T.D. Griggs (eds.). Sweet Potato. Asian Veg. Res. & Dev. Center, Tainan, Taiwan.
21. Magnuson, C.E., Y. Fares, J.D. Goeschl, C.E. Nelson, B.R. Strain, C.H. Jaeger, and E.G. Bilpuch. 1982. An integrated tracer kinetics system for studying carbon uptake and allocation in plants using continuously produced $^{11}CO_2$. Radiat. Env. Biophys. 21:51–65.
22. Pharr, D.M., S.C. Huber, and H.N. Sax. 1985. Leaf carbohydrate status and enzymes of translocate synthesis in fruiting and vegetative plants of *Cucumis sativus* L. Plant Physiol. 77:104–108.
23. Rufty, T.W. and S.C. Huber. 1983. Changes in starch formation and activities of sucrose phosphate synthase and cytoplasmic fructose-1,6-bisphosphate in response to source-sink alternations. Plant Physiol. 72:474–480.
24. Tukey, J.A. 1977. Exploratory data analysis, Addison-Wesley, Reading, Mass.
25. Upmeyer, D.J. and H.R. Koller. 1973. Diurnal trends in net photosynthetic rate and carbohydrate levels of soybean leaves. Plant Physiol. 51:871–875.
26. Yoshida, T., Y. Hozyo, and T. Murata. 1970. Studies on the development of tuberous roots in sweet potato (*Ipomoea batatas*, Lam. var. *edulis*, Mak.). The effect of deep placement of mineral nutrients on the tuber yield of sweet potato. Proc. Crop Sci. Soc. Jpn. 39:105–110.

J. Amer. Soc. Hort. Sci. 112(3):554–560. 1987.

Effect of Surfactants on Foliar Penetration of NAA and NAA-induced Ethylene Evolution in Cowpea

N.K. Lownds, J.M. Leon, and M.J. Bukovac
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Additional index words. *Vigna unguiculata*, Pace, Tween 20, Regulaid, absorption

Abstract. **Effects of the surfactants Pace, Regulaid and Tween 20 were determined on foliar penetration of NAA and on NAA-induced ethylene production by cowpea [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* cv. Dixielee]. All three surfactants decreased surface tension of NAA solutions, causing a marked increase in wetting and in droplet : leaf interface area. The greatest increase in NAA penetration was obtained with Regulaid followed by Pace and Tween 20. The surfactant effect was most pronounced during the droplet drying phase, but penetration continued to take place from the deposit after drying. The mode of action of surfactants in enhancing NAA penetration is complex. Regulaid-enhanced penetration closely paralleled the increase in interface area, but similar relationships were not found for Pace or Tween 20, particularly at concentrations above the critical micelle concentration. Surfactant-enhanced NAA penetration caused an increase in NAA-induced ethylene production. There was a strong correlation (r = 0.82) between NAA penetration and ethylene production for doses of 0.5 to 2.5 μg/disk. Above 2.5 μg/disk, ethylene production increased at a decreasing rate. The potential for using auxin-induced ethylene production as an index for quantifying auxin penetration is discussed. Chemical names used: 1-naphthaleneacetic acid (NAA), polyoxyethylene polypropoxypropanol dihydroxypropane (Regulaid), polyoxyethylene (20) sorbitan monolaurate (Tween 20), surfactant blend in paraffin base petroleum oil (Pace).**

Cuticular penetration is a prerequisite for the physiological action of foliar-applied systemic compounds (3, 17, 19). Thus, chemicals often are formulated specifically with surfactants to improve the physical : chemical characteristics of the spray solution to enhance wetting (8, 10, 13) and penetration.

The effects of surfactants on penetration have not been well-defined (18, 20). Surfactants that enhance penetration generally increase wetting as concentration is increased up to the critical micelle concentration (CMC). With a further increase in concentration, there is little or no additional enhancement of wetting or penetration. However, with some surfactants, penetration may be increased at concentrations above the CMC (18, 20, 22). This increase would suggest surfactant effects beyond that commonly attributed to improved wetting (2, 8, 17).

The effects of surfactant concentrations above the CMC on growth regulator performance and on plant processes largely have been ignored. Surfactant concentrations in the range of 0.1% to 1.0% are of particular concern in low-volume spray application where, while maintaining a constant pesticide dose, the concentration of constituents in solution is increased in proportion to the decrease in carrier volume (4). Therefore, when applying formulated agrochemicals, all constituents of the formulation are concentrated, including the surfactant, which may approach 1.0% (15).

NAA induces ethylene production in plants (1, 7, 11, 12), and this response may be useful as a means of quantifying NAA penetration (7). Data are not available on the direct relationship between NAA penetration and ethylene production. Further, the effects of surfactants on NAA-induced ethylene production have not been reported. Thus, the objectives of this study were to determine the effects of selected surfactants over a wide range of concentrations on foliar penetration of NAA and to relate these findings to NAA-induced ethylene evolution.

Materials and Methods

Plant material and culture. Cowpea seeds were pregerminated in the dark at 30°C on moist paper towels. Healthy seeds of uniform size and radicle length were selected, seed coats removed to facilitate epicotyl emergence, and planted into disposable AC-4-8 "Cell Paks" (Geo. J. Ball, W. Chicago, Ill.)

Received for publication 31 Oct. 1986. Michigan State Univ. Agricultural Experiment Station Journal Article no. 12148. These studies were supported in part by the USDA under Agreement no. 3607-20300-004-01 S and by a grant from the Shell Development Co., Modesto, Calif. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

using PROMIX BX (Premier Brands, New Rochelle, N.Y.) as a growing medium. Germination was completed and seedlings were held in a growth chamber at a day/night temperature of 25°/20°. Light was provided for 16 hr daily at 150–200 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (cool-white fluorescent, GE F48T12 CW-1500 supplemented with 15% incandescent). Relative humidity during the light period varied from 45% to 55% and 65% to 75% during the dark period.

NAA and surfactant solutions. NAA was selected as a model compound characteristic of weak organic acid growth regulators. NAA solutions of 50, 100, 250, and 500 $mg \cdot liter^{-1}$ were prepared, the pH adjusted to 6.5–7.0 using KOH, and surfactants were added (v/v) to give the desired concentrations. Three nonionic surfactants, Pace (Union Carbide Agricultural Products, Research Triangle Park, N.C.), Regulaid (Kalo Laboratories, Kansas City, Mo.), and Tween 20 (ICI Americas, Wilmington, Del.) were selected because they were nonphytotoxic, represent chemistries or blends that are agriculturally important spray additives, and are frequently used in formulating agricultural chemicals or added as a tank mix.

Surface tension of the treatment solutions was measured with a surface tensiometer (Fisher Model 20). Leaf wetting was indexed by calculating contact angles formed by 1-µl droplets of the NAA and surfactant solutions on the adaxial surface of 10-day-old cowpea leaves using Mack's formula (14). The contact angles were also used to calculate the relative droplet : leaf interface area (S_θ) using Eq. [1] (11) where: S_θ = interface area, V = droplet volume, and θ = contact angle.

$$S_\theta = \pi \left[\frac{6V}{\pi \left(3 \tan \frac{\theta}{2} + \tan^3 \frac{\theta}{2} \right)} \right]^{2/3} \qquad \text{Eq. [1]}$$

The CMC values for Pace, Regulaid, and Tween 20 are 0.18%, 0.008%, and 0.006%, respectively. The value for Pace was determined experimentally (9) and for Regulaid and Tween 20 from technical data sheets.

General treatment procedure and ethylene measurement. Primary leaves of cowpea, free of defects, were detached from 10-day-old plants, and two disks, centered on the midrib, were excised from each leaf using a sharp cork borer (23 mm in diameter). The disks were floated, abaxial side down, on distilled water in petri dishes held in a constant-temperature (25°C) water bath. Light was provided continuously (cool-white fluorescent) at 115 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

One hour after excision, 10 droplets (1.0 µl each) of NAA solutions were applied (using a microsyringe fitted with an automatic dispenser) to the adaxial surface, avoiding the midrib. Leaf disks were removed at the designated times and positioned, abaxial side outward, in 15 × 85 mm glass vials containing 0.5 ml distilled water. The vials were then flushed with air, sealed with serum stoppers, and incubated at 30°C for 1 or 4 hr. Ethylene was measured in a 1-ml air sample of the headspace by gas chromatography (Varian 1440, Varian Associates, Palo Alto, Calif.) using an activated alumina column fitted to a flame ionization detector. Conditions were: N_2 flow, 15 $ml \cdot min^{-1}$; injection port, column and detector temperature, 130°, 80°, and 150°, respectively.

Effect of leaf age, disk size, and surface. A series of experiments were performed using the previous general procedures to establish conditions of leaf age (4, 6, 8, 10, and 14 days after seedling emergence), leaf disk size (12, 16, and 23 mm in diameter), and surface of treatment on NAA-induction of ethylene production. NAA-induced ethylene production decreased linearly ($r = -0.96^{***}$, $\hat{Y} = 21.4 - 1.6x$) with leaf age, was maximum for leaf disk sizes of 16 and 23 mm, and treatment of the abaxial surface was slightly more effective than treatment of the adaxial surface (data not presented). Based on response to NAA and/or convenience in obtaining and using leaf disks in our assay, the remaining studies were performed by treating the adaxial surface of leaf disks 23 mm in diameter excised from primary leaves of 10-day-old seedlings. The fine structure of this surface is depicted in Fig. 1. The epicuticular wax appears as groups of vertically oriented platelets, some folded over, randomly distributed over the surface.

Surfactant effect on NAA penetration. Leaf disks were treated with varying doses of NAA (0.5 to 5.0 $\mu g/disk^{-1}$), labeled with 1-naphthalene[1-^{14}C]acetic acid (specific activity 592 $MBq \cdot mmol^{-1}$), in the presence or absence of Pace or Tween 20 as described above. Dose was varied by increasing concentration while holding droplet number and volume constant. Surfactant concentration was maintained at 0.1%. After a 12-hr penetration period, the NAA residue was removed by washing the treatment area with a jet of distilled water (≈5 ml). The leaf disks then were blotted dry, placed adaxial side down in 2.5-cm planchets lined with double sticky tape, and dried at 60°C for 24 hr. Radioactivity was measured using a Beckman Low Beta II proportional gas flow counter. Corrections were made for background and data expressed as dpm/disk.

Surfactant effects on NAA-induced ethylene production: Time-course. Leaf disks were treated with [^{14}C]NAA (ten 1-µl droplets, 250 $mg \cdot liter^{-1}$) in the presence or absence of Pace or

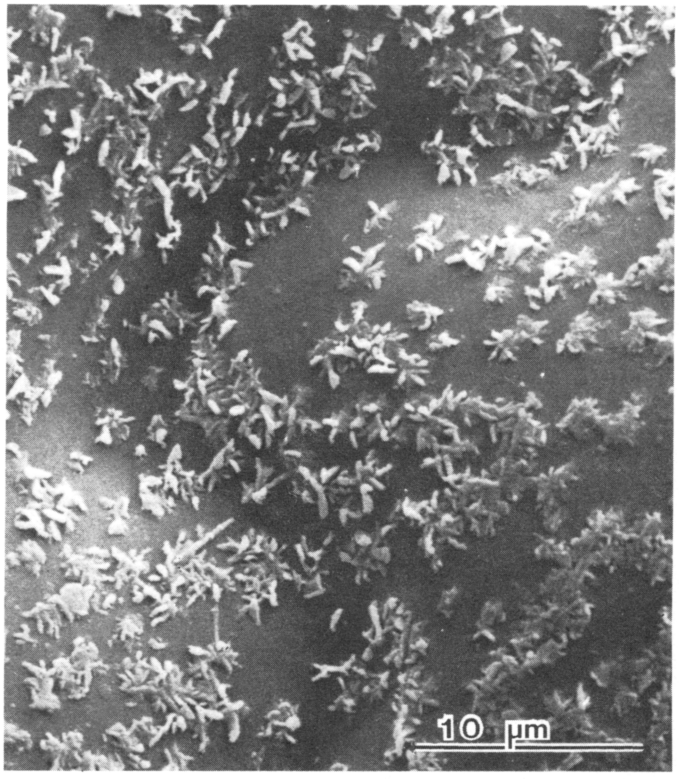

Fig. 1. Scanning electron micrograph of the adaxial surface of a primary leaf of 10-day-old cowpea seedling illustrating the nature and distribution of the epicuticular wax. Leaf tissue was quick-frozen in slush nitrogen, etched at −80°C, coated with gold (100 Å), and viewed on a cold stage.

Tween 20 (0.1%). Disks were removed after 3, 6, 12, and 24 hr for ethylene measurement as previously described. After ethylene determination, the NAA residue was removed and radioactivity determined as described previously. Disks treated with surfactants only (0.1%) served as controls.

NAA concentration response. Cowpea leaf disks were treated with NAA solutions of 50, 100, 250, and 500 mg·liter^{-1} with and without selected surfactants (0.1%) and ethylene evolution measured after 12 hr of penetration. Droplet number and volume were held constant (ten 1-µl droplets).

Surfactant concentration response. Pace, Regulaid, and Tween 20 were incorporated into NAA solutions (250 mg·liter^{-1}) to give final surfactant concentrations of 0.01%, 0.1%, 1.0%, and 10%. Ethylene measurements were made after 12 hr of penetration. Droplet number and volume were held constant as in previous studies.

Experimental design. Completely randomized experimental designs were used with eight observations (two disks per observation) for each treatment. Where appropriate, data were analyzed using linear regression analysis. The significance of coefficients of linear correlation (r) are expressed in the text as *, **, and *** at the 5%, 1%, and 0.1% levels, respectively.

Results

Physical : chemical characteristics. The surface tension of the NAA solution (250 mg·liter^{-1}) was 76.9 mN·m^{-1}. All three surfactants (Pace, Regulaid, and Tween 20) caused a marked reduction in surface tension (Table 1) with an increase in concentration up to the CMC. The most pronounced reduction occurred with the lowest concentration (0.01%) of Regulaid and Tween 20, while increases in surfactant concentration up to 10% resulted in little additional change in surface tension. Pace at 1.0% also reduced surface tension markedly and showed only a slight further decrease at higher concentrations (Table 1).

The decrease in surface tension was reflected in increased wetting, as indexed by contact angles formed by droplets on cowpea leaves. Droplet : leaf interface area was inversely related to surface tension and contact angle and was increased 2- to 3-fold over that of NAA alone (Table 1). While all three physical : chemical characteristics (surface tension, contact angle, and interface area) changed only slightly with concentration above the CMC, contact angle and interface area changed to a greater degree than surface tension (Table 1).

Table 1. Effect of surfactant concentration on selected physical : chemical characteristics of NAA (250 mg·liter^{-1}) solutions and droplets formed from those solutions.

Surfactant	Concn (% v/v)	Surface tension[z] (mN·m^{-1})	Contact angle[y] (°)	Relative area[x]
NAA (250 mg·liter^{-1})		76.9	116.4	1.1
Pace	0.01	75.5	120.0	1.0
	0.1	65.4	117.5	1.1
	1.0	42.6	71.0	2.6
	10	37.3	54.3	3.4
Regulaid	0.01	47.5	88.2	2.0
	0.1	42.3	76.3	2.4
	1.0	41.3	66.1	2.8
	10	40.7	64.5	2.9
Tween 20	0.01	47.9	93.1	1.8
	0.1	45.4	85.5	2.1
	1.0	44.4	83.1	2.2
	10	42.8	76.0	2.4

[z]Determined at 23°C.
[y]Determined on the interveinal area of the adaxial surface of 10-day-old primary cowpea leaves using 1-µl droplets (for surface fine structure, see Fig. 1).
[x]Relative area at the droplet : leaf interface (see Eq. [1]).

Surfactant effects on NAA penetration and NAA-induced ethylene production. There was a highly significant linear ($r \geq 0.87$**) increase in NAA penetration with increasing dose for NAA, NAA + Pace, and NAA + Tween 20 (Fig. 2). Tween 20 and Pace increased the slope of the uptake curve 2- and 3-fold, respectively.

NAA penetration increased linearly from 0 to 12 hr following treatment with NAA alone with little additional penetration between 12 and 24 hr (Fig. 3A). The addition of Pace and Tween 20 markedly increased penetration between 0 and 12 hr, resulting in a 2- to 4-fold increase in penetration compared to NAA (Fig. 3A). With Tween 20, there was evidence of continued NAA penetration from the surface residue through 24 hr, resulting in almost a 2-fold increase over NAA (Fig. 3A). Similar results were obtained for NAA at 25 mg·liter^{-1} (data not presented).

Corresponding ethylene evolution from leaf disks during NAA penetration (Fig. 3A) are presented in Fig. 3B. Significant increases in NAA-induced ethylene evolution occurred about 6 hr after treatment. The rate of ethylene evolution increased linearly (r = 0.99*) between 3 and 12 hr following treatment with NAA, NAA + Pace, and NAA + Tween 20 (Fig. 3B), then remained almost constant between 12 and 24 hr. Pace and Tween 20 increased NAA-induced ethylene evolution rates following 12 hr of penetration 1.5- and 1.2-fold, respectively, and 1.5- and 1.6-fold, respectively, at 24 hr (Fig. 3B). The general shapes of the NAA penetration and ethylene evolution time-course curves were similar (Fig. 3 A and B), except that ethylene evolution was evident about 3 hr after considerable quantities of NAA were absorbed. No significant ethylene evolution was induced with Pace, Regulaid, or Tween 20. Mean ethylene evolution rates for all surfactants (0.01% to 10%) were within 6.5% of the nontreated control. Further, there was no visual evidence of phytotoxicity (data not presented).

Surfactants enhanced NAA-induced ethylene production over a broad concentration (50 to 500 mg·liter^{-1}) range (Fig. 4). All surfactants gave significant (P = 0.01) curvilinear increases in rate of ethylene evolution with increasing NAA concentration.

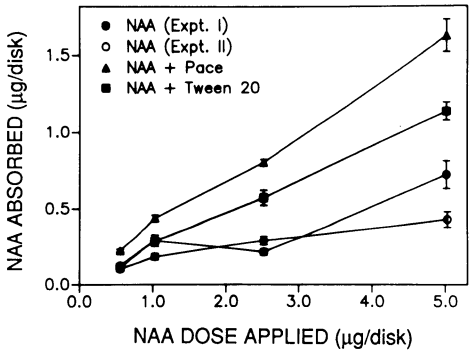

Fig. 2. The relationship between NAA dose applied and penetration into cowpea leaf disks in the absence and presence of Pace and Tween 20 (0.1%). NAA (50, 100, 250, and 500 mg·liter^{-1}) was applied as ten 1-µl droplets to the adaxial surface of cowpea leaf disks. NAA absorbed was determined following a 12-hr penetration period. Vertical bars represent SE.

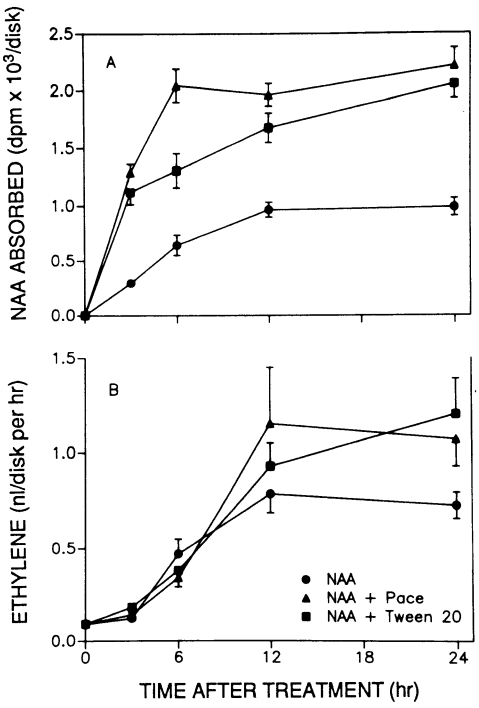

Fig. 3. Time-course of NAA absorption into the adaxial surface of cowpea leaf disks (**A**) and ethylene evolved from the same disks (**B**) in the absence and presence of Pace and Tween 20 (0.1%). NAA (250 mg·liter^{-1}) was applied as ten 1-µl droplets. Vertical bars represent SE.

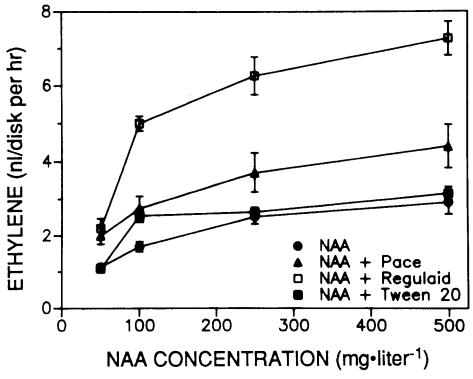

Fig. 4. Effect of concentration on NAA absorption by cowpea leaf disks (as indexed by ethylene evolution) in the absence and presence of Pace, Regulaid, and Tween 20 (0.1%). Ethylene was measured following a 12-hr penetration period. NAA (50, 100, 250, and 500 mg·liter^{-1}) was applied as ten 1-µl droplets to the adaxial surface. Vertical bars denote SE.

Regulaid was most active, Tween 20 the least, and Pace intermediate (Fig. 4). Maximum ethylene evolution rates were 2.9, 3.2, 4.4, and 7.3 nl/disk per hr for NAA, NAA + Tween 20, NAA + Pace, and NAA + Regulaid, respectively.

Increases in surfactant concentration (0.01% to 10%) had a marked effect on NAA-induced ethylene production, but the nature of the response differed for each surfactant (Fig. 5). Regulaid enhanced NAA-induced ethylene evolution about 2-fold at 0.01%, with little further change as the concentration was increased to 10% (Fig. 5). In contrast, ethylene evolution following NAA + Tween 20 treatment differed only slightly from NAA, except at a Tween 20 concentration of 10%. Pace

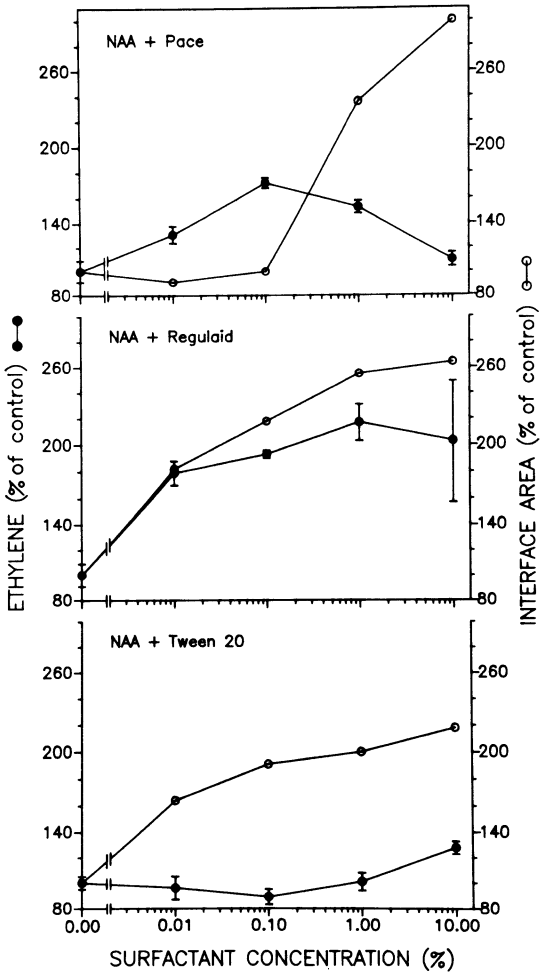

Fig. 5. Effect of surfactant concentration on NAA absorption (as indexed by ethylene evolution) and interface area. NAA (250 mg·liter^{-1}) was applied as NAA alone (control) or with surfactants (0.01%, 0.1%, 1.0%, and 10%) as ten 1-µl droplets to the adaxial surface of cowpea leaf disks. Ethylene evolution was measured following a 12-hr penetration period. Interface area was the calculated area of contact between the droplets and the leaf surface (control = 13 mm^2). Vertical bars represent SE.

induced a maximum response (1.7-fold) at 0.1%, whereas higher concentrations resulted in a log-linear decrease in ethylene evolution.

Relationship between NAA penetration and ethylene production. There was a strong relationship between the rate of ethylene evolution and the amount of NAA taken up by cowpea leaf tissue (Figs. 6 and 7). The slope (m) of the ethylene evolution curve differed between the two experiments (m = 1.01, Fig. 6A; 1.88, Fig. 6B) but remained linear (r = 0.98* and 0.92*). NAA penetration was enhanced by Pace and Tween 20, resulting in corresponding increases in ethylene evolution. However, the increase in rate of ethylene evolution decreased with further increase in NAA absorbed (Fig. 6). Combining data from six separate experiments showed a significant linear relationship (r = 0.82***) between the rate of ethylene evolution and NAA absorbed (Fig. 7). Correlation coefficients for the separate components NAA, NAA + Pace, and NAA + Tween 20 were r = 0.83**, r = 0.86*, and r = 0.78*, respectively.

A further comparison relating rate of ethylene evolution to quantity of NAA absorbed (i.e., nl/disk per hr per µg NAA) shows reduced ethylene evolution with increasing quantities of

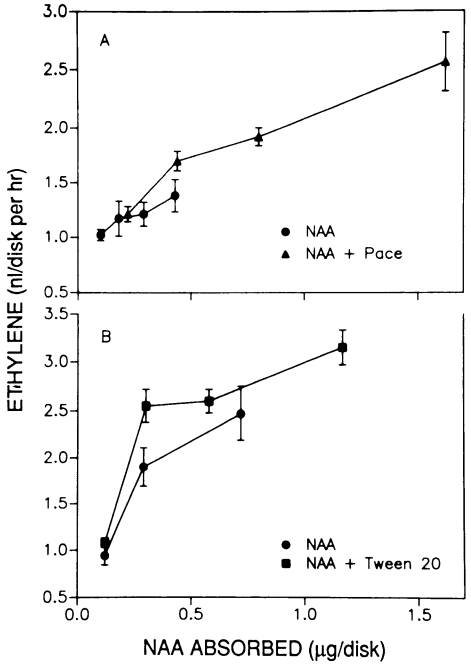

Fig. 6. Effects of Pace (**A**) and Tween 20 (**B**) on the relationship between NAA absorbed and ethylene production by cowpea leaf disks. NAA (250 mg·liter^{-1}) was applied as ten 1-µl droplets in the absence and presence of Pace or Tween 20 (0.1%). Measurements were made following a 12-hr penetration period. Vertical bars represent SE.

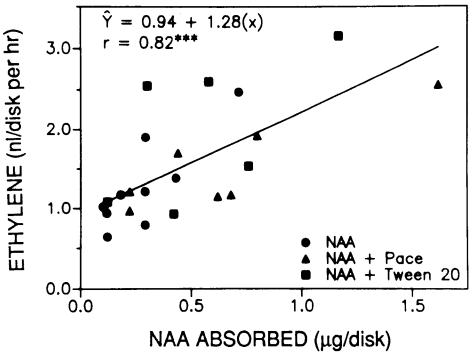

Fig. 7. Relationship between NAA absorption and ethylene production by cowpea leaf disks in the absence and presence of surfactants (0.1%). Data pooled from six separate experiments. Ethylene evolution and NAA absorption were measured following a 12-hr penetration period. ***Regression significant at the 0.1% level.

Table 2. Rate of ethylene evolution per quantity NAA absorbed (nl/disk per hr per µg NAA) from cowpea leaf disks following 12 hr of penetration.

	Ethylene evolution (nl/disk per hr per µg NAA)			
	NAA dose applied (µg/disk)			
Treatment	0.5	1.0	2.5	5.0
NAA	9.07 a[z]	6.53 a	4.25 a	3.36 a
NAA + Pace	5.45 b	3.86 b	2.41 b	1.58 c
NAA + Tween 20	9.31 a	8.53 a	4.51 a	2.70 b

[z]Means within a column followed by the same letter were not significantly different, Tukey's HSD, $P = 5\%$.

NAA absorbed (Table 2). This decrease was linear and significant, being $r = -0.88^*$, -0.91^*, and -0.96^* for NAA, NAA + Pace, and NAA + Tween 20, respectively. The magnitude of the surfactant effect varied with the dose applied. Pace enhanced NAA penetration (Fig. 2) and ethylene evolution (Fig. 4) at each application rate, but the efficiency (ethylene evolution per microgram of NAA absorbed) decreased (Table 2). Tween 20 increased NAA penetration (Fig. 2) and ethylene evolution (Fig. 4) at applications of 2.5 and 5.0 µg/disk, but the efficiency of ethylene evolution decreased at the 5.0 µg/disk level (Table 2).

Discussion

Our studies demonstrate that surfactants can enhance foliar penetration of NAA and that the response is related to the chemical nature of the surfactant (Figs. 2 and 3A). Enhancement of NAA penetration was most pronounced during the droplet drying phase (0 to 3 hr) for both Pace and Tween 20 (Fig. 3A). NAA continued to penetrate from the surface residue, but at a much slower rate, with the magnitude and time-course being dependent on the nature of the surfactant. Thus, the surfactant effect was not limited to the droplet drying phase but also modified penetration from the apparently dry residue on the leaf surface. This effect was most pronounced with Tween 20 and may be related to significant Tween 20 deposits formed on leaf surfaces on droplet drying (5, 6) and the hygroscopicity of the surfactant residue (21).

NAA penetration through the cuticle is believed to occur by diffusion (3, 17). The linear increase with increasing dose supports this view (Fig. 2). Although Pace and Tween 20 enhanced NAA penetration, the curves remained linear, indicating that the nature of the process was not altered. The marked differences in magnitude of enhancement with Pace and Tween 20 point to the importance of surfactant chemistry in the penetration process.

Although surfactant concentration is an important factor in facilitating penetration (20), the basis for its action remains unclear. Enhancement at concentrations below the CMC is most likely related to increased wetting, but the mode of action at high concentrations remains to be established. Differences in the shapes of the NAA-induced ethylene evolution curves for Pace, Regulaid, and Tween 20 (Fig. 5) suggest that the concentration effect is closely related to the chemistry of the surfactant. Such specific interrelationships may provide an explanation for conflicting reports in the literature concerning surfactant effects on foliar penetration (18–20). These data also point to the complex nature of the surfactant-enhanced penetration and the necessity of examining surfactant effects on the components of foliar penetration.

One important component of foliar penetration, readily affected by surfactants, is the droplet : leaf interface area (cross-section) through which penetration (diffusion) can occur. For NAA, a close relationship between droplet : leaf interface area and penetration has been demonstrated (11). Surfactants markedly reduced surface tension of the treatment solution, resulting in increased wetting and thus greater droplet : leaf interface areas (Table 1). If increases in interface area alone account for surfactant action, then surfactant-enhanced NAA penetration should closely parallel changes in interface area. This relationship existed for Regulaid (Fig. 5). For Pace and Tween 20, however, enhancement of NAA penetration did not parallel changes in droplet : leaf interface area, suggesting that other factors played an important role. Therefore, while droplet : leaf

interface area is important in foliar penetration, especially at surfactant concentrations below the CMC, its role at concentrations above the CMC depends on surfactant chemistry and is not, in itself, the general mode of action of surfactant-enhanced penetration.

All three surfactants at 1.0% and 10% significantly increased droplet drying time [NAA = 30 min, NAA + surfactants (1.0%, 10%) = 1–4 hr]. Maintaining the active ingredient in solution on the leaf surface would extend the time during which diffusion could occur and should increase penetration. There was, however, very little change in penetration associated with increased drying time. The potential increase in penetration with a longer drying period may have been offset by continued penetration from the surface residue.

Surfactants, regardless of their chemistry, induced similar qualitative changes in surface tension, contact angle, and droplet : leaf interface area (Table 1) that could have significant impact on penetration. The relationships between changes in surface tension, contact angle, and droplet : leaf interface area were not, however, straightforward. The overriding factor appears to be the nature of the surfactant. This factor raises the possibility of physical : chemical interactions between surfactant and a) the NAA in solution, b) the plant surface (cuticle) (6), and/or c) the NAA + plant surface (5, 6). Similar differences in penetration and activity associated with surfactant chemistry have been reported (18, 20), but, because these systems are so complex, few studies have addressed these interactions in detail.

NAA-induced ethylene evolution has been proposed as an index of auxin penetration into plant tissue and as a means of evaluating spray parameters (7). The utility of this assay for examining surfactant effects on auxin penetration required evaluation because some surfactants are biologically active (16), including inducing ethylene evolution from cowpea leaves (12).

Surfactant concentration studies established that Pace, Regulaid, and Tween 20 did not induce ethylene production in cowpea and could be used for studies on NAA-induced ethylene production. Within dosage levels of 0.5 to 2.5 μg/disk, NAA-induced ethylene evolution, with some exceptions, provided an acceptable index of auxin penetration in the absence and presence of Pace, Regulaid, and Tween 20 (Fig. 4). The shapes of the NAA uptake and ethylene evolution time-course curves were similar, with ethylene evolution lagging NAA penetration by several hours (Fig. 3 A and B). The strong linear correlation between NAA penetration and ethylene evolution from a number of different experiments, both in the absence and presence of surfactants (Fig. 7), provided strong evidence that increased NAA penetration in the presence of surfactants was reflected in an increase in ethylene evolution. However, there was an inverse relationship between NAA penetration and rate of ethylene production per unit of NAA absorbed (Table 2). The identical trends observed in the absence and presence of surfactants may reflect a saturation of the ethylene biosynthesis system. Further, results with Tween 20 show that NAA penetration was enhanced (direct radiotracer measurement, Fig. 2) to a greater extent than ethylene evolution (Fig. 6). The basis of this disparity is not clear; however, Tween 20 may facilitate NAA penetration into the cuticle, but not desorption and, thus, availability to site of action.

Because of these limitations and the recent observation that some surfactants can induce ethylene formation in leaves of several plants (11), care must be exercised in adapting auxin-induced ethylene responses to evaluation of surfactants for enhancement of penetration.

Literature Cited

1. Abeles, F.B. and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39:963–969.
2. Babiker, A.G.T. and H.J. Duncan. 1975. Penetration of leaves by asulam as influenced by adjuvants and humidity. Pest. Sci. 6:655–664.
3. Bukovac, M.J. 1976. Herbicide entry into plants, p. 335–364. In: L.J. Audus (ed.). Herbicides: physiology, biochemistry, ecology. Academic, New York.
4. Bukovac, M.J. 1980. The performance of growth regulators when applied in low-volume sprays. Mich. State Hort. Soc., 110th Annu. Rpt. p. 17–28.
5. Bukovac, M.J., R.E. Whitmoyer, and D.L. Reichard. 1983. Nature and effects of surfactant deposits on leaf surfaces. HortScience 18:618–619. (Abstr.)
6. Bukovac, M.J., D.L. Reichard, and R.E. Whitmoyer. 1986. The spray application process: Central for the efficient use of growth regulators in tree fruits. 5th Int. Symp. on Growth Regulators in Fruit Production. Acta Hort. 179:33–45.
7. Crabtree, G.D. and M.J. Bukovac. 1980. Studies on low-volume application of plant growth substances: I. Ethylene production, induced by 1-naphthylacetic acid, as a means of evaluating spray parameters. Pest. Sci. 11:43–52.
8. Foy, C.L. and L.W. Smith. 1969. The role of surfactants in modifying the activity of herbicidal sprays, p. 55–69. In: Pesticide formulations research. Physical and colloidal chemical aspects. Adv. Chem. Ser. 86, Amer. Chem. Soc., Washington, D.C.
9. Hsaio, L., H.N. Dunning, and P.B. Lorenz. 1956. Critical micelle concentrations of polyoxyethylated non-ionic detergents. J. Phys. Chem. 60:657–660.
10. Johnstone, D.R. 1973. Spreading and retention of agricultural sprays on foliage, p. 343–386. In: W. Van Valkenburg (ed.). Pesticide formulations. Marcel Dekker, New York.
11. Leon, J.M. 1980. Studies on low volume application of plant growth substances. A mathematical model for penetration from droplets and its experimental evaluation. PhD Diss., Mich. State Univ., East Lansing.
12. Lownds, N.K. and M.J. Bukovac. 1983. Surfactant enhanced penetration of growth regulators. Proc. 10th Annu. Plant Growth Regulat. Soc. Amer. p. 42.
13. Luckwill, L.C. and C.P. Lloyd-Jones. 1962. The absorption, translocation and metabolism of 1-naphthaleneacetic acid applied to apple leaves. J. Hort. Sci. 37:190–206.
14. Mack, G.L. 1936. The determination of contact angles from measurements of the dimensions of small bubbles and drops. J. Phys. Chem. 40:159–167.
15. McWhorter, C.G. 1982. The use of adjuvants, p. 10–25. In: R.H. Hodgson (ed.). Adjuvants for herbicides. Weed Sci. Soc. Amer., Champaign, Ill.
16. Parr, J.F. 1982. Toxicology of adjuvants, p. 93–113. In: R.H. Hodgson (ed.). Adjuvants for herbicides. Weed Sci. Soc. Amer., Champaign, Ill.
17. Robertson, M.M. and R.C. Kirkwood. 1969. The mode of action of foliage-applied translocated herbicides with particular reference to the phenoxyacid compounds: I. The mechanism and factors influencing herbicide absorption. Weed Res. 9:224–240.
18. Sands, R. and E.P. Bachelard. 1973. Uptake of picloram by eucalypt leaf discs: I. Effect of surfactants and nature of the leaf surfaces. New Phytol. 72:69–86.
19. Sargent, J.A. 1965. The penetration of growth regulators into leaves. Annu. Rev. Plant Physiol. 16:1–12.
20. Smith, L.W., C.L. Foy, and D.E. Bayer. 1966. Structure-activity relationships of alkyl-phenol ethylene oxide ether non-ionic surfactants and three water soluble herbicides. Weed Res. 6:233–242.
21. Stevens, P.J.G. and M.J. Bukovac. 1987. Studies on octylphen-

oxy surfactants: I. Effects of oxyethylene content on properties of potential relevance to foliar absorption. Pestic. Sci. (In press).
22. Westwood, M.N. and L.P. Bajter. 1958. Factors influencing absorption of dinitro-ortho-cresol and naphthaleneacetic acid by apple leaves. Proc. Amer. Soc. Hort. Sci. 72:35–44.

J. Amer. Soc. Hort. Sci. 112(3):560–565. 1987.

Refreshed Delayed Light Emission and Fluorescence for Detecting Pretreatment Effects on Chilling Injury in Coleus

Judith A. Abbott[1], Donald T. Krizek[2], Peter Semeniuk[3], Harold E. Moline[1], and Roman M. Mirecki[2]

Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

Additional index words. *Coleus blumei*, acclimatization, cold hardiness, temperature, physiological stress, photosynthetic photon flux, abscisic acid

Abstract. **Intact plants of a green-leafed strain of *Coleus blumei* Benth. (PI 354190) were exposed to 5°C for 48 or 72 hr after pretreatment for 48 hr at two levels of photosynthetic photon flux (PPF) (8 or 320 $\mu mol \cdot s^{-1} \cdot m^{-2}$) at two temperatures (13° or 20°). Plants were sprayed with two abscisic acid (ABA) levels (0 or 200 $g \cdot m^{-3}$) either 0 or 48 hr before chilling. Postchilling condition of the plants was assessed by comparing the time courses of refreshed (cyclically excited and measured) delayed light emission (RDLE) and fluorescence (FLU) from dark-equilibrated leaves. Greater suppression of RDLE and FLU indicates greater injury. Plants pretreated at 8 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PPF showed less suppression of RDLE and FLU, contained more chlorophyll, and showed less injury than did plants pretreated at 320 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PPF. Increasing the duration of chilling from 48 to 72 hr reduced the maximum RDLE and FLU slightly. Pretreatment temperatures and ABA concentration had negligible effects on RDLE and FLU levels. The maximum RDLE, the RDLE level at 7.5 sec, the maximum FLU, the FLU at 1.5 sec, and variable FLU were the measurement variables most responsive to individual and combined treatment effects. Maximum RDLE from upper leaf surfaces was the measurement most responsive to the combined effects of all treatments. Chemical name used: [*S*-(*Z*,*E*)]-5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentadienoic acid [abscisic acid (ABA)].**

Coleus and many other tropical and subtropical crops are susceptible to chilling injury at temperatures above freezing. In most chilling-susceptible species, the extent of injury caused by exposure to chilling depends on many factors, including temperature and duration of exposure, genotype (16), light intensity (14, 18), and prior hardening (7). Abscisic acid has been reported to ameliorate chilling injury in some species (18).

Chloroplasts progressively lose their photoreductive capacity (17) in chilling-susceptible plant tissues exposed to chilling stress and eventually chlorophyll content decreases; therefore, measurements of photosynthetic activity and chlorphyll content can provide information about chilling stress response. Measurements of chlorophyll fluorescence (FLU) or delayed light emission (DLE) can be used to estimate nondestructively the photosynthetic activity and chlorophyll content of leaves and other tissues (6, 9, 15, 19–21).

Melcarek and Brown (15) reported that temperature at the time of measurement affected the times of peak emission and the steady-state levels of both refreshed (cyclically excited) delayed light emission (RDLE) and FLU from intact leaves of chilling-sensitive species. Smillie and Nott (20) demonstrated that FLU maxima were sensitive to measurement temperature in chilling-susceptible species. Havaux and Lannoye (8) reported a maximum in the steady-state levels of RDLE near the temperature at which thylakoid membranes undergo a phase transition in chilling-sensitive species. Abbott and co-workers (1–3) exposed vegetables, fruits, leaves, and cotyledons to different temperatures, equilibrated them to ≈23°C in the dark, and then measured RDLE and FLU. They found that, in chilling-susceptible tissues, chilling exposure had caused a quantitative decrease in peak RDLE and, in some species, also caused a qualitative increase in the initial rise of RDLE.

The present study evaluated whether PPF levels, ABA, and temperature treatments before chilling exposure could condition plants against chilling stress and to determine the effects of these pretreatments on postchilling levels of RDLE and fluorescence from leaves. Coleus was chosen as the subject of this study because of its extreme sensitivity to chilling stress (16). RDLE and fluorescence data are presented here; morphological and physiological data have been reported previously (13).

Materials and Methods

Plant material and treatments. A strain of *Coleus blumei* (PI 354190) containing minimal anthocyanin was selected to eliminate potential interference by red pigments with detection of RDLE and FLU. [Chlorophyll emits at wavelengths between 660 and 800 nm and anthocyanins absorb in the range from 450 to 750 nm, depending on the anthocyanin and the pH, so some of the energy from DLE or fluorescence could be absorbed by some anthocyanins (K.H. Norris, personal communication).]

Plant materials and treatments summarized here are described in detail by Krizek et al. (13). Individual plants (experimental

Received for publication 14 Mar. 1986. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Horticultural Crops Quality Laboratory.
[2]Plant Stress Laboratory.
[3]Florist and Nursery Crops Laboratory.