Air Temperature, Humidity, and Leaf Age Affect Penetration of Urea Through Grapefruit Leaf Cuticles

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ABSTRACT. Effects of air temperature, relative humidity (RH), and leaf age on penetration of urea through isolated leaf cuticles of ‘Marsh’ grapefruit (*Citrus ×paradisi* Macfad.) trees on ‘Carrizo’ citrange (*C. sinensis* L. Osbeck × *Poncirus trifoliata* (L.) Raf. rootstock were examined. Intact cuticles were obtained from adaxial surfaces of ‘Marsh’ grapefruit leaves of various ages. A finite dose diffusion system was used to follow movement of ¹⁴C-labeled urea from urea solution droplets across cuticles throughout a 4-day period. Within the first 4 to 6 hours after urea application, the rate of urea penetration increased as temperature increased from 19 to 28 °C, but there was no further increase at 38 °C. Increasing relative humidity increased urea penetration at 28 °C and 38 °C. Cuticle thickness, cuticle weight per area, and the contact angle of urea solution droplets increased as leaves aged. Cuticular permeability to urea decreased as leaf age increased from 3 to 7 weeks, but permeability increased in cuticles from leaves older than 9 weeks. Contact angles decreased with increased urea solution concentration on leaf surfaces that were 6 to 7 weeks old, but solution concentration had no effect on contact angle on cuticles from younger and older leaves. Changing urea solution pH from 8.0 to 4.0 could have an effect on the amount of urea penetrating the cuticle through the loss of urea from breakdown possibly due to hydrolysis. Results from this study define leaf age, environmental conditions, and formulation for maximum uptake of foliar-applied urea.

Urea is a foliar-applied, nitrogenous fertilizer valued for efficiency and low cost. An advantage of foliar application of N is that it reduces soil applications and thereby potentially reduces leaching of nitrate into groundwater (Council for Agricultural Science and Technology, 1985). Foliar applications of urea on *Citrus* L. sp. in Florida are common because of potential benefits beyond elevated leaf N concentration. Recently, Albrigo (1999) offered initial evidence about such effects of urea sprays on fruit yield in *Citrus* trees in Florida. There are reports describing leaf damage in *Citrus* sp., however, these are due to application at high urea concentrations (Bondada et al., 1995). Mechanisms of urea uptake into the leaves and subsequent action of urea are still not fully understood.

The leaf cuticle is the first barrier that urea must pass through on its way into leaves. Cuticles function in protection and waterproofing of all aerial plant organs (Martin and Juniper, 1970). The cuticle is composed of soluble cuticular lipids (SCL), which are embedded into the matrix of lipid polyester cutin (Jeffree, 1996). Detailed study of chloroform-soluble cuticular lipids from sour orange (*C. aurantium* L.) leaves identified fatty acids, primary alcohols, esters, and hydrocarbons as major constituents (Haas and Schönherr, 1979). Layers of cuticular waxes influence the wettability and permeability of *Citrus* leaf cuticles (Haas and

Schönherr, 1979; Jeffree, 1996). Both quantity and chemistry of cuticular waxes change throughout the development of *Citrus* leaves (Freeman et al., 1979).

There have been studies of cuticular penetration and foliar uptake of plant growth regulators such as auxins (Black et al., 1995; Greene and Bukovac, 1971; Norris and Bukovac, 1969; Shafer et al., 1988), gibberellins (Knoche and Bukovac, 1999; Knoche et al., 1992), and cytokinins (Petracek et al., 1998). Effects of foliar application of urea on grasses (Bowman and Paul, 1992; Wesely et al., 1987) and fruit trees have been studied (Ali and Lovatt, 1992; Rabe, 1994) but mechanisms of urea uptake and effects of environmental variables on penetration rates were not described. Total uptake of urea through abaxial and adaxial surfaces of *Citrus* leaves was similar 24 to 30 h after application (Impey and Jones, 1960). Lea-Cox and Syvertsen (1995) reported that the uptake of urea into the leaves of ‘Redblush’ grapefruit (*Citrus ×paradisi* Macfad.) was dependent upon the total N content of the shoot. Studies of cuticular penetration of urea were performed in some species (Knoche et al., 1994; Yamada et al., 1965) but none of them dealt with the unique waxy cuticles of *Citrus*.

Therefore, our goal was to determine how different temperature, relative humidity (RH), and leaf age affect urea penetration through *Citrus* cuticles and to define a set of conditions under which penetration of urea would be maximized.

Materials and Methods

PLANT MATERIAL. Leaves of different ages were harvested from ten 15-year-old ‘Marsh’ grapefruit (*Citrus ×paradisi*) trees on ‘Carrizo’ citrange (*C. sinensis* × *Poncirus trifoliata*) rootstock. Trees were part of an unsprayed orchard at the University of

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Florida, Citrus Research and Education Center, Lake Alfred. Harvested leaves were the product of the late summer flush (September to October 1998) and early spring flush (February to March 1999) and were all from the south-facing portion of the canopy at a height of 1 to 2 m. Although harvested leaves came from 10 different trees, we did not test inter-tree variability.

CUTICLE ISOLATION. Circular disks (15 mm in diameter) without major veins were removed with a cork borer from leaves. Disks were soaked in an digestion solution of Na-citrate buffer (50 mM) adjusted to pH 4.0 to which 4% (w/v) of pectinase and 0.4% (w/v) of cellulase were added (Yamada et al., 1964). To prevent the growth of microorganisms, 1 mmol·L⁻¹ of sodium azide was added to the solution.

Incubation of leaf disks in the digestion solution lasted for 4 d at room temperature (23 °C). Throughout incubation, disks were exposed intermittently to a vacuum to facilitate infiltration of enzymes into the leaf tissue. After digestion of internal leaf tissue, cuticles were gently washed away from the internal tissue. Cellular debris remaining on cuticles was digested a second time in fresh enzymatic solution overnight and gently rinsed for 2 to 4 h with running distilled water. After rinsing, cuticles were extracted from the water on a piece of teflon and allowed to air dry. Adaxial cuticles were selected under the microscope for lack of stomata. Cuticles were tested for leaks by using a slight hydrostatic pressure (Petraček et al., 1998).

MEASUREMENT OF CUTICULAR PENETRATION. Cuticular penetration of urea was measured using a finite dose diffusion system (Petraček et al., 1998) that allowed repeated sampling of solution in contact with the cuticle. Radioactively labeled ¹⁴C-urea (specific activity = 2.05×10^9 Bq·mmol⁻¹, purity ≥98%, Sigma, St. Louis, Mo.), at 2.8 mmol·L⁻¹, was deposited onto the outer surface of a cuticle in three 1-μL droplets. This represented the activity of ≈60 × 10⁶ Bq. Following urea deposition, 100 μL subsamples were taken from the reservoir below the cuticle in specific time increments, added to the 4 mL of scintillation liquid (SX 18-4, Fisher Scientific, St. Louis, Mo.) and counted in the liquid scintillation counter (LS 6000SC, Beckman Coulter Inc., Fullerton, Calif.). The sample

volume was replaced with water. Ambient temperature and RH within a growth room were controlled using a combination of a refrigerated air conditioner, electric heater, dehumidifier, and vaporizer. Dataloggers (HOBO, Onset Computer Corp., Bourne, Mass.) were used to record air temperature and RH within the growth room. Temperature set points were: 19 ± 1.5 °C; 28 ± 2 °C; and 38 ± 2 °C. For the purposes of different experiments, RH was maintained in the ranges of: 5% to 20%, 20% to 35%, 35% to 50%, and 50% to 75%. Lowest range of humidity included levels (20% RH) that are not uncommon during dry spring months (March) in Florida. At the end of all experiments described above, cuticles were dried for 10 min, removed from the cuticle holder and radio-assayed for the presence of urea.

MEASUREMENT OF CONTACT ANGLES. Individual dried cuticles of different ages were positioned on a teflon slide by using vacuum grease, which was applied to the edges of the cuticles. Two to three 2-μL droplets of test solutions of zero (control) to 2.8 mol·L⁻¹ of urea were deposited onto the cuticle, and contact angles were measured with a Contact Angle Goniometer (Ramehart, Inc., Mountain Lakes, N.J.). Contact angles on two sides of each droplet were measured. Number of replications for each data point in these experiments was 16 to 25. When contact angles were measured on a teflon slide, droplets were deposited directly onto it.

MEASUREMENT OF UREA BREAKDOWN AT DIFFERENT pH. Na-citrate buffer solution (50 mM) was adjusted (with NaOH) to provide a range of pH from 4.0 to 8.0. Four milliliters of each solution of different acidity were put into open glass vials and placed in the fume hood. Into each vial, 3 μL of ¹⁴C labeled urea (activity ≈60 × 10⁶ Bq) was added and samples taken from the vial in specific time intervals over 144 h. The amount of radioactivity left in the vials was calculated from the number of counts measured by the scintillation counter for each sample. These experiments were performed at 23 ± 2 °C and 20% to 35% RH.

ELECTRON MICROSCOPY. For **transmission electron microscopy (TEM)**, digested cuticles were fixed in 1% potassium permanganate dissolved in 0.1 M potassium phosphate buffer at pH 7.2 for 1 to 2 h, followed by dehydration in acetone. The samples were

Fig. 1. Effect of temperature on penetration of urea through the (>12 weeks old) isolated 'Marsh' grapefruit leaf cuticles. (Inset: penetration rate within the first 6 h; Y-axis shows percentage of applied urea per hour). Data are means ± SE. Within each sampling time, means labeled with the same letter (or no letters) are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Fig. 2. Effect of RH ranges on penetration of urea through isolated 'Marsh' grapefruit leaf cuticles (>12 weeks old). Data are means ± SE. Statistical analyses were done within the 24 h and 96 h sampling times. Means labeled with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

then infiltrated and embedded in Spurr's resin (Spurr, 1969), thin sectioned with an LKB Huxley Ultramicrotome (LKB Produkter, Bromma, Sweden), and stained using uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963). Observations were made and photographed with a Philips 201 (Philips Corp., Eindhoven, The Netherlands) transmission electron microscope.

For **scanning electron microscopy (SEM)**, small (5 mm²) leaf tissue samples were cut from the midlamina area of fully expanded, mature, 2-month-old leaves. These samples received a droplet of urea solution on the adaxial surface. After deposition of urea, leaf samples were either left under the conditions of low humidity (RH = 20% to 35% at 23 ± 2 °C) or very high humidity (RH = 80% to 100% at 23 ± 2 °C) for 24 h. Samples were then attached to microscope metal stubs by using double-sticky copper tape and placed individually in small baskets. These baskets were plunged into liquid N and samples were freeze-dried using a modified version of the method described by Katoh and Matsumoto (1980), omitting the acetonitrile step. After drying, the leaf samples were remounted on different stubs, coated lightly with gold/palladium (Katoh and Matsumoto, 1980), and examined with a Hitachi S530 (Hitachi Ltd., Tokyo, Japan) scanning electron microscope.

DATA ANALYSES. All data presented are means of at least 10 replications (15 to 26 replications for contact angle measurements) ± SE. Each experiment was conducted at least twice, and the data

were pooled for analyses. Data were analyzed using analysis of variance procedures for a completely randomized design (SAS Institute, Cary, N.C.) with environmental conditions and leaf age as independent variables. Significant differences between treatment means were determined using Duncan's multiple range test at $P < 0.05$.

Results

Under almost all experimental conditions, penetration of urea was complete within the first 12 to 24 h after application and only insignificant amounts of urea diffused through the cuticles during the subsequent 3.5 d (Figs. 1 and 2). Increasing temperature from 19 to 28 °C resulted in an increase of total amount of urea which diffused through the cuticles within the period between the first 1 h and 24 h (Fig. 1). An increase in temperature from 28 to 38 °C did not increase the amount of urea penetrating through the cuticle. When compared to 19 °C, the amount of urea passing through the cuticle at 38 °C was higher in the period between 1 h and 12 h following application. Total amount of urea that diffused through the cuticles at 28 °C reached a maximum of about 50% 24 h after application. The time required for the amount of urea penetrated through the cuticles to reach the same level was at least twice as long at 19 °C (48 h) and about four times longer at 38 °C (96 h). At 19 °C, penetration rate decreased after the initial 20 min

Fig. 3. Scanning electron micrographs of the surface of 5- to 6-week-old 'Marsh' grapefruit leaves 24 h after (A) no urea application and kept at low RH (20% to 35%), (B) application of droplets of urea at low RH (20% to 35%), and (C) application of droplets of urea at high RH (80% to 100%).

increase and reached the level of $\approx 1\%/h$ 6 h after urea application. Penetration rate at 28 °C and 38 °C increased sharply for the first 20 to 40 min to a level of $\approx 12\%/h$ and then decreased slowly thereafter (Fig. 1 inset). Enhancement of penetration rate as a result of temperature increase from 19 to 28 °C continued for the first 4 h.

Cuticular penetration of urea was enhanced with an increase in RH at both 28 and 38 °C. At 28 °C, urea penetration was 50% greater for 35% to 50% RH than for 20% to 35% RH by 24 h and continued until 96 h after application (Fig. 2). Increasing RH from 5% to 20% to 20% to 35% at 38 °C yielded a similar although not statistically significant increase in urea penetration.

There were no signs of urea deposits on control leaf samples (Fig. 3A). As water evaporated, urea left a microscopic layer of a solid deposit throughout the area where a droplet was applied on the leaf surface (Fig. 3B and C). At 20% to 35% RH the droplet dried rapidly, and the deposit was thin. The resulting film of urea became detached and curled away in many places (Fig. 3B). Extended curling of detached pieces of urea residue resulted in irregularly shaped clumps (Fig. 3B). High RH (80% to 100%) allowed for the longer persistence of a layer of urea on the leaf surface because the droplet did not dry within 24 h. Under these conditions, the urea layer appeared thicker and well attached to the leaf surface (Fig. 3C). These deposits were undoubtedly urea and not epicuticular waxes since SEM of urea droplets on glass cover slips (not illustrated) appeared similar to deposits in Fig. 3C.

Cuticles from expanding (3 to 4 weeks old) leaves had a permeability to urea similar to cuticles from fully expanded but not yet fully thickened leaves (9 to 10 weeks old) between 12 to 96 h after urea application (Fig. 4). Cuticles from recently fully expanded leaves (6 to 7 weeks old) had permeability to urea lower than oldest (>12 weeks old) cuticles but similar to youngest (3 to 4 weeks old) cuticles throughout the 96 h period after urea application. We found the highest permeability to urea in cuticles from the oldest leaves (>12 weeks old; Fig. 4.)

SEM revealed a reticulate morphology for the young cuticles (Fig. 5A and B). Cuticles from the oldest leaves appeared amorphous (Fig. 5C). There were no apparent structural damage or

alterations that might have been related to the process of cuticle isolation or age. Very young cuticles were the thinnest whereas the old cuticles were the thickest (Fig. 5).

Fig. 5. Transverse sections through the cuticles of field-grown 'Marsh' grapefruit leaves (A) 3 to 4 weeks old, (B) 6 to 7 weeks old, and (C) >12-week-old leaves. Epicuticular wax = ecw.

Fig. 4. Effect of leaf age on total amount of urea penetrating through isolated 'Marsh' grapefruit leaf cuticles within three sampling times. Experiments were run at 28 °C and 35% to 50% RH. Data are means $\pm$ SE. Within sampling time, different letters denote significant differences according to Duncan's multiple range test ($P < 0.05$).

Fig. 6. Effect of urea concentration and leaf age of 'Marsh' grapefruit on contact angles of 2- μ L droplets. Data are means $\pm$ SE. Within a leaf age, means differed over concentrations only for leaves 6 to 7 weeks old according to Duncan's multiple range test ($P < 0.05$).

Contact angles of 2- μ L droplets deposited on cuticles >12 weeks old did not change with urea concentration (Fig. 6). Although angles were $\approx 15^\circ$ smaller on cuticles from youngest leaves than on old cuticles, angles did not change with urea concentration. However, increasing urea to $>0.028 \text{ mol}\cdot\text{L}^{-1}$ decreased the contact angles of droplets applied to cuticles that were 6 to 7 weeks old (Fig. 6).

Urea loss at various pH was measured as the loss of ^{14}C from the solution. Because loss of ^{14}C could have resulted only from volatilization of CO_2 as a product of urea breakdown, we can say that we indirectly measured urea breakdown at different acidities. At pH 4.0 and pH 5.0, urea breakdown approached 100% within 24 h (Fig. 7). Urea breakdown occurred at almost identical kinetics at pH 6.0 and pH 7.0; $\approx 20\%$ of urea was broken down within 1 d and complete breakdown required 2 to 4 d. Breakdown of urea was the slowest at pH 8.0; $\approx 20\%$ broke down within 2 d and up to 75% within 6 d (Fig. 7).

Discussion

Most urea penetrated the cuticle within the first 12 to 24 h after application (Figs. 1 and 2). When penetration of N-benzyladenine through the isolated cuticles of tomato (*Lycopersicon esculentum* Mill.) fruit was tested, diffusion of this compound continued at a slow rate for more than 10 d after application (Bukovac and Petrcek, 1993), and penetration of naphthaleneacetic acid through isolated cuticles of pear (*Pyrus communis* L.) leaves was linear during a period of 16 d (Norris and Bukovac, 1969). Yamada et al. (1965) reported nonlinear penetration of urea when applied at high concentration to tomato fruit cuticles and suggested that urea may affect its own uptake by changing cuticular permeability. However, Knoche et al. (1994) found a near-linear trend of penetration of urea for at least 7 h after the application to tomato fruit cuticles and refuted Yamada's suggestions. The similarities of initial penetration rates at 19°C , 28°C , and 38°C and then up to 2 h at 28°C and 38°C (Fig. 1), did not suggest an ability of urea to alter permeability of grapefruit leaf cuticles.

Foliar uptake and cuticular penetration of applied substances can increase with temperature (Baur and Schönherr, 1995; Black et al., 1995;

Fig. 7. Effect of solution pH on the breakdown of urea as estimated from loss of ^{14}C over a 144 h period. Data are means $\pm$ SE.

Greene and Bukovac, 1971; Knoche et al., 1994; Norris and Bukovac, 1969). We also found higher penetration of urea at 28°C than at 19°C within the first 12 h (Fig. 1). Increase in temperature from 28°C to 38°C did not affect urea penetration. We have three possible explanations for these findings. First, cuticular permeability (P) is a product of a diffusion coefficient (D) and a partitioning coefficient (K): $P = D \times K$.

Diffusion typically increases with temperature, but partitioning can decrease with increasing temperatures due to changes in solubility resulting from a phase transition in the cuticle (Shafer et al., 1988). The phase transition takes place in cuticles at specific temperatures. Eckl and Gruler (1980) reported that at $>37^\circ\text{C}$, parts of *Citrus* cuticles become fluid. They suggested that the phase transition was related to the soluble cuticular lipids which are considered the determining factor for permeability of the cuticle (Haas and Schönherr, 1979).

A second explanation for our findings is related to the measured decrease in apparent drying time of droplets with the increase in temperature. With the decrease in RH, droplet drying time decreased from 25 min at 19°C to 11 min at 38°C . Fast evaporation of water from droplets at 38°C (5% to 20% RH) probably promoted volatilization of ammonia from urea by concentrating urea and increasing the pH in the remaining deposit (Reynolds and Wolf, 1987; Wesely et al., 1987). At 28°C , RH was higher and evaporation was slower, which, together with beneficial deposition of urea (Fig. 3C), likely enhanced penetration. A third explanation relates to the rapidity of urea breakdown at high temperatures. With an increase in temperature, activity of urease is enhanced (Yadav et al., 1987) which could have resulted in less residual urea to diffuse across the cuticle.

After urea droplets are deposited onto the cuticle, penetration through the cuticle and the breakdown of urea begin at the same time. Only 1% to 3% of the initial radioactivity remained associated with the cuticles 4 d after urea application in all the treatments. After accounting for the amount that penetrated, this means that the breakdown and loss of urea after 4 d was between 15% for the most-favorable and 60% for the least-favorable conditions for penetration (Fig. 2). Urea breakdown reported after foliar application to ryegrass turf (*Lolium perenne* L.) and Kentucky Bluegrass (*Poa pratensis* L.) was $\approx 30\%$ and 31% to

35% after 2 and 4 d, respectively (Bowman and Paul, 1992; Wesely et al., 1987). Losses from soil application of urea can be as high as 60% depending on the environment (Reynolds and Wolf, 1987). It is generally believed that the enzyme urease is responsible for breakdown of foliar-applied urea to CO₂ and ammonia (Ferguson et al., 1984). The activity of soil urease is promoted by high RH (Reynolds and Wolf, 1987; Wesely et al., 1987; Yadav et al., 1987). Thus, we should have found higher urea penetration at low RH, because urease activity should have been low. On the contrary, we found that higher amounts of urea penetrated the cuticles under higher humidity (Fig. 2). There are two possible reasons for this.

First, a thick, well-attached layer of urea found on the leaf surface at high RH (Fig. 3C) may allow the microchannels within the cuticle to stay well hydrated and create a steep concentration gradient across the cuticle, thereby providing favorable conditions for urea diffusion. Schönherr and Schmidt (1979) proposed the existence of channels traversing the cuticle. When hydrated, these channels represent a way for water molecules to move across the cuticle, and urea molecules could move with them as well. Under the conditions of low RH, the layer of urea on the leaf surface was very thin and it detached from the leaf surface in many places (Fig. 3B). On a typical dry day during winter in Florida, minimum humidity can fall to <20%.

The second reason for higher urea penetration at high RH may be that the pool of urea deposited onto the cuticle where it could be the substrate for urease, was being depleted by diffusion into the experimental cell. It has been reported that urease activity has a lag phase of 24 h (Reynolds and Wolf, 1987), and within that time most of the urea already penetrated through the cuticles. We have not examined cuticles for the presence of urease activity.

When growers spray urea onto *Citrus* trees, they often mix it with other active compounds and adjuvants, some of which may change the chemistry of the solution mixture. Although we did not measure the direct effect of solution pH on cuticular penetration, we examined the stability of urea in the solutions of different pH with emphasis on acidic conditions. The breakdown of urea in droplets already applied onto leaves would, therefore, indirectly affect urea penetration through the cuticles. At low pH, urea breakdown proceeded very rapidly and was complete within 24 h (Fig. 7). Acidity increases the likelihood of urea degradation to ammonia and carbamate by hydrolysis (Morrison and Boyd, 1974). The fact that the breakdown of urea slowed as pH values approached neutrality, suggests a detrimental effect of acidity on urea (Fig. 7).

Cuticles grow and develop as leaves expand and thickness of the cuticles of grapefruit increased with age (Fig. 5). However, permeability did not exhibit the same trend. Within the first 6 to 7 weeks, permeability of cuticles decreased while the thickness increased (Fig. 4). As the cuticle thickens, more and more lipids are embedded into the developing cutin matrix resulting in decreased permeability of cuticles which are 6 to 7 weeks old compared to those which are 3 to 4 weeks of age (Jeffree, 1996; Fig. 4). Cuticles >12 weeks old were more permeable than those which are 6 to 7 weeks old (Fig. 4). Although all cuticles passed a leak test, we examined the structural integrity of isolated cuticles to exclude the possibility that physical damage might have been responsible for this increase in permeability. There was no apparent damage found on TEM sections of any cuticles. The appearance of grapefruit leaf cuticles was very similar to the leaf and fruit cuticles from other *Citrus* sp. (Fig. 5; Espelie et al., 1980).

There is good evidence for the existence of polar pores in the polymer matrix of *Citrus* cuticles. Their radii were estimated to be ≈ 0.46 nm, which is sufficient to allow the passage of urea molecules with radii of 0.26 nm (Schönherr, 1976). The existence of channels in cuticles depends on fluidity and arrangement of SCL within polymer matrix. Fluid SCL can be positioned in polymer matrix in an unorganized manner (as blobs), and can either cover or plug some, but not all of the pores existing in the polymer matrix (Schönherr and Schmidt, 1979). Channels may also result from a surface parallel arrangement of SCL such that their polar groups face each other and form pores or plane structures in the direction of transport (Schönherr and Schmidt, 1979). This idea would require highly regular organization of long chains of SCL. We suggest that as cuticles become fully developed and start aging they lose fluidity. As a consequence, their SCL become better organized within the polymer matrix and assume more stable positions, thereby allowing the establishment of more microchannels proposed by Schönherr and Schmidt (1979). This in turn, would result in higher permeability to water and urea. Eckl and Gruler (1980) showed that fully developed cuticles assume a rather rigid structure under specific environmental conditions, offering support for our idea.

The spread of a droplet and its contact angle with the cuticle are dependent on the epicuticular wax that develops as the cuticle matures as well as on the polarity of the leaf surface due to the chemical composition of waxes (Martin and Juniper, 1970). Larger contact angles of droplets of water were measured on older cuticles than on the younger cuticles (Fig. 6). The amount of epicuticular waxes increases with the age of *Citrus* leaves and, as a result, wettability decreases (B. Bondada, personal communication). The reason for the unique effect of high concentrations of urea decreasing the size of contact angles of droplets on leaves which are 6 to 7 weeks old is not clear. Freeman et al., (1979) reported that throughout development of Navel orange (*C. sinensis* L.) leaves, the proportion of primary alcohols within the pool of total waxes changes, rendering the cuticle less hydrophobic. When leaves of grapefruit are 6 to 7 weeks old, the chemical composition of their epicuticular waxes may enhance spreading of urea at higher concentration. Despite their possible composition change, cuticles can maintain their low wettability (expressed as a high contact angle in Fig. 6) due to the quantity and pattern of wax distribution. For 5-month-old Navel orange leaves, epicuticular wax coverage can crack leaving the cuticular surface exposed beneath crevices (Freeman et al., 1979). This would allow droplets applied onto leaves to have large contact angles and still get into direct contact with cuticles which may be the reason for the high urea penetration through the cuticles from oldest leaves we used in this study (Fig. 4). Because epicuticular waxes are difficult to remove from cuticles without affecting intracuticular waxes, it is extremely difficult to examine their direct effect on urea penetration.

In contrast to other species where urea uptake is greater through abaxial than adaxial surface of leaves (Cook and Boynton, 1952; Freiberg and Payne, 1957; Klein and Weinbaum, 1985) our data support those of Impey and Jones (1960) who found that absorption of N was similar through abaxial or adaxial surface of *Citrus* leaves 24 to 30 h after urea application. Similar to our results, by 30 h after urea application, Impey and Jones found that similar amounts of urea were absorbed by young and mature leaves (Fig. 4).

Cuticular penetration of urea depends on environmental factors and on characteristics of the cuticle itself. Our results suggest

that urea sprays of *Citrus* sp. trees should be done at 25 to 31 °C and high RH to achieve maximum penetration. Thus, spraying at night when diurnal temperature is low and humidity is high is an appropriate practice to increase urea penetration. *Citrus* sp. trees have mature leaves with relatively high permeability to urea present in the canopy throughout the year. Consequently, penetration of urea should be facilitated regardless of the season. Although newly developed leaves are slightly less permeable to urea, it should be noted that young leaves are more sensitive to urea phytotoxicity than mature leaves (Lea-Cox and Syvertsen, 1995) so rates should not be increased when young flush is present. Urea spray solution should be kept at pH 7.0 to pH 8.0 to prevent urea loss from breakdown during preparation and spraying.

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