Original Article

Effects of Polyoxyethylene Nonylphenyl Ether and Silicon Surfactants on Penetration of Propanil through Adaxial Epidermis of *Commelina communis*

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Effects of surfactants, polyoxyethylene nonylphenyl ether and the Silwet L-77, on penetration of propanil through *Commelina* adaxial epidermis were investigated in terms of concentration and EO molarity of the surfactants and stomatal condition of the epidermis. The initial penetration rates of propanil varied widely. In 70% of the epidermis they were below 70 pmol/mm²/hr. On microscopical observation, most stomata on the surface remained closed throughout the penetration experiment. There was no significant correlation between the initial penetration rates and the number of stomata. The promotive effect of surfactants was high when the initial penetration rate was low, approaching 1 as the rate increased. There was an exponential relationship between the initial penetration rate (X) and the penetration rate (Y) after surfactant addition. The relationship is expressed as, $Y = e^a \cdot X^b$. Surfactant efficiency to promote penetration was evaluated by two approaches: (i) by substitution and (ii) by plotting coefficients a and b . There was a parabolic relationship between the EO molarity of surfactant and the promotive effect, and surfactant NP(8) was the most effective in enhancing. There was a linear relationship when coefficient b was plotted against coefficient a , and that showed a difference in effectiveness in enhancing penetration among the surfactants. The Silwet L-77 significantly promoted non-pore penetration.

INTRODUCTION

Foliar-applied herbicides exhibit intrinsic activity after the active ingredient has reached the receptor site(s) in plant cells, and a mechanism working on the relationship among penetration membrane, surfactant and penetrant has been progressively studied by Schönherr *et al.*¹⁾ and Holloway *et al.*²⁾ Among many kinds of barriers to obstruct the passing of chemicals through leaf surfaces, the cuticle layer (wax+cutin) seems to be one of the major barriers.^{3,4)} Effects of surfactants on the penetration of chemicals have been studied by using plant cuticle (stomatal and astomatal),^{5,6)} epidermis (cuticle layer+epidermal cells),^{5,7,8)} leaf disks⁹⁾ and whole plant leaves.¹⁰⁾ Silicox & Holloway have pointed out that epidermis is useful for foliar uptake

studies.⁷⁾ We have independently developed a test system for penetration study that uses the adaxial epidermis of *Commelina communis* L. as a penetration membrane in the solution/membrane/solution penetration system, and have shown that penetration enhancement of propanil with surfactants through the epidermis was significant when non-pore penetration was predominant.⁸⁾ The present study was aimed to investigate how propanil penetration was enhanced by addition of polyoxyethylene nonylphenyl ether (NP(n), n : EO molarity) and silicon (Silwet L-77) surfactants through the adaxial epidermis in terms of the number of stomata, the EO molarity and concentration of surfactant, and to evaluate their efficiency by two analytical approaches.

MATERIALS AND METHODS

1. Chemicals

Propanil was synthesized from 3,4-dichloroaniline and propionyl chloride, and purified by column chromatography and recrystallization. The surfactants used were polyoxyethylene nonylphenyl ethers with ethylene oxide molarities of 6, 8, 10, 12 and 14, and Silwet L-77. Cellulose membrane (pore size: 0.24 μm , thickness: 30.5 μm) for dialysis was purchased from Union Carbide Corp.

2. Preparation of Adaxial Epidermis

Commelina communis L. plants were collected from a field. The leaves were washed with tap water and kept overnight in a vessel at room temperature in the dark. The detached leaves were washed again with tap water, and the leaf disks (15 mm in diameter) were punched with a cork borer. The adaxial epidermis was removed from the leaf disks with fine tweezers and placed on a wet filter paper in a Petri dish containing phosphate buffer ($\text{KH}_2\text{PO}_4 + \text{K}_2\text{HPO}_4$, 1/15 M, pH 7.2) and stored in the dark until use.

3. Penetration of Propanil through Epidermis or Dialysis Membrane with or without Surfactant

The technique described in the previous paper⁸⁾ was used with some modifications. Each adaxial epidermis or dialysis membrane was sandwiched with silicon rubber gaskets (1.0 mm in thickness) having a hole (5.5 mm in diameter) in the center. The area of the membrane exposed was 23.8 mm². The mounted epidermis was inserted between two glass cells, and the cells were clamped together. Five milliliters of phosphate buffer ($\text{K}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$, 1/15 M, pH 7.2) was poured into the donor cell and left for 20 min in the dark in order to check the integrity of the epidermis. If the buffer leaked out to the receiver cell, the epidermis was changed to a new one, and only no-leak epidermis was used for penetration study. After removing the buffer, fresh buffer (5 ml) was placed on the receiver cell, and propanil solution (100 ppm) was poured into the donor cell. The meniscus of the receiver cell was constantly adjusted to that of the donor

Table 1 Conditions of stomata on *Commelina* adaxial epidermis.

	Stomatal condition		
	Closed	Open	Closed ratio (%)
Epidermis	133	6	93.7
Stomata	24922	43	99.8

cell to equalize the hydrostatic pressure on the epidermis. Efficient stirring was provided with a stirring magnet in each cell. The apparatus was kept at 25°C in the dark. The receiver solution was collected after 3 hr of penetration, and the propanil content (an initial penetration rate: X) was measured by HPLC. After washing the receiver cell with fresh buffer, new buffer (5 ml) was poured into the cell, and the solution of the donor cell was changed to propanil solution with a surfactant. After an additional 3-hr penetration, the receiver solution was collected. The propanil content in the receiver solution (a penetration rate: Y) was determined by HPLC (HPLC: TOYO SODA HLC 803D, column Cica-MERK Hibar Pre-packed column ODS (RP-18), UV: TOYO SODA UV-8 monitored at 254 nm, solvent: acetonitrile/ H_2O =65/35, flow rate: 1 ml/min). The lower limit of detection was 0.5 ng. The penetration rates are expressed as pmol/mm²/hr. After the penetration test, the number of stomata (open or closed) distributed on the surface of the epidermis exposed to the solutions was counted microscopically at 150 times magnification.

RESULTS AND DISCUSSION

1. Relationship of Initial Penetration and Stomata

The initial penetration rate (X) of propanil without surfactant through the *Commelina* adaxial epidermis varied widely between 3.8 and 279 pmol/mm²/hr (a mean: 52.8 ± 45.0) (Fig. 1). In 70% of the epidermis used the initial penetration rates were below 70 pmol/mm²/hr. On microscopical observation after 6 hr of penetration, 93.7% of the epidermis had no open stomata, and 99.8% of the total number of stomata were closed (Table 1). The penetration rates of propanil through the epidermis remained constant for a long time in

Fig. 1 Frequency distribution of the initial penetration rate of propanil through *Commelina* adaxial epidermis.

Fig. 2 Relationship between the number of stomata and the initial penetration rate of propanil through *Commelina* adaxial epidermis.

this penetration system under dark condition.⁸⁾ These results indicate that most stomata on the surface (23.8 mm²) exposed to solutions were closed during the experiment, and that the character of the adaxial epidermis remained unchanged throughout the experiment.

Figure 2 shows the relationship between the number of stomata and the initial penetration rate. The distribution patterns of stomata on the epidermis were divided into two groups: one with a small number of stomata (less than 100) and the other with a large number (more than 100). The former was from leaves of plants in the shade, and the latter from those exposed to sunlight. The initial penetration rates of both groups, however, showed very similar variations: the rates of the former group ranged 9.9–96 pmol/mm²/hr and those of

the latter 8.9–102 pmol/mm²/hr. There was no correlation between the initial penetration rates and the number of stomata in both groups, and the level of significant difference (LSD) was at 5%. These results indicated that there was no significant relationship between the wide variation in the initial penetration rates and the number of stomata. Since most of the stomata exposed to the chemical solutions were closed (Table 1) and the thickness of the epidermis was between 45 and 55 μ m (unpublished data), variations in the initial penetration rates in this study seemed to be attributable to leaf-surface conditions such as difference in the wax content and undetectable fine cracks.

2. Effect of EO Molarity of NP Surfactants

Comparison in the penetration rates with and without surfactants showed marked contribution of the surfactants to penetration enhancement, but the promotive effects ($E = Y/X$) of the surfactants also varied similarly to the case of the initial penetration rates. As described in the previous paper,⁹⁾ the promotive effect was high when the initial penetration rate was low, being close to 1 when the rate increased. Schönherr *et al.* have reported a similar result: the effect of surfactant on solute mobility was maximum when initial resistance was great.¹⁾ The regression equation between the penetration rate (Y) after surfactant addition and the initial penetration (X) could be expressed as the following linear relationship,⁹⁾

$$\ln Y = a + b \ln X \quad (1)$$

and Eq. (1) can be converted to an exponential Eq. (2).

$$Y = e^a \cdot X^b \quad (2)$$

The relationship between both penetration rates at different EO molarities was very significant (Table 2). Both penetration rates (X and Y), however, varied in each penetration experiment (Fig. 1). It seemed difficult to evaluate surfactants according to penetration enhancement such as an index of promotive effect ($E = Y/X$). We therefore evaluated surfactant efficiency by two approaches: (i) substitution and (ii) plotting coefficients a and b .

Table 2 Estimated coefficients in Eq. (2) $Y=e^a \cdot X^b$ by EO molarity and concentrations of NP surfactant and by concentrations of the Silwet L-77 surfactant on propanil penetration through *Commelina* adaxial epidermis.

Surfactant	Concentration (ppm)	Coefficient		r^2 ^{a)}	n ^{b)}
		a	b		
NP (6)	100	1.96 (0.487)	0.760 (0.0773)	0.740	36
NP (8)	100	3.44 (0.373)	0.384 (0.0756)	0.462	32
NP (10)	100	2.56 (0.444)	0.486 (0.0810)	0.461	44
NP (12)	100	2.21 (0.298)	0.615 (0.0695)	0.697	36
NP (14)	100	0.85 (0.0442)	0.883 (0.0836)	0.852	23
NP (10)	10	2.00 (0.408)	0.621 (0.123)	0.444	34
NP (10)	1000	2.43 (0.297)	0.587 (0.0772)	0.652	33
Silwet L-77	100	3.31 (0.198)	0.509 (0.0526)	0.690	44
Silwet L-77	1000	2.80 (0.534)	0.549 (0.114)	0.326	50
Silwet L-77	5000	2.55 (0.405)	0.508 (0.108)	0.410	36

Standard deviations in parentheses.

^{a)} Regression coefficient.

^{b)} The number of experiments.

(i) Substitution

Many researchers have pointed out that there is very little possibility of pore penetration when herbicides formulated with surfactants with a surface tension above $30 \text{ mN}^{-1} \text{ m}$ are sprayed on leaf surfaces.^{8,4,11,12)} Further, in a penetration system of solution/membrane/solution, solute can penetrate freely through open stomata. Under those conditions, penetration through pores is greater than that through the membrane. Norris & Bukovac have reported that the penetration of naphthaleneacetic acid was approximately 10-fold greater in the isolated stomatal lower cuticle than in the astomatal upper cuticle of pear leaves.¹³⁾ In numerous studies,^{8,18,14)} propanil mainly penetrated through non-pore penetration routes when the initial penetration rate was low, that is, where pore penetration such as through open stomata and large cracks was limited. If the lower initial penetration rate is substituted for X of Eq. (2), the penetration rate (Y) and the promotive effect (E) on non-pore penetration of propanil are obtained, and efficacy of surfactants can consequently be evaluated. From the variations in the initial penetration rates found in this study (Fig. 1), we selected $3.0 \text{ pmol/mm}^2/\text{hr}$ as X on non-pore penetration, and showed the relationship between the promotive effect and EO molarity in

Table 3 Estimated promotive effect derived from a regression equation substituting $3.0 \text{ pmol/mm}^2/\text{hr}$ for the initial penetration rate (X).

Surfactant	Concentration (ppm)	Promotive effect ($E=Y/X$)
NP (6)	100	5.5
NP (8)	100	16
NP (10)	100	7.4
NP (12)	100	6.0
NP (14)	100	2.1
NP (10)	10	4.9
NP (10)	1000	7.2
Silwet L-77	100	16
Silwet L-77	1000	10
Silwet L-77	5000	7.5

a NP(n) series, as shown in Table 3. Surfactant NP(8) showed the highest promotive effect approximately 16 times, followed by NP(10), NP(12) and NP(6), and an addition of NP(14) resulted in the lowest effect. There was a parabolic relationship between the EO molarity and the promotive effect, and this order was also consistent with the results of the pot test using barnyard grass, in which an addition of NP(8) to propanil resulted in the most herbicidal activity, and NP(14) the least.¹⁵⁾

(ii) Plotting coefficients a and b

The coefficients obtained by regression analysis according to Eq. (1) of NP(n) series are summarized in Table 2. Coefficients a and b were linearly related.

$$y = -4.50x + 5.02 \quad (3)$$

(0.737) (0.297)

$$n = 5, r^2 = 0.925$$

Coefficient a ranking of the surfactants was in order of NP(8), NP(10), NP(12), NP(6) and NP(14). This order was consistent with that in the result of analysis by substitution. These results show that surfactants on the left in Fig. 3 were more active in enhancing non-pore penetration. Variations in coefficients a and b , which were intrinsic values derived from the regression analysis, would reflect diverse inter-relationships among physiological properties of epidermis, physicochemical properties of surfactants and a penetrant, but their physical meaning is not yet clear.

3. Effect of Concentration of Surfactant NP(10)

The effect of NP(10) on penetration enhancement was dependent on its concentration. The promotive effects at 100 and 1000 ppm were almost the same, 7.4 and 7.2 times, respectively, and that at 10 ppm was the lowest (Table 3). Plots at 10 and 1000 ppm concentrations were fitted on the regression line Eq. (3) at EO molarities of the NP surfactants (Fig. 3), and a regression analysis combining data both on the concentration and on the EO molarity was carried out. A new relationship was found as follows,

$$y = -4.51x + 5.00 \quad (4)$$

(0.620) (0.251)

Table 4 Initial penetration rate (X), penetration rate (Y) after surfactant addition at 100 ppm and promotive effect (E) through dialysis membrane.

Surfactant	Penetration rate Mean (pmol/mm ² /hr)		Promotive effect E (mean)	$n^{a)}$
	X	Y		
NP (6)	744 (96.6)	778 (107)	1.05 (0.107)	13
NP (10)	784 (88.5)	796 (96.8)	1.01 (0.06)	19
NP (14)	803 (130)	870 (151)	1.09 (0.13)	12
Average	775 (111)	815 (129)	1.05 (0.104)	44

Standard deviations in parentheses.

^{a)} The number of experiments.

Fig. 3 Relationship between coefficient a and b derived from regression analysis of propanil penetration rate through *Commelina* adaxial epidermis with/without NP surfactant.

●: NP(6–14) at 100 ppm concentration, ■: NP (10) at 10 ppm concentration, ▲: NP(10) at 1000 ppm concentration.

$$n = 7, r^2 = 0.914$$

There was no significant difference between Eqs. (3) and (4): the LSD remained at 1% level. This result may indicate that an analytical approach by plotting coefficients is capable of evaluating the efficiency of penetration enhancement at different concentrations of the surfactant NP series.

4. Effect of Surfactant through Dialysis Membrane

In the penetration study using a cellulose dialysis membrane (pore size: 0.24 μ m, thickness: 30.5 μ m), the mean initial penetration rate was greater than that of *Commelina* epidermis penetration, and variations in the rates were relatively narrow (mean: 775 ± 111 pmol/mm²/hr, Max: 999, Min: 527) (Table 4). This result indicates that the initial penetration rate was great when pores were present on

the surface of the membrane. The promotive effects varied between 1.04–1.09, but their values remained almost the same regardless of the EO molarities (Table 4). Moreover, there was no difference in the penetration rate (Y) and the promotive effect (E) due to the EO molarity in the LSD test ($p=0.01$). This result shows that the surfactants did not promote penetration through pores under the solution/membrane/solution system.

5. Effect of Silicon Surfactant on Membrane Penetration

The Silwet L-77 significantly promoted the penetration of propanil at all the concentrations tested, and there were a good relationship between the initial penetration rate ($\ln X$) and the penetration rate ($\ln Y$) (Table 2). According to an analysis by substitution, the promotive effect at 100 ppm was the highest at 16 times, and that at higher concentrations (1000 and 5000 ppm) was 10 and 7.5 times, respectively (Table 3). It has been known that the Silwet L-77 has an ability to induce stomatal infiltration.¹⁶⁻¹⁸⁾ There is a possibility that the Silwet L-77 promotes penetration through undetectable fine cracks on the leaf surface and/or on closed stomata, but the above results have made it clear that it is progressively involved in the non-pore penetration of propanil.

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要 約

プロパニルのツユクサ表皮層透過に及ぼすポリオキシエチレンノエルフェニルエーテル系およびシリコン系界面活性剤の作用

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ツユクサ表皮層を透過膜とする液/膜/液系での界面活性剤の効果を初期透過量 (X) と界面活性剤添加後の透過量 (Y) の変動から解析した。使用した 70% 以上の透過膜の X 値は $70 \text{ pmol/mm}^2/\text{hr}$ 以下で、透過膜の気孔はすべて透過実験中、閉じた状態にあった。 X と気孔数の間には有意的な相関関係は存在せず、本透過系は膜透過系での薬剤透過に対する界面活性剤の効果を判定することができることが示された。 X と Y は指数関係 ($Y=e^a \cdot X^b$) にあり、係数 a と b は直線関係にあった。界面活性剤の添加効果を (1) X 値として $3 \text{ pmol/mm}^2/\text{hr}$ を代入する方法と、(2) 係数 a, b を比較する方法で検討した。(1) 法では、エチレン付加モル数 8 モルが最も効果が大きく、14 モルが低かった。(2) 法でも (1) 法での結果を反映したグラフとなった。これは同系統の界面活性剤の膜透過に対する効果の判定が、この実験系での a, b の比較によりできることを示している。シリコン系活性剤は、これまで言われていた気孔等の開孔部からの透過以外に、膜透過でのプロパニル透過をも積極的に促進することが示唆された。