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Studies on Octylphenoxy Surfactants: IX. Effect of Oxyethylene Chain Length on GA_3 Absorption by Sour Cherry Leaves

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Abstract. The effect of a homologous series of octylphenoxy surfactants, α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly-(oxy-1,2-ethanediyl), condensed with 5, 7–8, 9–10, 16, and 30 oxyethylene (EO) units on enhancement of gibberellic acid (GA_3) absorption by leaves of *Prunus cerasus* cv. Montmorency was studied. Increasing EO chain length (5–30 EO) increased surface tension (27.5–35.3 mN m^{-1}) and contact angles on adaxial (21–36°) and abaxial (28–49°) leaf surfaces. With increasing EO content, the form of GA_3 deposits from droplets on the leaf surface changed from an annulus shape (5 and 7–8 EO) to globular forms covering increasingly smaller interface areas (9–10 to 30 EO). The surfactants increased GA_3 uptake, the magnitude decreased with an increase in oxyethylene chain length. Similar trends were found for both the adaxial and abaxial surfaces. Penetration through the abaxial surface was linearly related to the logarithm of the oxyethylene content of the surfactant molecule (r^2 = 0.934**) and to the hydrophilic:lipophilic balance (r^2 = 0.926**). Absorption by the abaxial surface was approximately one order of magnitude greater than by the adaxial surface.

Gibberellic acid (GA_3) inhibits flower initiation and promotes spur formation in cherry yellows-infected sour cherry trees (Bukovac et al. 1986, Parker et al. 1969). The practice of annual foliar application of GA_3 is rapidly being adopted to increase fruiting potential and cropping efficiency (Bukovac et al. 1986). Field observations (unpublished data) suggest that some commonly used surfactants may have a marked effect on GA_3 performance in sour cherry, and recent reports for *Citrus* (Coggins et al. 1989, Greenberg et al. 1987) and sour cherry indicate surfactant-enhancement of GA_3 penetration. We found striking differences among some commonly used surfactants and surfactant blends on GA_3 absorption by sour cherry leaves (Knoche et al. 1990). However, no data are available on the relationship between surfactant chemical structure and the enhancement of GA_3 absorption.

The objective of this study was to establish the effect of a homologous series of nonionic surfactants (commonly used in formulations and surfactant blends) of varying oxyethylene chain length on GA_3 penetration into and deposit characteristics of GA_3 from spray droplets on sour cherry leaves. Furthermore, we relate surfactant chemical structure to physical properties of the spray solution.

Materials and Methods

Newly expanded leaves were detached from the third to fifth node (from the apex) of current season's shoot growth from sour cherry (cv. Montmorency) trees grown without pesticide application at the Horticulture Research Center, East Lansing, Michigan. Discs (21 mm in diameter) were excised from the lamella on both sides of the midrib and floated on deionized water in petri dishes positioned in a water bath at 25°C. Photosynthetically active radiation was maintained at 230 μmol · s^{-1} · m^{-2} during a 16-h photoperiod.

Spray solutions were prepared with [1,7,12,18-^{14}C]GA_3 (251 MBq · $mmol^{-1}$ specific activity, 99% pure, Amersham, Arlington Heights, IL, USA) at a concentration of 0.5 mM in 20 mM citric acid buffer (pH 3.0) and a surfactant concentration of 0.1% (wt/vol). Surfactants used were commercial preparations of octylphenol condensed with 5, 7–8, 9–10, 16, or 30 moles ethylene oxide (trade name Triton TX-45, TX-114, TX-100, TX-165, and TX-305, Rohm and Haas Co., Philadelphia, PA, USA). Spray solutions were applied to the leaf surface using a microsyringe fitted with a Teflon-coated needle and an automatic dispenser.

Surface tension of spray solutions was determined using the Du Nouy ring method and a Fisher Model 20 tensiometer (Fisher Scientific, Pittsburgh, PA, USA). Solutions were equilibrated for 1 h prior to measurement. Measurements were replicated five times. Wetting of the adaxial and abaxial leaf surfaces was evaluated by determining the contact angle formed with each treatment solution. Base width and height of droplets were measured

Table 1. Physical properties of octylphenoxy oxyethylene surfactants and their aqueous solutions.

Surfactant (Triton X series)	Avg. EO[a]	HLB[b]	Avg. mol. weight	CMC[c] ($g \cdot L^{-1}$)	Surface tension[d] ($mN \cdot m^{-1}$)	Contact angle[d] (degree)	
						Adaxial	Abaxial
Control	—	20[e]	—	—	71.0 a[f]	59 a[f]	82 a
TX-45	5	10.4	426	0.05	27.5 e	21 d	28 e
TX-114	7–8	12.4	536	0.12	28.3 f	25 d	31 de
TX-100	9–10	13.5	628	0.19	29.5 d	31 c	35 d
TX-165	16	15.8	910	0.39	33.7 c	29 c	45 c
TX-305	30	17.3	1526	0.99	35.3 b	36 b	49 b

[a] Represents the average number of ethylene oxide units (EO), the oligomer distribution follows a Poisson distribution. Values for EO, HLB, and molecular weight from Anonymous (1988).
[b] HLB: Hydrophilic:lipophilic balance.
[c] CMC: Critical micelle concentration, data from Stevens and Bukovac (1987b).
[d] Solution for measurements of surface tension and contact angle contained 0.1% wt/vol surfactant, 0.5 mM GA_3, and 20 mM citric acid in distilled water.
[e] Assigned value.
[f] Means within columns followed by the same letter are not significantly different at $p = 0.05$, Duncan's multiple range test.

and contact angles calculated according to Mack's equation (Mack 1936). Each determination consisted of a minimum of five measurements (one 1 µl droplet each) per leaf segment replicated 10 times.

Deposit formation on the abaxial leaf surface on droplet drying was investigated by scanning electron microscopy (SEM). Leaf tissue was excised, mounted on alumina stubs with carbon adhesive, and spray solution was applied as discrete droplets (0.24 µl). Specimens were then held in a chamber at 25°C and 45% relative humidity for about 15 min while droplets dried. Immediately after droplet drying, the fresh uncoated samples were studied by SEM (ISI 40, International Scientific Instruments Inc., Pleasanton, CA, USA).

GA_3 absorption was measured 24 h after application of 15 × 0.24 µl droplets per disc according to the procedure described by Stevens and Bukovac (1987a). Briefly, the nonabsorbed residue was removed from the leaf surface with 5 ml of acetone:water (3:2). Epicuticular wax with associated radiolabel was removed by stripping with cellulose acetate. The remainder of the leaf tissue was oxidized (model OX-400, Harvey Inc., Hillsdale, NJ, USA) and the $^{14}CO_2$ trapped with Carbo-Sorb II in toluene-based scintillation cocktail. Radioactivity was determined by liquid scintillation spectrometry (model 1211, Rackbeta, LKB Wallace, 20101 Turku 10, Finland). Counts were corrected for background, quenching, and efficiency where necessary. Data presented were calculated from the sum of the radioactivity in the wax and the tissue fraction on a percent applied basis. Average recovery of the radioactivity was 91%. Each treatment was replicated 10 times.

Data were subjected to analysis of variance where appropriate. Comparison of means was undertaken using Duncan's multiple range test at $p = 0.05$. Regression lines were calculated from treatment means. Significance of coefficients of determination (r^2) of linear regression analysis was indicated by * and ** at $p = 0.05$ and 0.01, respectively.

Results and Discussion

Surface tension of the spray solutions and hence contact angles of spray droplets on adaxial and abaxial leaf surfaces increased as oxyethylene content of the surfactant molecules increased (Table 1). Striking differences in deposition pattern were observed among surfactants (Fig. 1). Droplets of spray solution containing TX-45 (5 EO) formed scattered deposits throughout the droplet/leaf interface area, and preferentially at the periphery (Fig. 1B). With an increase in EO chain length, the deposit form changed from annulus-shaped deposits (7–8 EO, Fig. 1C) to globular deposits covering increasingly smaller interface areas (9–10 to 30 EO, Fig. 1D–F). Form of deposits from solutions containing surfactants with 9–10 or more EO units (TX-100, TX-165, and TX-305) was similar to those from solutions without surfactant (Fig. 1D–F vs. 1A). Deposition occurred frequently adjacent to veins suggesting variation in wetting characteristics of the leaf surface. The deposit/leaf interface area was generally smaller than the droplet/leaf interface area. The qualitative relationship between EO chain length and interface area appeared to remain the same (i.e., the droplet and deposit interface area decreased with increase in EO content).

Surfactants significantly enhanced GA_3 uptake into sour cherry leaves through the abaxial leaf surface (Fig. 2). Enhancement of penetration was greatest with surfactants having a hydrophile chain length of 9–10 EO units or less (TX-45, TX-114, and TX-100, Fig. 2A), followed by TX-165 with 16 EO units. TX-305 (30 EO) did not affect GA_3 penetration compared to the control. Uptake through the adaxial surface followed a similar trend (Fig. 2C), but was approximately one order of magnitude lower (Fig. 2A). Absorption of GA_3 was best described by a linear regression model where the logarithm of the number of EO units or the hydrophilic:lipophilic balance [HLB, represents the approx-

Fig. 1. Deposits from spray solutions (0.24 µl, calculated inflight diameter 772 µm) containing GA_3 in citric acid buffer and surfactants of varying oxyethylene (EO) chain length on the abaxial surface of sour cherry leaves. **(A)** Control (without surfactant); **(B)** TX-45 (5 EO); **(C)** TX-114 (7–8 EO); **(D)** TX-100 (9–10 EO); **(E)** TX-165 (16 EO); **(F)** TX-305 (30 EO). See text for details.

imate weight percent of EO in nonionic surfactants divided by 5 (Griffin 1954)] was the independent variable (Table 2). Similar findings were reported for DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane] and atrazine (6-chloro-*N*-ethyl-*N'*-isopropyl-1,3,5-triazinediyl-2,4-diamine) by Stevens and Bukovac (1987a). Sorption of GA_3 by the wax fraction never exceeded 1.5% of the total amount applied, and was significantly greater for the abaxial than the adaxial surface (data not presented).

Since the flux across the cuticle into the leaf tissue is proportional to the solution/cuticle interface area, GA_3 absorption was calculated on a droplet/leaf interface area basis (Fig. 2B). For a first approximation, the assumptions were made that penetration occurred from the liquid phase and that differences (range of 1.3 min for abaxial and 3.8 min

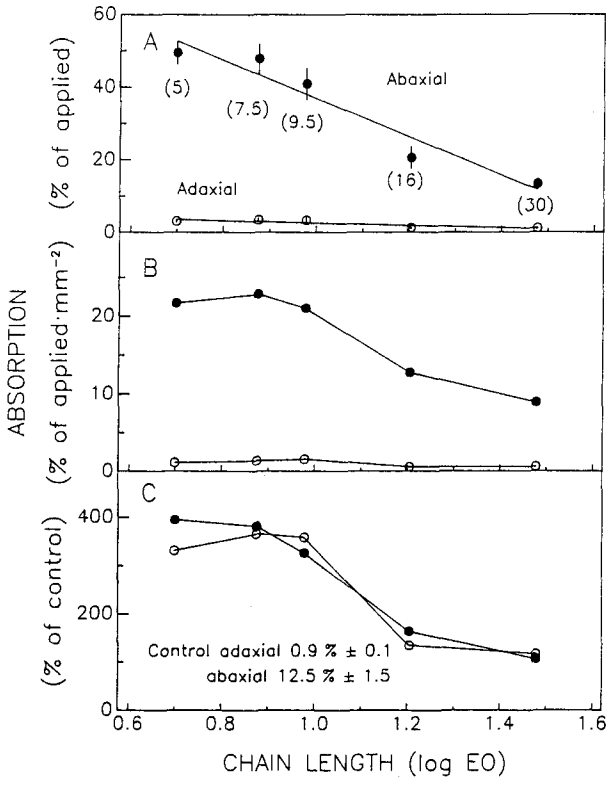

Fig. 2. Effect of oxyethylene (EO) chain length of nonionic octylphenoxy (Triton-X) surfactants on GA_3 absorption through adaxial (○) and abaxial (●) sour cherry leaf surfaces. **(A)** Absorption was calculated as percent of the amount applied. **(B)** Absorption calculated on a droplet/leaf contact area basis, representing the area effective for GA_3 absorption. **(C)** Absorption calculated as percent of the control without surfactant addition (Control = 100%).

for adaxial surface) in the length of drying time among surfactants of the spray droplets did not significantly affect GA_3 penetration. If the surfactant effects were limited to improved wetting [i.e., enhancement of droplet spreading (contact angles, Table 1)], uptake of GA_3 per unit interface area would be independent of the EO content of the surfactant molecule. Our data demonstrate that the surfactant effect can be only partially attributed to improved wetting. The surfactants with short (5 to 9–10 EO) EO chains were still more effective on an interface area basis than those with long (16 and 30 EO) chains, the relative difference in effectiveness, however, declined (Fig. 2A vs. B).

The reason for this phenomenon is not completely understood. Mass flow of the spray solution through open stomata cannot be completely excluded, since the critical surface tension [the surface tension at which the surface is wetted (zero contact angle) and stomatal pore infiltration may

Table 2. Relationship between oxyethylene (EO) chain length of octylphenoxy surfactants and GA_3 uptake by sour cherry leaves.

Surface	Regression equation ($\hat{Y} = b + aX$)	Coefficient of determination (r^2)
Adaxial	Uptake (%) = 5.9 − 3.3 (log EO[a])	0.757
Abaxial	Uptake (%) = 89.5 − 52.7 (log EO[a])	0.934**
Adaxial	Uptake (%) = 7.4 − 0.4 (HLB[b])	0.736
Abaxial	Uptake (%) = 115.0 − 5.8 (HLB[b])	0.926**

[a] EO: number of oxyethylene units.
[b] HLB: Hydrophilic:lipophilic balance.

occur] was approached in solutions containing TX-45 (5 EO), TX-114 (7–8 EO), or TX-100 (9–10 EO) (Schönherr and Bukovac 1972). However, no symptom of stomatal infiltration (i.e., no appearance of water-soaked areas) was observed and the surfactant effect on GA_3 penetration was similar for both the astomatous adaxial and the stomatous abaxial surfaces (Fig. 2C). These data imply that the surfactants studied may have a direct effect on the cuticle. Surfactants may alter cuticular penetration by increasing either the partitioning of the active ingredient (a.i.) into the cuticle or the mobility of the a.i. within the cuticle. Increased sorption of other growth-regulating compounds has been demonstrated in the presence of surfactants with low oxyethylene content (Shafer et al. 1989). However, we are not aware of any reports relating a.i. mobility in the cuticle to chemical structure of surfactant molecules.

Little is known about which surfactant properties are critical for enhancement of cuticular penetration. In such studies, surfactants with different critical micelle concentrations (CMC) and molecular weights (Table 1) are compared on a weight per volume basis as is practiced in formulation chemistry. Consequently, number of surfactant molecules, monomer and micelle concentration, size, shape, and number of micelles differed among surfactants. Additionally, concentration of a.i. and surfactant change as the spray droplet dries, the rate of drying varying with surfactant. Finally, a "dry" deposit is obtained retaining different amounts of water according to the hygroscopic properties of the surfactant (Stevens and Bukovac 1987b). Moreover, deposition pattern varied markedly among surfactants. We do not know whether GA_3 is uniformly distributed within the bulk of the deposit. If the distribution is homogenous, the microenvironment (i.e., crystallinity, degree of hydration) within an annulus-shaped scattered deposit is very likely to be different from globular deposits of smaller surface area and could be of importance for penetra-

tion from deposits remaining on the surface after the solvent evaporates. The integration of all of these effects resulted in the data presented. Clearly, further studies evaluating single factors under controlled conditions are critical for a better understanding of the role of surfactants in foliar penetration.

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