons) become associated with the micelle core, whereas progressively more polar solubilizates become localized at an appropriate location in the palisade layer (*31*). By using pyrene as a fluorescence probe, micelle solubilization of ECW was followed by fluorescence quenching and the effect of the solubilized ECW on the polarity of the microenvironment of the micelles by changes in the fluorescence intensities of the III′/I′ vibronic peaks (Figure 4, see Materials and Methods).

The kinetics of TX-100 solubilization shows a strong linear relationship with time for the first 12 h (I′/I =

1.01−0.03 (time), $r^2 = 0.951$**). Recognizing that these data were generated at one concentration (0.03%) of Triton X-100, we would expect higher solubilization rates for higher concentrations because solubilization by detergent solutions is generally proportional to micelle concentration (*32*). An additional factor enhancing micelle solubilization at high concentrations is that micelles formed at high concentrations have a higher aggregation number, which is associated with greater solubilizing capacity (*18*).

Our data on the effect of temperature and POE chain length on solubilization of ECW were consistent with general models published for micelle solubilization of nonpolar compounds (*33*). An explanation of increased TX-100 solubilization with an increase in temperature is complex. First, an increase in temperature can affect intermolecular interactions between the solvent and solute (e.g., H-bonding) so that solvent properties for the ECW as well as for the surfactant may be altered. We have no evidence that this was a factor in our system. Second, it is well established that a number of temperature-induced changes occur in nonionic surfactants of the POE type which enhance solubilization, namely, a decrease in the cmc and an increase in micelle size/aggregation number and dehydration of the palisade layer leading to a less polar environment and thus greater solubilizing capacity for nonpolar substances (*30, 34*). Similarly, the inverse relationship between micelle solubilization of ECW and POE chain length agrees with the generalized concept that for POE surfactants the amount of aliphatic hydrocarbon solubilized increases with an increase in length of the hydrophobic tail and decreases with an increase in length of the POE chain (*21, 30*).

A critical question remains: Does the solubilization of ECW described in our model system occur during spray application of pesticide chemicals under field conditions? We can visualize a number of conditions existing at the spray droplet and deposit/plant surface interface favoring surfactant solubilization of epicuticular wax. The ECW is not covalently bound and can be easily solubilized by appropriate solvents, and some wax can be readily removed mechanically. Waxes with extensive fine-structure present a large surface area for surfactant attack. Spray droplets frequently contain nonionic surfactants at concentrations above their cmc, along with other formulants and active ingredients, at time of impaction/retention. The aqueous phase evaporates, usually at greater rates from the wax surface/droplet/air interface, causing preferential deposition at the periphery in the form of an annulus. This deposition pattern coincides with areas of greatest ECW softening and phytotoxicity (*4, 35*).

During droplet drying, surfactant monomers may be adsorbed by and aggregrate on the plant surface to form admicelles (hemimicelles) with solubilizing characteristics identical to or greater than those of micelles in solution (*21, 36*). They are (most likely) mixed admicelles, which usually have greater solubilizing capacity than true micelles (*17, 21*). Furthermore, the surfactant concentration increases during evaporation, forming various liquid crystal phases (*37*) in intimate contact with the ECW. Concomitantly, monomers of the surfactant are sorbed by the wax and cuticle and there may alter the physical and permeability characteristics (*7, 38*−*42*) of the cuticle. Once the admicelles, micelles, and liquid crystal phases are formed, the wax (components) is solubilized and removed from the surface as proposed for crystal solubilization by Chan et al. (*43*). Surfactants may facilitate transfer of solute molecules from crystal to solution by decreasing the activation energy (*44*). This may be particularly important in plant species, where the epicuticular waxes are crystalline because they represent a barrier to penetration (*42*). Also, because waxes are not covalently bound, surfactants may remove ECW from the surface by detergent action (*32*), much like the removal of greases from a polyester fabric.

To what extent solubilization of epicuticular wax constituents affect transcuticular penetration needs to be researched, particularly because the embedded waxes are considered to be the prime barrier to penetration (*42, 45*). Aside from penetration, solubilization of epicuticular wax may have an impact on the performance of foliarly applied chemicals by altering surface wax physical properties causing changes in wettability, spray retention, and distribution and thus dose per unit area available as a surface crop protectant or for penetration.

It should be noted that factors of time, concentration, temperature, and POE chain length on surfactant solubilization of ECW closely parallel their effects on phytotoxicity (*26, 35*), ECW softening (*46*), and cuticular membrane permeability (*6, 7, 40*).

Although the above associations are of a general nature, they represent well-documented events that occur at the spray droplet interface during evaporation and between the resulting droplet residue and the plant surface.

ABBREVIATIONS USED

ECW, epicuticular wax; OE, oxyethylene; POE, polyoxyethylene; TX, Triton X; cmc, critical micelle concentration; HLB, hydrophilic/lipophilic balance.

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Registry No. (supplied by the author): 1-eicosanol, 629-96-9; octadecane, 593-45-3; 2-octadecanone, 7373-13-9; octadecanoic acid, 57-11-4; methyl octadecanoate, 112-61-8; pyrene, 129-00-0; *n*-tricosane, 638-67-5; Triton X-45, Triton X-114, Triton X-100, Triton X-165, Triton X-305, Triton X-405, all 9036-19-5.

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