Surfactant-Enhanced Penetration of Benzyladenine through Isolated Tomato Fruit Cuticular Membranes

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The effect of Triton X-100 (TX-100) on the penetration of benzyladenine (BA) through isolated tomato (*Lycopersicon esculentum* Mill.) fruit cuticular membranes was studied using finite and infinite dose diffusion and sorption/desorption systems. Finite dose penetration of BA (18 and 160 μM, pH 2 and 6) from donor droplets (3 μL) was characterized by an initial time lag, a maximum rate of penetration, and total penetration. TX-100 (0.1% w/v) increased maximum BA penetration rates 1.5−40-fold (pH 6, 18 and 160 μM) and total penetration 1.5−3-fold (pH 2 and 6, 18 and 160 μM BA). In infinite dose studies, rates of BA penetration (20 μM, pH 6.0) were increased ∼2-fold by TX-100. Surfactant effects were greatest when the surfactant was presented to the outer morphological surface of the cuticle (both native and dewaxed) regardless of whether TX-100 was penetrating in the same or opposite direction to BA. Sorption/desorption of BA was not affected by TX-100.

Keywords: *Cuticle; cytokinin; diffusion; Triton X-100; sorption; Lycopersicon esculentum*

INTRODUCTION

Surfactants are used in spray solutions to enhance the performance of foliar-applied chemicals. This enhancement has been attributed to (1) increased active ingredient (ai) solubility or dispersion in the spray solution, (2) improved wetting of the leaf surface, (3) modified physicochemical characteristics of the spray solution, and (4) enhanced cuticular penetration. Because the cuticle is the rate-limiting step for foliar uptake, studies of isolated cuticle/penetrant interactions have been the focus of numerous investigations [for reviews see Bukovac and Petracek (1993), Bukovac et al. (1990), and Schönherr and Riederer (1989)].

The effect of an additive on ai penetration of the cuticle may be assessed, in part, by using infinite dose diffusion systems. Recent studies established that a nonionic surfactant, Triton X-100 (TX-100), increased steady-state cuticular permeation of the growth regulator naphthaleneacetic acid (NAA) and that the surfactant appeared to modify permeability of the cuticular waxes (Knoche and Bukovac, 1993a,b; Knoche and Bukovac, 1994). This observation was supported by studies on Triton X-45-enhanced penetration of NAA (Petracek et al., 1993).

In this study, surfactant-enhanced penetration of benzyladenine (BA) through isolated tomato fruit *Lycopersicon esculentum* Mill. cuticle was examined both in the presence and in the absence of epicuticular and cuticular waxes. A finite dose diffusion system (Bukovac and Petracek, 1993) was used to determine the effects of surfactant on BA penetration from simulated spray droplet deposits, whereas the mechanisms of surfactant-enhanced penetration were then examined in infinite dose diffusion (Knoche and Bukovac, 1994) and sorption (Bukovac and Petracek, 1993) systems.

MATERIALS AND METHODS

Cuticle Isolation. Disks (17 mm diameter) were excised from mature tomato (*L. esculentum* Mill. cv. Pik Red) fruit grown without pesticide application. The cuticular membranes (CM) were enzymatically isolated (Orgell, 1955; Yamada et al., 1964), rinsed in deionized water, air-dried, and stored at room temperature. Dewaxed CM (DCM) were prepared by batch extracting CM with solvent (chloroform/methanol 1:1 v/v) 10 times over a 7-day period at 45 °C.

Chemicals. Solutions of [^{14}C]BA [*N*-(phenylmethyl)-1*H*-purin-6-amine, specific activity = 2.04 GBq mmol^{-1}, radiochemical purity 97%] were prepared in 20 mM citrate buffer containing 1 mM NaN_3 to prevent microbial growth. NaN_3 was present in all citrate buffer solutions. For source of BA, see the Acknowledgment. The pH was adjusted to 2.0 and 6.0 using HCl and NaOH, respectively. Calculated ratios of cationic/nonionic BA were 19:1 for pH 2.0 and 1:500 for pH 6.0 as determined by the Henderson−Hasselbalch equation (Segel, 1976) and using 3.3 for the pK_a of BA (Shafer, 1990). Therefore, BA was primarily cationic at pH 2.0 and nonionic at pH 6.0 (Figure 1). Surfactant solutions of 0.1% w/v (∼1.59 mM) TX-100 [α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl)], a commercial polydisperse preparation by Rohm and Haas (Philadelphia, PA; critical micelle concentration, cmc, 0.019% w/v), were prepared in 20 mM citrate buffer.

Experimental Procedure. *Finite Dose Diffusion.* BA penetration from a droplet/deposit was measured using a system previously described (Bukovac and Petracek, 1993; Figure 2A). Isolated CM disks were weighed and checked for leaks by mounting (with vacuum grease) the CM disks in holders (plexiglass washers with 10 mm i.d.), sealing the mounted CM between two glass half-cells of the infinite dose diffusion apparatus (Figure 3A) with vacuum grease, and applying a slight hydrostatic pressure (∼3 cm of water) for 24 h. The mounted CM disks were transferred to finite dose diffusion units and sealed with vacuum grease. The wax-rich,

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S0021-8561(97)01093-5 CCC: $15.00
Published on Web 05/28/1998

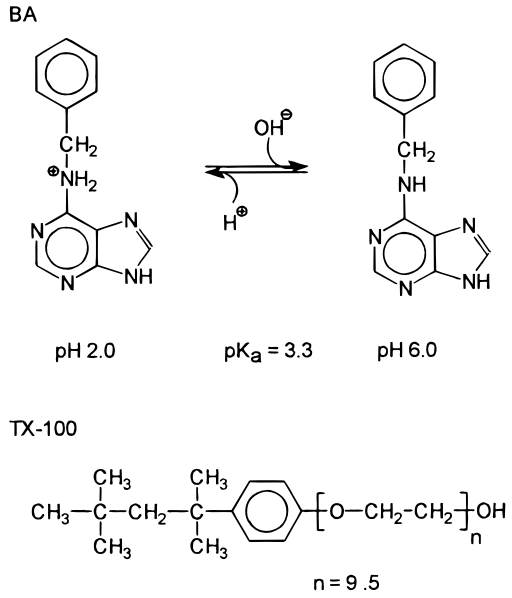

Figure 1. Chemical structures for BA and TX-100. Effect of pH on BA dissociation is from Shafer (1991).

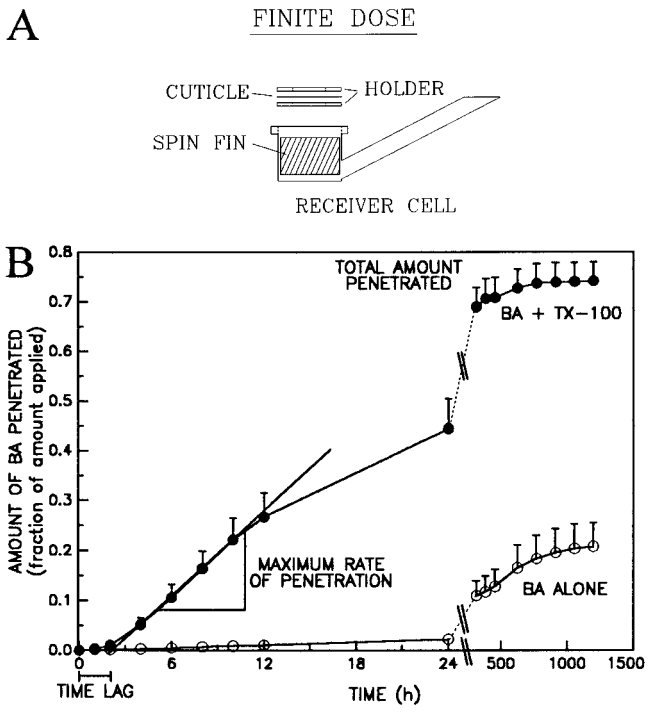

Figure 2. Finite dose diffusion cell (A) and example of the effect of surfactant (TX-100, 0.1% w/v) on cuticular penetration of BA (160 mM in 20 mM citrate, pH 6) from a 3-μL droplet (B). Apparent drying time was ~20 min.

outer morphological surface of the CM was exposed to air, whereas the inner morphological surface (cell wall side) was oriented toward the receiver cells containing 2.9 mL of buffer at pH 6.0.

After the CM were allowed to equilibrate for 24 h, one 3-μL droplet of [^{14}C]BA in buffer was applied on the surface at the center of the exposed CM. Droplets dried in ~20 min. Cells were held at 25.0 ± 1.5 °C, and the receiver solutions were stirred continuously. Receiver cell solutions were sampled (0.5 mL) periodically through a side port and radioassayed by liquid scintillation spectrometry (model 1211 Rackbeta, LKB Wallac, Turku, Finland). The sample volume was replaced with buffer solution. Example time courses are shown in Figure 2B. The effects of BA concentration (18 and 160 μM) and pH (2 and 6)

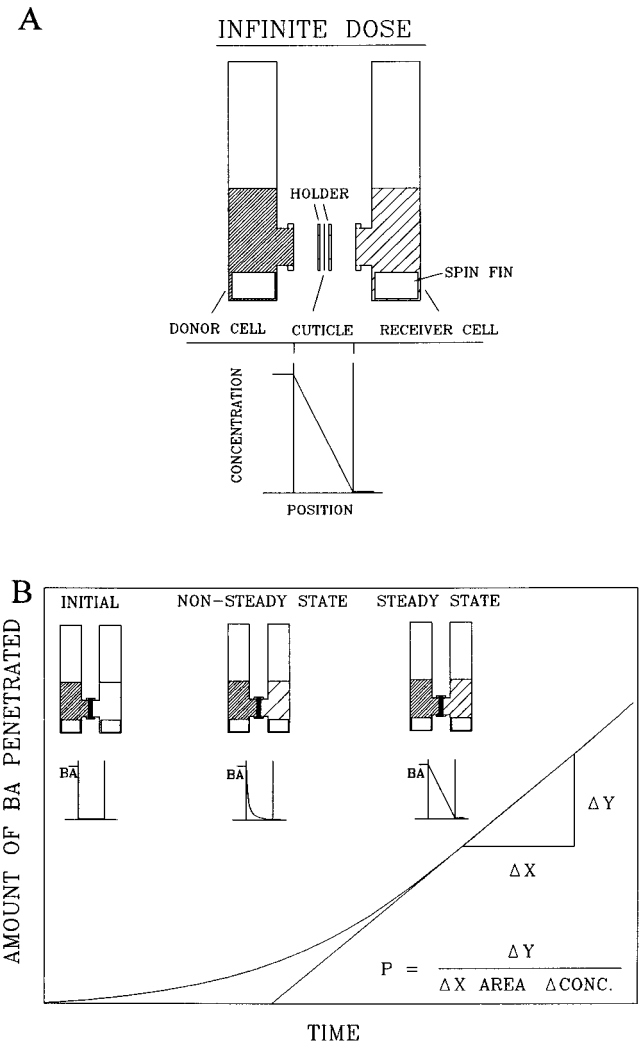

Figure 3. Diagrammatic representation of infinite dose diffusion cell and simplified steady-state concentration profile across the cuticle (A) and time course of BA penetration of isolated cuticle after addition of BA to donor cell with corresponding concentration profiles during non-steady-state and steady-state penetration (B).

of the droplet solution and TX-100 (0.1% w/v) were investigated using this system.

Infinite Dose Diffusion. The infinite dose diffusion apparatus (Figure 3A) has been previously described (Kerler et al., 1984). Briefly, CM and DCM were mounted in the diffusion cells and checked for leaks as previously described. Diffusion cells were emptied, and then 5 mL of buffer solution (pH 6.0) was added to each receiver cell and 5 mL of [^{14}C]BA-containing donor solution (20 μM BA, pH 6.0) was added to each donor cell. Receiver cell solutions were sampled (0.5 mL) periodically, radioassayed by liquid scintillation spectrometry, and refilled with buffer solution until steady-state penetration (constant penetration rate, Figure 3B) was established. Diffusion units were held at 25.0 ± 0.5 °C in a thermostated water bath, and solutions were stirred continuously. Donor cell solutions were sampled (0.1 mL) after steady-state penetration of BA was attained to determine the concentration gradient across the cuticles.

Surfactant effects on BA penetration were determined by adding sufficient TX-100 to either donor or receiver cell to attain 0.1% w/v surfactant. Equal volumes were maintained in both cells. Receiver cell solutions were again sampled periodically, and sample volume was replaced with the appropriate buffer solutions until steady-state penetration was re-established (Figure 4). An additional determination of surfactant effect on BA penetration was made by adding TX-

100 to the cell of each diffusion unit that did not previously receive surfactant. The second addition of TX-100, sampling, and radioassaying were performed as previously described.

Steady-state permeance (P) was defined as the amount of BA penetrated (ΔY) per unit time (ΔX), cross-sectional area (A), and concentration gradient (ΔC) when flow rate was constant (Figure 3B). The effect of TX-100 on penetration was described by the ratio of permeance after the first TX-100 addition (P_a) to the permeance before TX-100 addition (P_b) or P_a:P_b (Figure 4) (Geyer and Schönherr, 1988). The effect of TX-100 addition to the previously non-surfactant-containing diffusion cell on penetration was described by the ratio of permeance after the second TX-100 addition ($P_{a'}$) to P_b or $P_{a'}$:P_b (Figure 4).

Sorption and Desorption. Sorption and desorption studies were performed as described previously (Bukovac and Petracek, 1993). Briefly, cuticles (~5 mg) were weighed in 15 × 45 mm (1 dram) glass vials. Buffer solution was added to hydrate the cuticles and then removed by Pasteur pipet and replaced with 1.5 mL of sorption solution containing [^{14}C]BA (20 μM, pH 6.0, 20 mM buffer) with or without 0.1% w/v TX-100. Control vials (vials without cuticles) were used to correct for any BA sorbed to the vials. Studies were conducted in a water bath held at 22.5 ± 0.5 °C. Samples (0.1 mL) were taken at 10, 20, and 30 min and 24, 500, and 1000 h and radioassayed. After sorption equilibrium was established, desorption was initiated. In these assays, BA sorbed to the CM was desorbed into the desorption solution containing no BA. Time courses of desorption were established using sampling schedules identical with those for sorption. At desorption equilibrium, the next desorption cycle was initiated by replacing the desorption solutions with fresh desorption solutions, and the sampling schedule was repeated. Buffer solution with or without TX-100 (0.1% w/v) was used as the desorption solution. For these studies the following three treatments were used:

sorption	desorption
(1) BA/buffer	buffer
(2) BA/buffer, 0.1% TX-100	buffer, 0.1% TX-100
(3) BA/buffer	buffer, 0.1% TX-100

Rates of sorption and desorption were expressed as the time required to reach half of the equilibrium BA concentration in the cuticle (Bukovac and Petracek, 1993). The extent of sorption and desorption was expressed as a partition coefficient (K) or the ratio of equilibrium concentration in cuticle (mmol of BA/g of cuticle) per equilibrium concentration in solution (mmol of BA/mL of solution).

Statistical Analysis. *Finite Dose Diffusion.* The effects of TX-100, pH, and BA concentration of the droplet solution on the time lag, maximum rate of penetration, and total amount penetrated were examined by a three-way factorial experiment with a randomized complete block design. Factorial analysis was performed by analysis of variance. Multiple regression analyses were performed to determine correlations among parameters. Six replications were used per treatment.

Infinite Dose Diffusion. The effects of cuticular waxes, cell to which TX-100 was first added, CM orientation to BA donor solution, and pH on BA permeance before and after TX-100 addition were examined by three- and four-way factorial experiments with randomized complete block designs. Factorial analysis was performed by analysis of variance. Six replications were used per treatment.

Sorption and Desorption. Treatment means were compared using Duncan's new multiple-range test (P = 0.01). Five replications were used per treatment.

RESULTS AND DISCUSSION

Finite Dose Diffusion. The time course of cuticular penetration of BA from a droplet/deposit was characterized by three phases: (1) lag, (2) quasi-linear, and (3) plateau. The lag phase corresponded to the time required for detectable levels of BA to diffuse through the cuticle from the donor to the receiver cell. The duration of the lag phase was estimated by the time lag, a quantity determined by extrapolating a line fitted through a minimum of four data points in the quasi-linear phase back to the x-axis (Figure 2B). Time lags for BA penetration ranged from 1.4 to 57.3 h (Table 1). Time lag and cuticle thickness (estimated by cuticle weight and specific gravity of 1.21 g/cm^3; Petracek, 1991) were positively correlated for replications within the treatment and coefficients of determination ranged between 0.05 and 0.30. Time lags were greater for the cationic species of BA (pH 2.0) than for the nonionic species (pH 6.0), and the pH effect increased with concentration (Table 1). The presence of TX-100 did not affect time lag.

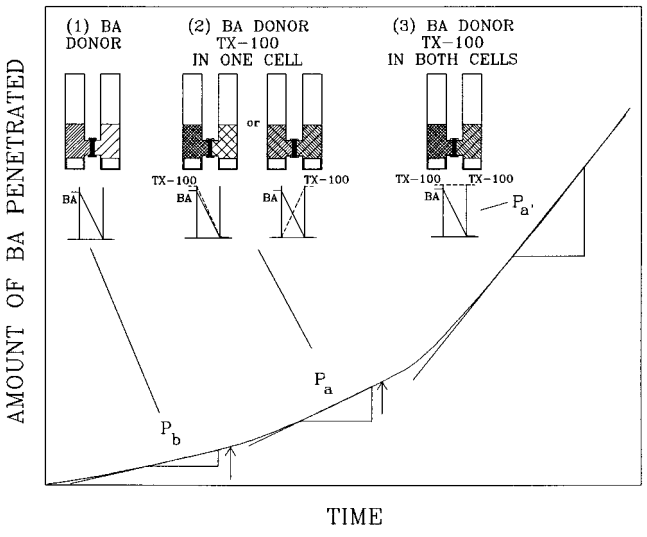

Figure 4. Illustration of penetration of BA before and after TX-100 addition to BA donor cell, receiver cell, or both (left cell is donor and right cell is receiver in all cases): (1) BA added to donor cell; (2) TX-100 added to donor cell or receiver cell; (3) TX-100 added to donor or receiver cell not previously containing surfactant. BA and TX-100 concentrations are represented in the diffusion cell diagrams by +45° and −45° diagonal cross-hatching, respectively. Relative BA and TX-100 concentrations are represented in concentration profiles by solid and dashed lines, respectively. Surfactant-enhanced permeance (P) is signified by an increased ratio of steady-state permeance after surfactant addition to one (P_a) or both ($P_{a'}$) diffusion cells to steady-state permeance before addition of surfactant (P_b). Arrows represent time of surfactant addition.

Table 1. Effect of TX-100 on BA Penetration of Isolated Tomato Fruit Cuticles from a Finite Dose Deposit

treatment		parameter		
deposit[a]	pH	time lag,[b] h	max rate of penetration,[c] % of applied/h	total amt penetrated,[d] % of applied
18 μM BA				
BA alone	2.0	10.1	0.04	23
BA + TX-100	2.0	8.8	0.21	41
BA alone	6.0	2.2	0.72	38
BA + TX-100	6.0	2.0	1.14	58
160 μM BA				
BA alone	2.0	57.3	0.04	18
BA + TX-100	2.0	32.5	0.06	43
BA alone	6.0	1.4	0.07	21
BA + TX-100	6.0	2.4	2.85	62

[a] BA in 20 mM citrate buffer (3 μL droplet) with and without 0.1% w/v TX-100 (25 ± 1.5 °C, n = 6). [b] Significant interaction between BA concentration and pH (P = 0.01). [c] Significant interaction among BA concentration, surfactant, and pH (P = 0.01). [d] Significant main effect of surfactant (P = 0.01).

The quasi-linear phase corresponded to the period of near constant penetration rate. The maximum rate of penetration was estimated during this phase by a line fitted through a minimum of four data points ($r^2 \geq 0.91$; Figure 2B). The maximum rate of penetration (range for all treatment means = 0.04−2.85% of BA applied/h) was affected by a three-way interaction among BA concentration, TX-100, and pH (Table 1).

The plateau phase corresponded to the final stages of penetration in which the rate of penetration approached zero and was quantified by the total amount of BA that penetrated the cuticle (Figure 2B). The total amount penetrated (range for all treatment means = 18−62% of BA applied) was significantly increased by the addition of TX-100. Correlation between the maximum rate of penetration and total amount penetrated was significant ($r^2 = 0.37$; $P = 0.032$), whereas correlations between maximum rate of penetration or total amount penetrated and time lag were not ($r^2 < 0.04$).

Several observations on finite dose penetration of BA should be emphasized. First, solution pH affected all three measured parameters regardless of the presence of surfactant and BA concentration. In general, the nonionic species of BA (pH 6.0) penetrated the cuticle with shorter time lags, higher maximum rates, and greater total penetration than the cationic species (pH 2.0). The effect of pH on penetration may be related to the concentration of nonionic species. Previous studies have shown that sorption (Shafer and Bukovac, 1989) and infinite dose penetration (Bukovac et al., 1971; Schönherr, 1976; Riederer and Schönherr, 1984) of the predominantly hydrophobic cuticle by charged species are much less than those by the noncharged molecule. This study shows a similar pH effect for finite dose penetration, which, in contrast to infinite dose and sorption systems, followed penetration from a concentrated, apparently dried down, droplet deposit. During the droplet drying process, numerous changes take place such as (1) precipitation and possibly crystallization of the ai from a concentrated solution, (2) a change in pH, and (3) a decrease in droplet-deposit/leaf interface area. If the pH effect was limited to an effect on dissociation only, driving forces adjusted for differences in concentration of the nondissociated species should have been similar. However, the calculated initial concentration of nonionic BA of pH 6.0 in the droplet was ∼20 times greater than at pH 2.0, suggesting that pH may have other effects.

Second, BA penetration from a deposit continued over a surprisingly long period of time and at a similar rate. Despite the apparent drying of the droplet in ∼20 min, significant quantities of BA did not accumulate in the receiver for several hours and penetration continued in some cases for several weeks. Thus, BA must remain relatively mobile in the deposit for a substantial period. This may be due to the deposit remaining in a sufficiently hydrated state throughout the study. The similar rate of penetration during the quasi-linear phase indicated the presence of a relatively constant driving force.

Third, the addition of TX-100 generally increased the rate and total amount of BA that penetrated. Surfactant-enhanced diffusion from a deposit may result from effects both on the deposit and on the cuticle. One possible role of surfactants in the deposit is that they enhance penetrant mobility within the apparently dry deposit after the aqueous phase evaporates. Since ethoxylated surfactants such as TX-100 may act as humectants (Stevens and Bukovac, 1987), they may sequester sufficient water in the deposit to improve mobility of the penetrant in the deposit to the deposit/cuticle interface and thus maintain the driving force for cuticular diffusion.

Infinite Dose Diffusion. The possibility of direct effects of surfactant on the cuticle was examined by infinite dose diffusion studies. BA permeance (P_b) before TX-100 addition was affected by an interaction between pH and the presence of cuticular waxes (Table 2). Permeance was greater at pH 6.0 than at pH 2.0, regardless of the presence of wax. Increasing pH increased permeance 14- and 20-fold in the presence and absence of wax, respectively. These responses closely paralleled the change in nonionic BA concentration for the low and high pH and suggested preferential penetration of the nonionic BA species (see Materials and Methods for calculations). Permeance was 5-fold less in the presence of waxes. Cuticle orientation to BA/buffer only (outer morphological versus cell wall side) did not affect permeance, and therefore cuticular penetration of BA was not asymmetric. Additionally, BA penetration was not affected by receiver solution pH (Petracek et al., 1991). BA time lags (infinite dose diffusion) were greater for CM than for DCM (5.1 versus 3.2 h) and were not significantly affected by solution pH or orientation (data not shown).

In comparison to BA permeance, previous infinite dose studies (Petracek, 1991) indicated that TX-100 permeance of isolated tomato fruit cuticles was less dependent on the presence of waxes (TX-100 permeance = 1.2×10^{-10} and 3.8×10^{-10} m/s; time lag = 7.9 and 5.9 h for CM and DCM, respectively) and not dependent on pH or orientation (Petracek, 1991). Since time lags were similar for TX-100 (7.9 and 5.9 h for CM and DCM, respectively; Petracek, 1991) and nondissociated BA (5.1 and 3.2 for CM and DCM, respectively; see above), the two compounds would be expected to reach steady-state penetration in a similar time period.

TX-100 increased BA penetration of the cuticle (P_a: $P_b > 1$, Table 2) regardless of cuticular orientation (outer morphological or cell wall side exposed to BA donor solution), location of TX-100 addition (addition to donor or receiver cell), pH (2.0 or 6.0), or presence of wax (CM or DCM). Several mechanisms of TX-100-enhanced penetration may be operative. They include (1) surfactant−penetrant copenetration by which surfactant molecules essentially "escort" the penetrant through the cuticle (Holloway et al., 1992), (2) surfactant effect on the wax (Knoche and Bukovac, 1993a), and (3) surfactant effect on the cutin matrix.

Surfactant ai copenetration requires an interaction between surfactant and ai. When surfactant is added to the receiver, surfactant concentration in the donor is negligible compared to when the surfactant is added to the donor cell. Thus, the probability of surfactant−penetrant interaction for the case of surfactant addition to the donor cell is great compared to when the surfactant is added only to the receiver cell. In general, BA in the donor cells (20 μM initial concentration) was ∼900−1800 times greater than in the receiver cells at steady state. Similarly, [^{14}C]TX-100 penetration studies showed that steady-state levels of TX-100 in donor cells (0.1% w/v or 1.6 mM initial concentration) were ∼800−1600 times greater than in receiver cells (Petracek, 1991). Thus, if copenetration of TX-100 with BA was

Table 2. Effect of TX-100 on Infinite Dose Penetration of BA through CM and DCM of Tomato Fruit

treatment			permeance (P)		
cuticle orientation to BA donor solution[a]	initial cell of TX-100 addition	cuticle orientation to initial addition of TX-100	P_b,[b] 10^{-10} m/s	P_a:P_b[c]	$P_{a'}$:P_b[d]
		CM, pH 2.0			
outer surface	donor first	outer surface	5 ± 1	1.46	1.62
outer surface	receiver first	cell wall side	2 ± 1	1.12	1.30
cell wall side	donor first	cell wall side	4 ± 1	1.07	1.98
cell wall side	receiver first	outer surface	3 ± 1	2.93	2.24
		CM, pH 6.0			
outer surface	donor first	outer surface	32 ± 17	2.32	2.66
outer surface	receiver first	cell wall side	59 ± 21	1.22	2.76
cell wall side	donor first	cell wall side	44 ± 12	1.41	4.02
cell wall side	receiver first	outer surface	57 ± 27	2.58	3.45
		DCM, pH 2.0			
outer surface	donor first	outer surface	25 ± 9	2.28	1.85
outer surface	receiver first	cell wall side	7 ± 2	1.58	2.64
cell wall side	donor first	cell wall side	9 ± 2	1.19	2.25
cell wall side	receiver first	outer surface	14 ± 6	2.47	2.01
		DCM, pH 6.0			
outer surface	donor first	outer surface	420 ± 84	1.61	1.77
outer surface	receiver first	cell wall side	180 ± 93	1.62	3.66
cell wall side	donor first	cell wall side	320 ± 79	2.09	4.09
cell wall side	receiver first	outer surface	150 ± 74	5.66	8.79

[a] 20 μM BA in 20 mM citrate buffer with and without 0.1% w/v TX-100 (25.0 ± 0.5 °C, $n = 6 \pm$ SE). [b] BA permeance before TX-100 addition. Significant interaction between pH and the presence of cuticular wax ($P = 0.01$). [c] Ratio of BA permeance after TX-100 addition to permeance before addition. Significant interaction between cuticular orientation to BA donor solution and initial cell of TX-100 addition ($P = 0.01$). [d] Ratio of BA permeance after TX-100 addition to permeance before addition to both donor and receiver cells. Significant main treatment effect of cuticle orientation to BA donor solution ($P = 0.01$).

Table 3. Effect of TX-100 on BA Sorption to and Desorption from CM and DCM of Tomato Fruit

treatment solution[a]		sorption		stepwise desorption 1		stepwise desorption 2	
sorption	desorption	$t_{1/2}$,[b] h	K[c]	$t_{1/2}$,[b] h	K[c]	$t_{1/2}$,[b] h	K[c]
			CM				
BA + buffer	buffer	7.0	506 b	0.10	1071 a	0.08	1764 a
BA + buffer	buffer + TX-100[d]	7.1	502 b	0.11	940 a	0.15	1677 a
BA + buffer + TX-100	buffer + TX-100	6.8	449 a	0.11	958 a	0.09	1522 a
			DCM				
BA + buffer	buffer	7.2	499 ab	0.10	1036 a	0.08	1672 a
BA + buffer	buffer + TX-100	7.0	494 ab	0.10	1074 a	0.14	2284 a
BA + buffer + TX-100	buffer + TX-100	8.2	484 ab	0.10	1020 a	0.08	2123 a

[a] 20 mM citrate buffer, pH 6.0 (22.5 ± 0.5 °C, 20 μM BA). [b] Rate constant, time to half completion. No significant treatment effect. [c] Partition coefficient (cuticle/solution). Means of five replications followed by the same letter are not significantly different by Duncan's new multiple-range test ($P = 0.01$). [d] TX-100, 0.1% w/v.

essential, the probability of TX-100−BA interaction in the donor cell would be much greater when the surfactant was added to the donor cell. Furthermore, from a simplistic model the total amount of surfactant in the cuticle at steady state is not expected to depend on the cell to which the surfactant is added. The middle two concentration−position profiles (illustration 2 in Figure 4) illustrate this point. The total area under the surfactant concentration curve (dashed line) is the same regardless of whether the surfactant is added to the donor or receiver cell. Neither argument supports an advantage of donor cell surfactant addition on BA transport and thus does not support copenetration as the operative mechanism.

The interaction between CM orientation and location of TX-100 was significant ($P = 0.01$). When TX-100 was added to the cell facing the outer morphological surface (cuticular surface with epicuticular wax), surfactant-enhanced penetration was greater than when it was added to the diffusion cell facing the cell wall side. These data confirm those of Knoche and Bukovac (1993a) and support the hypothesis that the surfactant interacts with epicuticular wax. However, since our studies showed a similar effect on BA penetration with DCM, the penetration enhancement by TX-100 must also have had an effect on the cutin matrix. These findings support the hypothesis that the surfactant may act as a plasticizer of the cuticle. Surfactant interaction with the cutin matrix may consist of loosening the polymer backbone of the polyester matrix or side chains. The loosening of the matrix may facilitate the opening up of small pores, increasing polymer elasticity, or decreasing tortuosity and thus allowing the penetrant molecule to more easily pass through the cuticle.

With few exceptions, the second addition of TX-100 (addition to the remaining cell not containing TX-100) enhanced BA permeance beyond that of first addition of TX-100 ($P_{a'}$:P_b > P_a:P_b, Table 2). Concentration−position profile 3 in Figure 4 illustrates that a maximum level of TX-100 is present in the cuticle when TX-100 is added to both donor and receiver cells. Ideally, comparable treatments in which TX-100 was added to donor or receiver cell initially should become equivalent if TX-100 is acting simply as a plasticizer of the cuticle. This was typically the case in this study. The only significant treatment effect was that enhancement was significantly greater when the orientation of the BA donor solution was toward the cell wall side of the cuticle. This

is in contrast to the surfactant effect after the first addition, when BA penetration was significantly greater when the TX-100 was added to the cell facing the outer morphological surface. This contrast may be an artifact or result from the two molecules targeting different sites in the cuticle.

Sorption and Desorption. The rates of sorption and desorption were not affected by the presence of TX-100 (Table 3). However, the extent of BA sorption, as indicated by sorption partition coefficients, is reduced by ~10% in the presence of 0.1% (w/v) TX-100. This depression corroborated a previous observation that TX-100 above its cmc reduced nonionic BA sorption (Shafer et al., 1988).

The surfactant depression of sorption of nonpolar ai by the cuticle has been attributed to competition among solution and cuticle with the surfactant micelles for the ai molecules, thus decreasing the effective ai concentration (Shafer and Bukovac, 1988). By analogy, surfactant micelles should compete with the cuticle for BA molecules and thus increase desorption. However, desorption was not increased by the presence of TX-100 (Table 3). The lack of surfactant effect may be due to the inability of this approach to measure small treatment effects or differences in mechanisms between sorption and desorption.

Cuticular sorption/desorption of and penetration by solutes are diffusion-controlled processes characterized by diffusion and partition coefficients. The effects of an adjuvant on cuticular penetration, in principle, should be exhibited by changes in sorption characteristics (Bukovac and Petracek, 1993). This model is supported by studies showing that cuticular sorption of and penetration by NAA were similarly enhanced by the surfactant TX-45 (Petracek et al., 1993). However, TX-100 had either relatively little or no effect on BA sorption and thus did not predict the substantial enhancement of BA penetration observed in studies on infinite dose diffusion. The differences in response of cuticular sorption and penetration to surfactant incorporation in these studies suggest that sorption and penetration sites may differ. Although cuticular penetration and sorption may undergo similar processes (sorption, diffusion, and desorption), penetration may be effectively targeted to only a relatively small number of sorption sites. Effects of an adjuvant on these specific sites may be profound, whereas the majority of sorption sites may be unaffected. Thus, substantial surfactant effects on penetration may be masked by indiscriminate sorption. Studies on the localized effects of surfactant–cuticle interactions may better define the effect of adjuvants on solute penetration of the cuticle.

Our findings have significant implications. The finite dose system permits critical, detailed studies on factors affecting penetration from simulated spray droplets during droplet drying and from the remaining droplet deposit. TX-100, an extensively used agricultural surfactant, directly enhances penetration of BA from droplet deposits. On the basis of infinite dose studies, this enhancement appears to be related to interaction between surfactant and cuticle rather than copenetration with BA. The surfactant enhancement of BA penetration was not related to sorption/desorption characteristics, and thus partition and diffusion coefficients derived for BA from sorption studies may be of limited value in extrapolating to other cuticles not suitable (e.g., stomatous) for direct transport studies or intact leaf/plant systems.

ACKNOWLEDGMENT

We thank Dr. Warren E. Shafer, Abbott Laboratories, for the generous gift of benzyl [8-^{14}C]adenine.

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Registry No. Supplied by the Author: BA, 1214-39-7; Triton X-100, 9036-19-5.

Received for review December 23, 1997. Revised manuscript received April 14, 1998. Accepted April 15, 1998. This paper is based on research supported by Grants CAN 58-3607-1-118 and CAN 58-5114-7-1002 from the Agricultural Research Service, USDA.

JF971093F