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A Study of Foliar Absorption of Urea in Peach and Apple Trees Influenced by Plant and Environmental Factors

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A STUDY OF FOLIAR ABSORPTION OF UREA IN PEACH AND APPLE TREES AS INFLUENCED BY PLANT AND ENVIRONMENTAL FACTORS

by

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Ataollah Yazdaniha, Doctor of Philosophy

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Major Professor: Dr. David R. Walker
Department: Plant Science

Studies were conducted under greenhouse conditions to investigate the relative efficiency of urea absorption by 1-month-old peach and apple leaves. A 4 percent solution of urea containing .1 percent Colloidal X-77 was applied to the leaves in the form of a fine spray. To aid in this procedure, an improved microsprayer with a 1 milliliter capacity was developed. Accuracy of the sprayer was $\pm$ 1 percent.

Under greenhouse conditions, the upper and lower surface of peach and apple leaves absorbed urea. More urea was absorbed through the lower than the upper surface. Peach lower surface absorbed nearly as much as apples after 48 hours. In another experiment using a controlled environmental growth chamber, the effect of temperature, humidity and surfactant (Colloidal X-77) on absorption of 1 percent ^{14}C urea solution by apple and peach leaves were studied. Uptake was again much greater

from the lower surface of the leaves as compared to the upper surface. Low relative humidity (25 percent) reduced absorption substantially. High temperature (24 centigrade) under low humidity (25 percent) decreased absorption. Uptake was increased substantially with the high temperature (24 centigrade) and relative humidity (85 percent). Peach leaves were more sensitive to temperature than apple, in regard to the amount of absorption that occurred. In peach, a 5 to 10 fold decrease in absorption was observed when the temperature was lowered from 24 to 10 centigrade. Surfactant increased absorption through the lower surface within a short period after application but decreased it afterwards. Urea absorption through 45-day-old leaves at 85 percent relative humidity and 24 centigrade indicated that within 48 hours over 90 percent of the urea applied to lower surfaces was absorbed by both species of leaves.

A cuticular permeability experiment indicated that upper cuticles from both species of leaves were permeable to urea. It seemed that permeability of peach cuticle increased with time at the higher temperature. After 48 hours, the amount of urea, which penetrated through the peach cuticle at 24 centigrade, was 2.7 fold as much as at 10 centigrade.

Urea absorption within 1 hour and translocation after 4 hours were observed under favorable conditions (24 centigrade and 85 percent relative humidity). Radioautograms of ^{14}C urea treated apple and peach leaves indicated that the ^{14}C urea and/or its metabolites had been translocated within a large portion of the leaf within 8 hours after application.

Studies were also performed on these species utilizing microradioautography and histochemistry techniques. Microradioautograms prepared from treated leaf sections demonstrated that adsorption and absorption of radioactive urea occurred on the epidermal hairs of apple leaves. Urea entry occurred in both apple and peach leaves as evidenced by high activity of ^{14}C compounds within the leaf tissue. Microscopic observations of freshly sectioned leaves of both apple and peach demonstrated a relatively high amount of pectinaceous substances between the cell walls and especially the bundle sheath and bundle-sheath extension cells. Pectinaceous substances were present more in apple cuticle than in peach cuticle.

(137 pages)

INTRODUCTION

Nutrients have been applied to the foliage of plants for many years. Iron, zinc, copper, boron, manganese, molybdenum, phosphorus, potassium, sulfur, nitrogen, calcium and magnesium have been applied as foliar sprays. Some forms of these nutrients, however, are of limited value commercially because of their burning effects, low absorption rates or physiological effects associated with translocation and assimilation into the plant other than at the site applied. It has been reported that environmental conditions, e.g. temperature, light, relative humidity and water tension affect absorption rates. The absorption rate of some nutrients may vary when applied in conjunction with other nutrients or at different pH's.

Foliar application of urea has been successful with many species of plants. Prior to the last decade, extensive investigations were performed pertaining to foliar sprays of urea on apple trees, especially with the McIntosh variety. Commercial applications have been fairly common with apple trees. Some studies, however, have indicated that urea absorption by peach foliage is rather limited and does not provide a significant nitrogen response. It has been suggested that there may be inefficient utilization of urea by peach leaves as a result of a possible lack of the enzyme urease. This possibility, however, has been studied and the urease activity in some cases was even greater in peach than in apple

leaves. In connection with this, (^{14}C, ^{15}N) labeled urea was reported as being incorporated into the various amino acids in peach, as well as apple leaves, when applied through the petioles of excised leaves. Studies on the foliar absorption of urea, in peach leaves, particularly in comparison with apple leaves have not been done. Material and structural differences of the cuticle and epidermal cell walls of peach and apple leaves may be contributing factors accounting for the difference in foliar absorption.

Many reports have indicated that stomatal differences in plants may not be an important factor in foliar absorption, since internal suberization would prevent water soluble substances from entering freely. Recent studies by German workers, however, have demonstrated that spray materials penetrate into the foliage through ectodesmata in the guard cells and not through the stomatal openings. Conical hairs as well as anticlinal walls of epidermal cells contain a number of ectodesmata, functioning as pathways of entry. Apple leaves contain a large number of epidermal hairs which are relatively wettable; however, peach leaves lack hairs.

Several techniques including microscopic, radioautography and cuticular permeability tests may help in studying some of the problems of foliar absorption. More information on the low absorption rate of urea in peaches as compared with apples may lead one to find blocked pathways which prevent penetration. This investigation, therefore, seemed

important, since foliar sprays of nutrients are becoming more and more popular.

Objectives

1. Compare the absorption rates of urea in apple and peach leaves under similar greenhouse conditions.

2. Determine the effect that temperature, relative humidity and surfactant have on the uptake of ^{14}C urea by apple and peach leaves.

3. Determine urea translocation rates using radioautography of the ^{14}C urea-treated leaves.

4. Make cuticular permeability comparisons under different temperature conditions.

5. Determine the movement patterns of ^{14}C labeled urea in the leaf tissue using a microradioautographic technique.

6. Determine the location and extent of cutin and pectinaceous substances in leaves and search for possible differences in the two species.

The above studies were performed in an attempt to understand more about some of the plant and environmental conditions which may influence differential response to foliar sprays of urea applied to apple and peach leaves.

LITERATURE REVIEW

This review is concerned primarily with literature pertaining to foliar applications of urea on plants in general but with special emphasis on apple and peach trees. Some of the plant and environmental factors affecting foliar absorption and the methods of investigating these problems will be reviewed and discussed. Numerous papers are available for both specific and general information on the subject, though only the main areas concerned with this study are reviewed in this report. For a general review of foliar absorption, the reader is referred to Boynton (1954), Franke (1967), Wittwer (1957) and Wittwer and Teubner (1959).

Foliar Applications of Urea and the Plant Response

Nitrogen fertilization through foliar application was first reported by Hamilton, Palmiter and Anderson (1943). A variety of nitrogen carriers such as urea and sodium and potassium nitrate were used on apple foliage. Urea foliar sprays of 5 pounds per 100 gallons of water induced higher chlorophyll and nitrogen contents in the treated leaves than in the untreated foliage. No apparent leaf injury was observed with urea sprays. Sprays in the early part of the season had a rather short-term effect, resulting in leaf nitrogen becoming low in late summer which was desirous for good fruit color.

Fisher, Boynton and Skodvin (1948) studied the effects of several

foliar and soil-applied urea treatments on the chlorophyll content of leaves and some of the fruit characteristics of McIntosh apples. Either soil or foliar urea treatments increased the chlorophyll content of the leaves but reduced the fruit color. The authors suggested that the yield and fruit quality depends on the number, dosage and timing of the urea spray. Fisher and Cook (1950) reported that three sprays of urea (calyx, first and second cover; a total of 2.4 pounds of urea per tree) increased the yield as much as did 6 pounds of urea applied per tree through the soil. With three spray treatments, the size of the fruits were similar to those which resulted from a soil application of the same poundage. In the following year, those trees which received the three-spray treatment had a reduced bloom but a higher percent of fruit set as compared with the trees treated with the same amounts of urea by soil.

Fisher (1952) suggested the following three principles: (a) apple trees receiving urea sprays yield at least as good as those obtaining their nitrogen from the soil. (b) Within the time period of pre-blossom to the second cover spray, the later sprays had more effect in increasing the nitrogen content. (c) Although the effects of sprays are better or at least as good as the soil applications, they are more temporary.

Rodney (1952) experimented with 1-year-old Richared apple trees to determine the amount of urea absorbed by the foliage. He covered the plant growing medium in order to prevent spray from dripping on the roots; then he determined the nitrogen content of leaves after a period of time. The leaves of sprayed trees showed an increase in nitrogen content as

compared with untreated trees. He observed that both upper (stomata free) and lower surfaces absorbed the solute. From this, he concluded that the spray materials penetrated the upper cuticle.

Cook and Boynton (1952) studied a number of factors which affected the absorption of urea by McIntosh apple foliage under greenhouse conditions. Using a spray and washing technique, they found that the upper surfaces of the leaves absorbed much less than the lower ones. The lower/upper absorption ratio was 10.5 after 2 hours but decreased to 1.7 in 72 hours. Within a pH range of 5.6 to 8.0, it was noticed that the addition of a phosphate buffer to the solution caused a change in absorption. The direct or indirect effect of the buffer is not known. The surfactants Tween 80 and Tween 20 at a .1 percent to .01 percent level generally increased absorption. An increase of temperature from 70 to 90 F decreased urea uptake. The authors interpreted this reduction as being due to the increased vapor pressure gradient between the spray droplets and the atmosphere.

Weinberger, Prince and Havis (1949) were the first to report the application of urea solutions on peach foliage. The experiments were performed at Fort Valley, Georgia, and Beltsville, Maryland. Spray solutions ranging up to 10 pounds of urea per 100 gallons of water were used. Leaf analyses indicated that no significant amounts of nitrogen were absorbed by the leaves. The sprays were made in early to mid spring, and they were repeated three times. Limited tests with 25 and 50 pounds per 100 gallons caused no leaf color changes (greening), but

did cause some leaf injury. Contrary to these findings, Walker (1952, working under Utah conditions, found that two sprays of urea (1 pound and 1 1/2 pounds urea applied at each application per tree) at a concentration of 20 pounds per 100 gallons increased the nitrogen content of Elberta peach fruit flesh and leaves significantly. These trees were fertilized each year, and a nitrogen deficiency was not apparent at the time of spraying.

In Wenatchee, Washington, Bullock, Benson and Tsai (1952) reported that three sprays of 5 pounds per 100 gallons of Nu Green (urea), without a wetting agent, did not increase the percentage of nitrogen in leaves. In another experiment under greenhouse conditions, urea sprays increased the nitrogen levels of the foliage significantly. The authors concluded that peaches were able to absorb urea at 15 pounds per 100 gallons but that they did not receive a nitrogen effect when lower concentrations were applied.

Experiments involving foliar absorption of urea by 1-year-old Elberta peach trees during both the dormant and active seasons were performed by Eckert and Childers (1954). They observed that even with 100 pounds of urea per 100 gallons of water no significant differences in the nitrogen level occurred when the trees were sprayed during their dormant season. Trees sprayed with 10 to 20 pounds of urea per 100 gallons in combination with 6 pounds of sulfur bentonate and 6 pounds of lime had a significantly higher nitrogen level than unsprayed trees. Leaf samples were collected July 12, at which time the trees had received four urea foliar sprays.

It has been the general opinion of research workers that peach trees are unable to utilize urea efficiently; therefore, commercial applications have not been recommended. Studies by Harley et al. as quoted by Dilley (1960) indicated that absorption of urea by peach leaves as measured by a standard washing technique (quantity sprayed minus the quantity recovered equals the amount absorbed) was in some instances higher than in apples. Other experiments by Harley et al. showed that growth responses to foliar sprays of urea were apparent with apple but not with peach seedlings. This paradox was explained by the possibility that peach foliage did not absorb urea, but that it remained on the cuticle as an insoluble compound. Dilley suggested that benzaldehyde which has been reported as present in the cuticle of Prunus armeniaca may also be present in peach cuticle, causing precipitation of urea after it is applied.

Walker (1955) and Walker and Fisher (1955) studied the effects of urea sprays on three sour cherry orchards in Western New York. Data obtained from three year's work suggested that the nitrogen treatment did not increase the foliar content of nitrogen enough to be statistically significant, but the sprays tended to increase terminal growth and fruit size and decrease the soluble solids content of the fruit. They reported that a biuret impurity in urea was associated with injury on the foliage.

Another plant which efficiently absorbs and translocates urea and its metabolites is tobacco. Volk and McAuliffe (1954), using ^{15}N labeled urea sprays, observed that within 24 hours all nitrogen that was applied

had been absorbed. Within a 6-hour period, ^{15}N nitrogen was detected in every part of the plant. It was also noted that absorption was 3 to 10 times greater at night than during daytime and three times greater in the morning than in the afternoon. It was suggested that the internal change within the plant during the night may play an important role in absorption.

Coffee, cacao and banana leaves have been reported to absorb urea efficiently. In regards to absorption rates, all urea applied virtually entered the leaf tissues in less than 24 hours for coffee and cacao and less than 30 hours for banana. The amino acids in the leaf tissues increased following urea applications, but it has not been verified whether the increase came from the urea or from hydrolysis of protein in the plant (Cain, 1956).

Factors Affecting Absorption and Translocation

Environmental factors

Temperature, light and humidity are reviewed together since they are interrelated, and many researchers have not separated one from the other. Light and temperature have profound effects on the life processes of the plant, while atmospheric humidity may become influencial if the plant is under water stress conditions.

Variations in absorption rates of urea during day and night periods as reported by Volk and McAuliffe (1954) most likely resulted from interaction effects of light, humidity and temperature rather than as a single factor. The authors explained these findings as follows: (a) the relative

humidity and temperature may have interacted to alter the drying period of the spray solution. Permeability of the cuticle and the cell membranes might also have been changed, as the temperature varied from 70 F during the night to a maximum of 98 F during the day. Similarly, the relative humidity changed from 72 percent during the night to a minimum of 30 percent in the afternoon.

(b) Another variable may have been the effect of low temperature and darkness on some of the plant constituents. Organic acids may have accumulated during the night and, as a result, enhanced urea metabolism. Foliar absorption of urea under such conditions might have occurred rapidly.

Observations on streptomycin-^{14}C and DL-leucine-^{14}C absorption by the lower surface of Jonathan apple leaves indicated that the entry of both compounds in the leaf is dependent on temperature and light (Kamimura and Goodman, 1964). In these studies, the relative humidity was kept at a high level and the chemicals, which were applied, were kept in solution throughout the course of the study. Applications of .5 ml were applied using a glass tube sealed to the leaf between the veinlets. Results were based on the radioactivity count from the leaf discs removed from the leaf where the treatment was made.

Illumination at 528 ft-c for 24 hours during the uptake period increased absorption of leucine five times and streptomycin 2 fold as compared to controls. Both light intensity and quality affected the uptake. The most effective light colors increasing absorption were blue

and red. It was concluded that foliar absorption of organic compounds is in part mediated by photosynthetic and respiration high energy compounds (Kamimura and Goodman, 1964).

Mechanisms of foliar absorption of phosphate and rubidium in bean leaves were studied by Jyung and Wittwer (1964) using leaf-emersion and leaf washing techniques. Using the temperatures of 5 to 25 C, a temperature coefficient of 1.82 and 1.55 for absorption of phosphate and Rb, respectively, was observed. Increased light intensity promoted mineral uptake. The light saturation occurred at 320 ft-c for rubidium, while intensities up to 1400 ft-c did not cause saturation (no response to light beyond this light intensity) for phosphate uptake. Decreased uptake by metabolic inhibitors such as 2, 4-dinitrophenol (DNP) and chloramphenicol as compared to controls, accumulation against a concentration gradient, and light and temperature dependency suggest that absorption is an active process, the authors concluded.

According to Cook and Boynton (1952), a pretreatment of darkness for a period of one-half hour to 6 days did not affect absorption of urea by apple leaves. Contrary to these findings, the uptake of ^{60}Co by bean and cucumber plants was enhanced by light and higher temperatures (Gustofson, 1956). The uptake was measured at two different temperature ranges, 70 to 76 F and 87 to 100 F.

Spray timing affected uptake of magnesium by apple leaves. Foliar applications 1 hour before dark had a greater magnesium effect than when applied at other times of the day (Oland and Opland, 1956). An

increase in atmospheric humidity and a drop in temperature may have resulted in the spray material staying in solution on the leaf for a longer period of time, thus increasing absorption. However, another possibility is that internal changes (due to the lack of light and reduced temperature) may have favored increased absorption.

Thorne (1958) studied phosphorus uptake by bean leaves under a variety of external conditions. He reported that phosphorus uptake was inversely related to the drying rate of the solution. The addition of glycerine to the spray solution decreased the drying rate, and increased absorption in his studies.

It is interesting to encounter new theories about the properties of water at various temperatures, and the possible influence water has on biological activities and permeability of the membranes. In an article "The Puzzle of Water," Drost-Hansen (1966) explained that the properties of water changed according to the temperature, but not linearly as the temperature was increased. As an example, instead of having a more or less straight line relationship with temperature, the viscosity change of water consistently showed "kinks" or inflection points. Within the range of 0 to 100 C, anomalies appeared approximately at 15, 30, 45 and 60 C. This is believed due to a transition in the structure of water at these points, therefore causing abrupt changes in the properties of water. Although several theories are available for the structure of complex water $(H_2O)n$, no theory gives enough information about the fundamental structure and explains the many varied and peculiar properties of this fluid.

Other articles by Drost-Hansen (1965a, 1965b and 1967) provided more information about the subject. In this review, some selected parts are as follows:

> We believe it is safe to insist that the observed anomalies temperature and concentration dependencies of the surface and interfacial tension of water and aqueous solutions are real; likewise that the surface tension of pure water is a very complicated function of temperature. . . . The addition of salts lead to marked anomalies in the surface potentials at more or less discrete concentrations. . . . The essential elements of the surface structure of water are probably clusters or "cages" which may serve as sites for solutes and possess individual stability and discreteness. The size of the units involved are probably similar to those postulated by many authors as occurring in bulk water--the order of 20 to 200 molecules per cluster. (Drost-Hansen, 1965b, p. 18-37)

Experiments concerning the effect of temperature on diffusion rates of salts through simple membranes as well as variations of potential energy across biological membranes are discussed and interpreted by Drost-Hansen and Thorhaug (1967).

Diffusion of sodium and potassium chloride across a thin layer of a 1-butanol membrane separated by two aqueous phases showed an abrupt change between 30 to 39 C. Within this range of temperature, the rate of diffusion did not increase while it did from 17 to 30 C and 30 to 45 C. In other studies, multilayer membranes of barium stearate demonstrated the same trend in respect to electrical conductance of the membrane.

Studies on the natural membranes of alga Valonia macrophysa and Valonia utricularis revealed that the potential difference across the membrane was almost constant between 15 to 30 C regardless of temperature changes. An abrupt increase occurred at 30 C in both species, while at

below 15 C a decrease occurred in V. utricularis. Lowering the temperature caused a very sharp peak in electromotive force across the membrane around 10 C in the other species.

It is suggested that these changes observed in artificial and living cell membranes are most probably associated with water phase transitions. According to the studies mentioned above, one may speculate that the arrangement of water phase molecules as well as the amount of water within a membrane of a living organism may manifest a great influence on solution penetration at critical phase transition temperatures.

Regarding temperature effects on cuticular permeability, still a great gap is present in our knowledge about the water status, degree of hydration or hydration sites of this polylayer structure. It could be assumed that the water movement paths in various cuticles are different in size; therefore, the temperatures at critical points do not influence penetration of solutions equally. In this respect, it may be expected that the temperature would not influence diffusion through the cuticle with large size water paths, while great anomalies may occur in those with small entry avenues.

Spray solution characteristics

Addition of surfactants to the spray solution may greatly influence penetration. These compounds may affect ionization of nutrients, alter cuticular permeability and help the spreading or sticking of the spray solution on the foliage. In general, it would be expected that with the

addition of proper amounts of surfactant, foliar absorption would increase. However, some side effects and interactions with plant and spray solutions make this prediction uncertain. Klingman (1966) outlined five important effects of wetting agents, as follows:

(a) They cause a uniform spreading of the solution over the foliage.

(b) They cause better sticking and decrease bounce-off and run-off of the spray solutions.

(c) They increase intimate contact with the leaf surface, epidermal hairs, etc.

(d) They may solubilize non-polar plant materials available in the cuticle and lipoidal cell walls, therefore enchance absorption.

(e) Finally, they may have harmful effects, such as protein precipitation, inactivation of enzymes and suppression of some biological activities.

Klingman described surfactants as chemicals having a hydrophilic group on one side and a lipophilic group on the other side of the molecule. Because of this, the molecules would orient themselves at the interfaces. Orientation properties of these molecules between water and lipoidic substances cause better spreading and sticking and facilitate emulsification.

Surfactants are commonly classified into four groups: anionic, cationic, non-ionic and ampholytic. Ampholytic surfactants are compounds having the properties of becoming cationic in acidic medium and anionic in alkaline solutions. The non-ionic surfactants have a rather wide application in biological systems. These compounds are expected

to be rather chemically inert, hence possess less biological side effects (Parr and Norman, 1965).

A mixture of non-ionic and ionic surfactants are often used. Development of full surface active properties of ionic surfactants depends on the extent of ionization. The degree of ionization controls overall behavior of the chemical mixture and often becomes an important factor in spray effectiveness. In non-ionic surfactants, however, the lipophilic and hydrophilic balance in a single molecule controls the character of a surfactant. The ratio of the strength of hydrophilic to lipophilic is commonly called HLB or hydrophilic/lipophilic balance. Low HLB surfactants promote water in oil emulsions, while those with a high HLB facilitate oil in water emulsions (Behrens, 1964).

Phosphorus penetration into apple foliage was enhanced by addition of Triton X100 but opposite effects were observed using magnesium with this surfactant (Fisher and Walker, 1955). The authors reported that only a small quantity of surfactant was needed for a satisfactory spread over the leaf surface. High concentrations of surfactant were found undesirable because of increasing run-off of the spray solution. Observations by Swanson and Whitney (1953) using Tween 80 in ^{32}P solutions showed that this surfactant decreased foliar absorption of phosphorus by bean plants. Measurements were based on the translocated amounts. Similarly, Teubner et al. (1957) used a number of surfactants to evaluate their influence on foliar absorption of $H_3{}^{32}PO_4$ by bean plants. They reported that all of the tested compounds with the exception of B-1956 and Sterox AJ

reduced uptake. The additives tested were at concentrations of .01, .1 and 1 percent. Only .01 percent Sterox AJ enhanced absorption. Adherance of phosphates to the leaves was reduced significantly by addition of surfactants. In another report, Koontz and Biddulph (1957) studied the effects of some anionic, cationic and non-ionic surfactants on phosphorus foliar absorption. They indicated that none of the compounds tested were effective in absorption, but that Vatsol OTB and Tergitol 7 suppressed uptake.

Studies by Cook and Boynton (1952) revealed that both Tween 80 at .1 percent and Tween 20 at .01 percent increased absorption of urea through the lower surface of apple leaves three and two times, respectively, compared with absorption of urea solutions not containing surfactants. The main effect of these surfactants is assumed to be due to the reduction of surface tension. The addition of a wetting agent decreased surface tension about 45 percent.

Many surfactants show their maximum effects pertaining to reduction of interfacial tensions at concentrations of .01 and .1 percent. With the addition of more surfactant, there is very little, if any, change in effectiveness. The point of maximum efficiency is termed the critical micellar concentration. At higher strengths, colloidal micells form which are not active. Most organic substances modify the energy relationship of the solvent; surfactants, however, do this in extreme fashion. In addition to changes in free energy, surfactants also modify the electrical potential of the two phases (Jansen, Gentneer and Shaw, 1961).

Studies by Cook and Boynton (1952) revealed that urea uptake by apple leaves was affected by the pH of the spray solution. Using a buffer system, by mixing Na_2HPO_4 and KH_2PO_4 in varying proportions, they observed increased uptake at pH 5.6, as compared with pH 8. In five experiments, comparisons were made between different pH values of 5.6 vs 8, 5.6 vs 7.2, 7.2 vs 8 and 5.4 vs 6.6 vs 7.3 vs 8. Absorption was at a maximum when the spray solutions were acidic, intermediate at basic pH 8 and minimum at basic pH values of 7.2 and 7.3.

Different buffer systems were used by Volk and McAuliffe (1954) to study the effect of the hydrogen ion concentration on the absorption of urea by tobacco leaves. Potassium dihydrogen phosphate-sodium hydroxide buffer was used for pH values of 6, 7 and 8. Minimum absorption occurred at 6 and a maximum at 8. In the same experiment, sodium hydroxide-potassium acid phosphate and sodium hydroxide-boric acid buffers were used with pH values of 5 and 9, respectively.

Swanson and Whitney (1953) demonstrated that phosphate uptake was increased as the pH decreased. A negligible amount was absorbed at pH 7. Teubner et al. (1957) also observed that absorption of phosphate was highest at pH 2 to 3. At a pH below 3, necrotic spots occurred with the treatment. This was not evident at higher pH values. Further work by Teubner et al. showed that the effect of the hydrogen ion concentration varied considerably, depending upon the accompanying cations. Double peaks for absorption of some phosphates were observed when the pH of the spray solutions varied from 2 to 7. Ammonium phosphate and sodium

phosphate were absorbed at the highest rate at pH values of 2 to 3. These rates decreased at 4, but increased again at pH 5. Absorption of potassium phosphate increased at pH 2 to 3 and 7. Low absorption rates were observed at pH 4, 5 and 6. In another experiment ammonium phosphate at pH 8 was absorbed more by bean plants than by tomatoes. The reverse was true when potassium phosphate was used.

General studies by various investigators have confirmed that the herbicide 2,4-D and weak organic acids are absorbed better in an acidic medium. It is believed that these weak acids penetrate at a higher rate when they are ionized (as when in an acidic solution).

Orgell and Weintraub (1957) conducted experiments to determine the effects of hydrogen ion concentrations as well as those of other cations and anions in buffer systems used for 2,4-D foliar applications. A response to 2,4-D was observed with alkaline solutions containing ammonium and triethanolammonium phosphate. These cations were surprisingly more effective at a pH range of 8 to 8.5.

An interaction between Tween 20 and the ammonium phosphate buffer was observed at pH 8.5 and when surfactant concentrations were higher than .01 percent. This was not evident with other buffer systems. It seems logical to conclude that although some properties of many chemicals (e.g. solubility) are affected by the hydrogen ion concentration, the constituents of a particular buffer system may also influence cuticular properties and subsequent biological activities which eventually will

influence metabolic uptake of chemicals and/or reactions associated with assimilation of that particular compound.

Plant factors

Cuticle. The cuticle is a relatively impermeable layer over the leaf surface, composed of fatty substances and waxes, pectins, cellulose and cutin. The cellulose and pectins are hydrophylic components of the cuticle which may have a role in the passage of water-soluble substances through the cuticular layer.

Scott, Schroeder and Franklin (1948) studied the internal suberization of the leaf by using the IKI-H_2SO_4 test. Tissues stained with IKI, which contained small amounts of suberin, swelled and gradually turned blue when irrigated with H_2SO_4. In highly suberized tissues, they remained brown and swelling did not occur. In young leaves, suberin appeared as a thin film in the intercellular spaces, but completely impregnated the middle lamella in the mature leaves of some plants.

Increasing hardiness of the leaves, as they mature, is related in part to their thickness and in part to the internal suberization. The age, the habit and the habitat of the plant also determines the extent of the internal suberization of leaves. Therefore, it is expected that in all young leaves and mature leaves of hydrophytes and some shade mesophytes, the degree of suberization will be limited. On the other hand, the leaves of zerophytes and certain mesophytes may be highly suberized (Scott, 1950).

Skoos (1955) investigated the effects of age of the leaf, temperature, and water stress of the plant on the development of cuticle and wax depositions in the leaves of some plant species. Those grown at higher temperatures produced thicker cuticles. This was brought about by an increased production of lipoidal materials which made up the cuticle. Greater amounts of wax were also synthesized at higher temperatures. Water stress had marked effects on cutin development. Tree tobacco almost doubled its leaf cuticle thickness under water stress.

The major components of the cutin of Agave americana L. have recently been isolated and identified by Crisp (1965). Half of the cutin constituents were made of 9, 10, 18-trihydroxyoctanoic acid, while the rest were composed of 17 identified hydroxy acids ranging in a chain length from tridecanoic to octadecanoic. The linkages representing the types of bonding in cutin were ester, alkylperoxide and ether, with a ratio of 7:2:.12. Ultraviolet light irradiation enhanced formation of peroxide linkages in polymerization of procutin to cutin.

Studies of cuticle structure involving cytochemistry, polarizing microscopy and electron microscopy techniques were performed by Sitte and Pennier (1963). The thickening of the cuticular layer was due to the interposition of cutin and wax between layers that had been deposited earlier. It was found that the cellulose contents of the cuticular layers were very small except in the inner layer which had a considerable amount of cellulose. The outer layer which did not contain cellulose was positively charged. In no case did they find any microscopically

detectable pore over the cuticle, although cuticular transpiration and photosynthesis have been known for a long time.

Waxes. The fine structures of wax deposits on the leaves and herbaceous stem surfaces of several plants were studied by Mueller, Carr and Loomis (1954). The waxes were observed in various patterns of rod-like, semicrystalline and amorphous in shape. Differences in physical or chemical properties of waxes are present, and these differences are evidenced by the patterns of wax deposits on the leaf surface. The factors causing pattern irregularities are not constant, and pattern variations may even occur on a single leaf. Surface waxes are generally observed in a discontinuous layer rather than a uniform covering. According to Mueller, Carr and Loomis, surface waxes may not play an important role in foliar penetration. Addition of surfactant causes the spray solution to cover the cuticle areas on which wax particles are not in immediate contact with cuticle.

Schieferstein and Loomis (1956) investigated the sites of wax extrusion using the leaves of 50 species of plant. They observed that the waxes are not protruded from channels through the cuticle, but they are deposited from the margins of outer epidermal cell walls. The surface wax accumulation process is active during the cell growth and leaf expansion. The subcuticular wax infiltration in leaves may occur during later stages of growth and generally becomes a factor of more importance than surface waxes. Schieferstein and Loomis indicated that possibly a greater susceptibility of growing leaves to the herbicides is due to the

presence of a more permeable immature zone in the cuticle (between adjacent epidermal cell walls).

Further work by Schieferstein and Loomis (1956) showed that enzymatically isolated cuticles of old heavily-cutinized leaves usually gave a positive reaction to the cellulose test. They interpreted these observations as being that subcuticular wax deposits gradually impregnated epidermal cell walls, and much of the epidermal cell wall remains with the cuticle after separation. Wax accumulation on the older leaves when expansion stops is mainly subcuticular. Penetration properties of cuticle may change considerably with age. Permeability of the cuticle of Hedera helix to water was increased with leaf age, but permeability to 2, 4-D was decreased 50 times.

Epidermal hairs. Epidermal hairs may partially prevent nutrient entry into the leaf if a spray does not have enough surfactant to wet the leaf surface; on the other hand, it may be beneficial and enhance spray penetration when the surface is thoroughly wet. Epidermal hairs could cause more spray retention and also function as one of the absorptive sites. Franke (1961) demonstrated that at the basal part of epidermal hairs a large number of structures described as ectodesmata are present. Crystal-like bodies of water-soluble spray material were localized below the structure. Ectodesmata also were present in large numbers in guard cells and in some areas of the anticlinal walls of epidermal cells.

The presence of ectodesmata has been formerly reported by other German workers, but was studied in more detail by Schenpf (1959).

Schenpf studied a number of fixing and staining methods and found that fixing with Gilson solution and staining with pyoktanin was one of the better methods for demonstrating ectodesmata. Certain environmental and plant conditions, such as wilting, exposure to poisons as ether and KCN, high concentration of CO_2, and the application of IAA and histidine, etc., may break, deform or completely disintegrate ectodesmata. The effects of some of these factors may be partly reversible.

More recent studies concerning the function of ectodesmata in relation to the entry of tobacco mosaic virus into the leaf tissues were performed by Brants (1965). He inoculated the leaves of Nicotina tobacum L. and Daturus stramonium L. with ^{14}C labeled TMV and made microradioautograms of the plant sections. Heavy silver grains were accumulated along the basal portion of epidermal hairs corresponding to the higher density of ectodesmata. From these observations, he concluded that the portal of entry of viruses very likely would be the ectodesmata.

Stomata. There has been considerable attention given to the role of stomata in foliar absorption. According to Scott (1950), stomata and most of the cell walls of the leaves gradually become covered with materials called suberin. Highly suberized stomata will reduce penetration of water-soluble materials. It has been generally agreed that water will not penetrate through the stomatal pores unless surfactants are added to the solution. Oil-like compounds may easily penetrate through the stomatal pores. Surfactants, however, facilitate diffusion of water-soluble

substances into the stomata and intercellular spaces. After this step, gradual cellular absorption or translocation takes place.

The work done by Sargent and Blackman (1962) has shown that absorption of 2,4-D through the lower surfaces of leaves with a high number of stomata was greater than through the upper surfaces. They concluded that absorption does not take place through the stomatal pores but through the other walls of guard cells and the adjacent accessory cells. This statement was based on the observations that the relative rates of penetration of 2,4-D into the upper and lower surfaces of a leaf both in light and dark were proportional to the stomatal numbers. Franke (1964) applied droplets of ^{14}C labeled sucrose to the leaves of Spinacea oleracea and Viola tricolor and prepared microradioautograms from the treated spots by which he showed guard cells to be favored sites of absorption.

Epidermal cell walls and cell membrane. Epidermal cell walls are composed of materials such as pectins (highly hydrophilic), cellulose (relatively hydrophilic), cutin (semi-hydrophylic because some of its polar groups remain free during polymerization), various compounds such as hemicellulose, suberin, and waxes, and a variety of other organic and inorganic materials may be present. Water is the major constituent of the cell wall, and pectins and cellulose are the main compounds that keep the cell wall hydrated (Esau , 1962). Spray materials translocated through the phloem have to enter into a living cell around which a semi-impermeable membrane is a barrier. The presence of ectodesmata and

plasmodesmata with their protoplasmic nature facilitate transport of certain substances into the cell.

Van Overbeek and Blondeau (1954) described the cell membrane as having a bimolecular layer of lipidic compounds such as fatty acids, steroles and the glycerides. Lipophilic groups are connected together, while the hydrophilic groups were stabilized by two protein layers on both sides. The cell membrane at the stable state is almost impermeable to water-soluble compounds, unless mediated by metabolic energy of the cell to become permeable to certain ions or ruptured by fat solvents.

Franke (1959) diagramatically showed the possible pathways of foliar penetration as follows:

(a) Through the stomata, absorbed by the inner surface of the subsidiary cells or palisade parenchyma.

(b) Through the cuticle, moving into the intercellular spaces to reach the xylem.

(c) By the epidermal hairs, following the same pathway as in (a) and (b), above.

(d) By the epidermal hairs, entering into the intercellular spaces (inside the cell) by means of ectodesmata and moving from cell to cell through plasmodesmata.

(e) Through the same pathway as (d), with the initial entry via ectodesmata through the epidermal cells.

All of the above pathways are operative more or less, depending on the plant and the nature of the spray materials.

SECTION I

ABSORPTION OF UREA BY APPLE AND PEACH LEAVES UNDER GREENHOUSE CONDITIONS

Introduction

Various reports have indicated that apple leaves are capable of absorbing and utilizing urea efficiently. Nitrogen has been increased in peach foliage by urea sprays, but since the usual nitrogen effects were often limited, evidence is lacking as to whether it was absorbed through the leaf tissue or absorbed on the cuticle.

Dilley and Walker (1961a) reported that the urease enzyme had nearly the same activity in peach as in apple foliage. Labeled urea (^{14}C, ^{15}N) absorbed through the petiole was readily incorporated into different amino acids, amides and protein materials within 20 hours (Dilley and Walker, 1961b).

The purpose of this investigation was to evaluate the relative efficiency of urea absorption by apple and peach leaves under similar environmental conditions. During the course of the research work, a microsprayer was developed which is also described in this section.

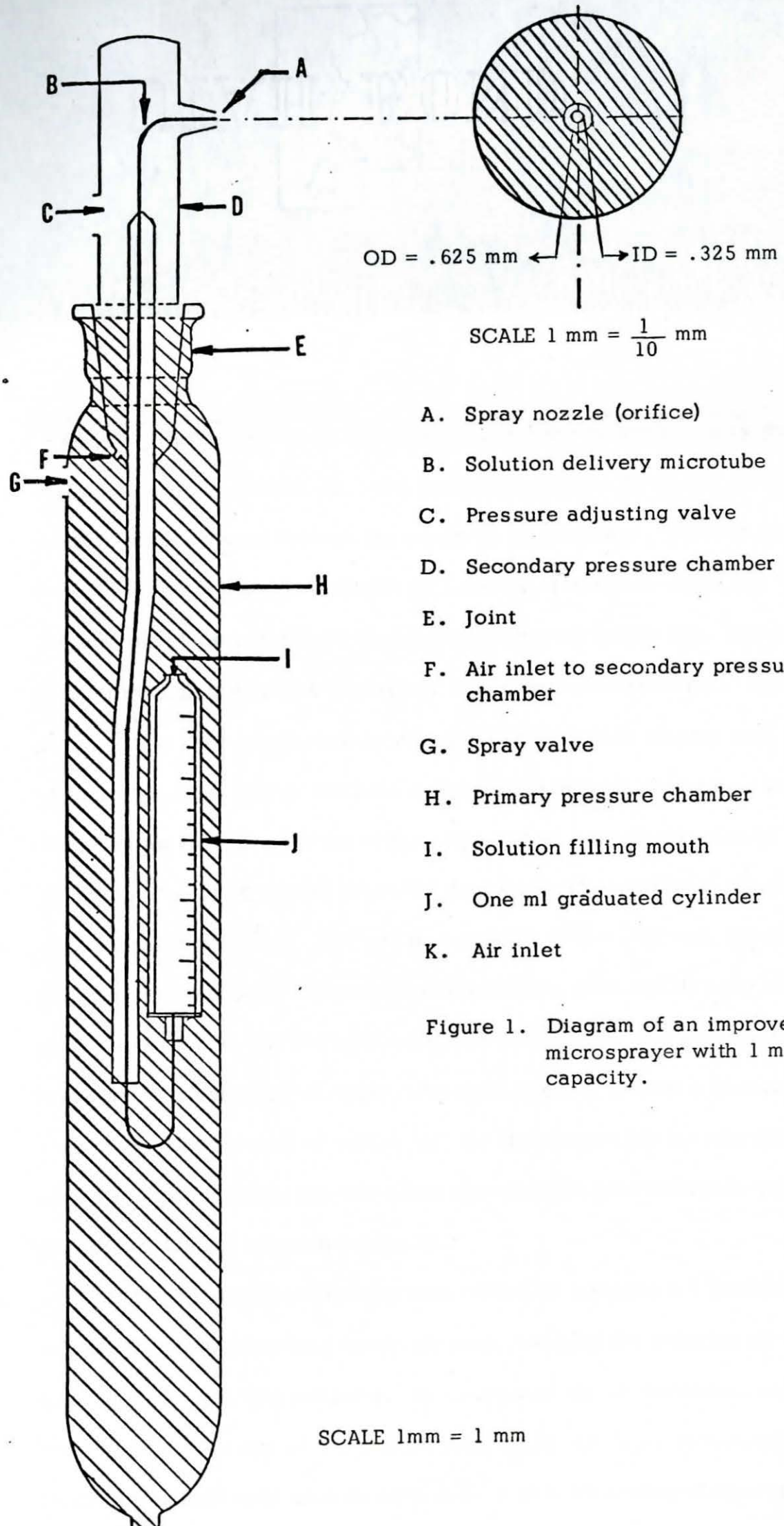
Materials and Methods

One-year-old apple trees, Pyrus malus var. McIntosh and Prunus persics var. Redskin, were obtained from a local nursery. After they had

received their chilling requirement, the trees were cut back to five or six buds from the rootstock union and were planted in sand in 1-gallon tin cans. Hoagland nutrient solution was supplied to the plants twice a week, and water was flushed through the containers in a day or two after the nutrients were added. Pests were controlled with Dibrome fumigation. Only one or two shoots of the trees were allowed to grow in order to obtain large leaves. The mid shoot leaves were tagged and dated, as soon as they appeared, in order to measure their age. The trees were kept in a greenhouse with a temperature of 60 to 65 F at night and 75 F in the daytime. The temperature was occasionally above 75 F on some sunny days. A photoperiod of 14 hours was supplied by natural and artificial light.

Small sprayers of different types have been used for applying foliar sprays in research work. Cook and Boynton (1952) used a perfume hand atomizer for their study of urea absorption. A Paasche air brush atomizer was used later (Boynton, Margolis and Gross, 1953; Fisher and Walker, 1955). Fisher and Walker reported an accuracy of measuring the solution sprayed of + .01 g. The measurement was made by weighing the sprayer before and after the spraying was done. A 1 ml microsprayer was developed during this study, which the author feels is superior to the previous types used.

Figure 1 shows a diagram of the sprayer. It is basically the same as a chromatography atomizer except the unit is smaller and has two additional features, a 1 ml graduated cylinder (J) and a pressure adjusting



A. Spray nozzle (orifice)

B. Solution delivery microtube

C. Pressure adjusting valve

D. Secondary pressure chamber

E. Joint

F. Air inlet to secondary pressure chamber

G. Spray valve

H. Primary pressure chamber

I. Solution filling mouth

J. One ml graduated cylinder

K. Air inlet

Figure 1. Diagram of an improved microsprayer with 1 ml capacity.

5 to 6 minutes with continuous spraying. This standard adjustment was maintained throughout the course of the experiment. Extreme precautions were made for passing clear air through the sprayer. These precautions were taken in order to keep out the atmospheric dust and the oil droplets coming from the air pump. These impurities could cause errors in the experiment or plug the sprayer microtube. In this experiment, air was supplied by a pressure pump and bubbled into three successive bottles of water containing .1 percent Colloidal X-77 surfactant solution before it entered the sprayer.

After the shoots had grown and there were three to four leaves 28 to 32 days old in the mid shoot region, the leaves were randomly selected for the experiment. Treatments consisted of measuring the absorption of urea through upper and lower surfaces of peach and apple leaves 1, 6 and 48 hours after application. One ml of 4 percent urea in deionized distilled water containing .1 percent Colloidal X-77 was used on the large apple leaves, but only one-half ml of this solution could be applied on the peach leaves because of the size and waxy surface. Five leaves, one leaf from each of five trees, were used as a replicate. Four replications were used for each treatment in this experiment. The data were analyzed using a completely randomized block design. At first, a small portion of sprayed solution ran down the petiole, but it was prevented by placing silicon grease around the petiole where it was attached to the blade. The early data obtained before this error was eliminated were not used. The treatment of each replication was done within a 10-minute

period. The leaves were held horizontally until the solution had partially dried and there was no danger of dripping. Spraying was performed between 8 to 10 A.M. After spraying, care was necessary to prevent run-off loss. The spray treatments were applied during a 6-day period.

The amount of urea absorbed after a specific period of time was determined using basically the leaf washing method reported by Fisher and Walker (1955). Each leaf was washed thoroughly with approximately 30 ml of distilled water containing .1 percent Colloidal X-77. The wash water from five leaves (a replicate) was combined.

The wash water was diluted to 200 ml; a 25 ml aliquot was then analyzed for nitrogen using a micro-kjeldahl procedure. The modified kjeldahl method recommended by researchers at the Utah State University Soils Laboratory (1961) was followed. The amount absorbed was determined by substracting the recovered from the applied nitrogen. The data are expressed as percentage absorption.

Results and Discussion

The largest difference was observed between the absorption of upper and lower leaf surfaces. Considerably higher amounts of urea were absorbed by the lower surfaces of the apple and peach compared with their respective upper surfaces. There was little or no uptake from the upper surface after 1 hour in either species. The lower surface continued to absorb urea throughout the remainder of the 48-hour period at which time the experiment was terminated. Absorption was much faster during the

first hour than during other periods of the experiment (Figure 2).

The lower/upper ratio of absorption after 48 hours was 2.1 for apple and 5.5 for peach leaves. Analysis of variance of the data indicated that the differences in absorption between the species, the upper and lower surfaces and the period of absorption, were statistically significant at the 1 percent probability level. The interaction effects of species X absorption period, treatment surface X absorption period and species X absorption period X treatment surface were also significant at the 1 percent level. The species X surface effects were significant at the 5 percent probability level.

The uptake of 84.9 percent of the urea by lower surfaces of peach leaves during the 48-hour period was rather surprising. Absorption of such a large quantity of urea under field conditions should result in a positive nitrogen response. Brown spots on lower surfaces appeared on both apple and peach leaves 24 hours after they had been sprayed. Within 48 hours, an average of four to five nectrotic spots of 2 to 4 mm in diameter was evident on each leaf. Upper surface-treated leaves did not show such symptoms. The urea used was of reagent grade and was low in biuret content; therefore, the appearance of necrosis on the leaves most probably was the result of large quantities of urea entering the leaf. Different opinions have been presented in the literature as to whether such injury is due to the accumulation of urea in the leaf or one of its metabolites such as ammonia. Marginal injury of leaves observed on sour cherry leaves under field conditions (Fisher and Walker, 1955) was

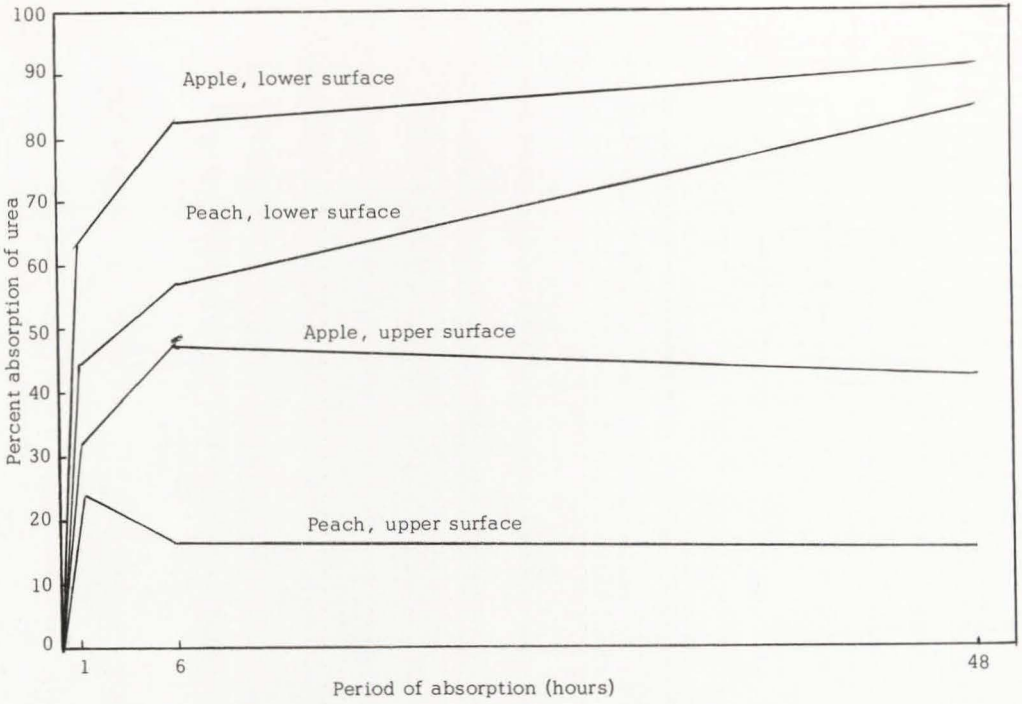


Figure 2. Urea absorption by the upper and lower surfaces of 1-month-old apple and peach leaves.

not evident in this experiment.

After the spray solutions had dried for an hour, absorption continued from the lower surfaces though at a much lower rate during the next 5-hour period. An additional 30 percent of the amount absorbed during the first hour was absorbed during the next 5 hours in both species. Figuring on the basis of total urea absorbed, the difference in the percent absorption between 1 and 6-hour periods was 18.9 and 13.2 for apple and peach, respectively. Hence, apple leaves absorbed urea at a faster rate shortly after application than peach leaves. Conversely, peach leaves absorbed urea more rapidly than apple leaves later on, though the peach leaves did not absorb as much as the apple leaves during the 48 hours this experiment was conducted.

After 1 hour (or less) when the spray material on the leaf surface has dried out, additional absorption may occur by either of the two possibilities below:

1. There may still be a semi-fluid phase present between the cuticle and the dried crystals on the leaf surface. This semi-fluid mixture contains a very high concentration of the applied material, and although it may not actually have been absorbed by the plant tissue, it is most likely to be connected to the fluid phase in the plant. A portion of the semi-fluid material, together with that which has dried on the surface, may be washed off before absorption has taken place.

2. After the early period of absorption, rehydration likely occurs which increases absorption. Observations by Bald (1952) may explain

why absorption of urea was continued, though it should have been stopped after a few hours because of drying. Bald indicated that when stomata open in early morning, they may exudate droplets of water over the leaf surface (stomatal guttation). These droplets may become larger in size if plant and environmental conditions are favorable and may be reabsorbed in case water deficit develops in the plant. Conditions of cool air, high root pressure and warm soil are favorable for stomatal guttation. Skoss (1955) emphasized stomatal penetration of water-soluble compounds and possible involvement of stomatal exudate in continued absorption. Continued uptake of urea by peach and apple leaves in the greenhouse is assumed to be connected someway with rehydration of the leaf. It seems that factors in favor of rehydration have been stronger for peach leaves as evidenced by the absorption data presented.

SECTION II

ABSORPTION OF ^{14}C LABELED UREA UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

Materials and Methods

Young McIntosh apple and Redskin peach trees were obtained from a local nursery for the experiments reported in this section. Growing conditions for the trees were similar to those described in Section I. Thirty-day-old and 45-day-old leaves were used for the first and second experiments, respectively, as described in this section. The leaves were detached from the shoots, and the petioles were immediately placed in water where they were kept for a period of about 1 hour before being used. All leaves were selected randomly and detached between 9 and 10 A.M.

Labeled urea (^{14}C) was obtained from the Nuclear-Chicago Corporation, Des Plaine, Illinois. The specific activity of the urea was 650 μc/mg. Five hundred μc of urea were dissolved into 10 ml of deionized distilled water and blended with reagent grade unlabeled urea to make a 1 percent solution. This solution was divided into 2 ml portions and preserved in sealed glass ampules. The ampules were kept in the refrigerator until used. A surfactant solution of .3 percent Colloidal X-77 was used with the urea solution in some of the experiments though it was applied separately.

Five ml capacity plastic test tubes with tight caps were used to support the leaves for treatment. A hole slightly larger than the diameter of a petiole was made on the side of a tube near the cap. The petiole was then placed in this hole for leaf support. Four of these test tubes were then arranged approximately 8 cm apart by placing them in holes drilled into a piece of board which acted as a test tube support. The tubes were filled with distilled water, then the petioles of the leaves were inserted into them. The leaves were positioned with either the lower or the upper surface facing up. To hold a leaf blade firmly in a horizontal position, small pieces of transparent adhesive tape were used to stick the edges of the leaf to the board. A ring of lanolin 5 mm in diameter was placed on the leaf, as shown in Figure 3. The lanolin ring was applied by placing a holed rubber stopper in the petri dish containing a thin layer of lanolin and then stamping the rubber stopper on the leaf.

In all experiments, 10 microliters of urea solution was applied inside the lanolin ring. Prior to placement of the urea solution, the inside of the ring was moistened with 5 microliters of either distilled water or .3 percent Colloidal X-77, depending on whether or not the surfactant was used for that particular treatment. Hence, the total volume of the solution in the ring was 15 microliters and, as a result, diluted the urea solution to .67 percent and the surfactant to .1 percent.

As soon as droplet application was completed, the treated leaves were placed in a Sherer model CEL 25-7HL (with humidifier unit) plant growth chamber at a distance of 3 feet from the light source. The leaves

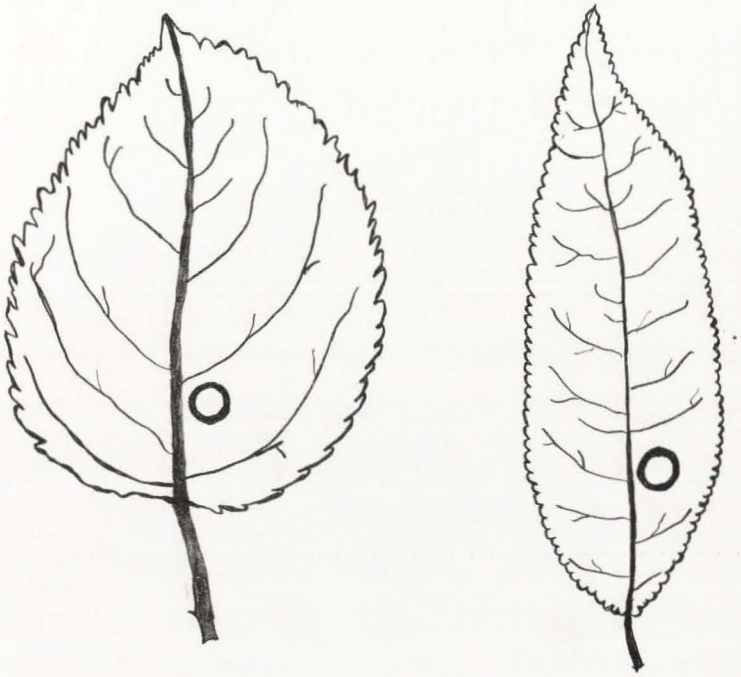


Figure 3. Drawing of apple and peach leaves showing the areas where urea droplet was applied.

were illuminated throughout the period of absorption. Ten 20-watt fluorescent and only four of the eight 50-watt incandescent lights were on to allow a safe operation at the minimum temperature of 10 C used in the experiment. The temperature variations were between $\pm$ 1 C. The relative humidity was maintained at $\pm$ 5 percent of the desired humidity.

In the first experiment, two replications and six factors were used in a $2X2^6$ factorial design. Absorption was measured as affected by the following variables: apple leaf vs peach leaf, upper surface vs lower, 24 vs 10 C temperature, 25 percent vs 85 percent relative humidity, no surfactant vs .1 percent Colloidal X-77, 1 hour absorption period vs 8 hours. In the second experiment, a factorial design of 2X2X2X2X4 was used. All variables were studied as in experiment one, except the surfactant and relative humidity factors were held constant and were not part of this experiment. The surfactant level was .1 percent and the relative humidity, 85 percent. Measurements were made after 1, 4, 16 and 48 hours of absorption.

The treated area of a leaf (inside the lanolin ring) was washed with deionized distilled water after termination of the absorption period. This was done by placing a drop of water on the spot and removing it with a small piece of filter paper at the end of 1 minute. This washing procedure was repeated five times in order to remove the unabsorbed urea. The filter papers were then washed with distilled water which was diluted to 10 ml and analyzed to access the percentage urea unabsorbed. A

similar procedure but with a watch glass instead of a leaf gave a 97 to 100 percent recovery. The average of five replications was 98 percent.

The radioactivity of the wash water was measured by a Tri-Carb liquid scintillation spectrometer. A 1 ml aliquot of the unabsorbed urea solution was mixed with 19 ml of scintillation solution, similar to the method reported by Bruno and Christian (1961). The scintillation solution contained 1 percent PPO (2,5-diphenyloxazole), .05 percent Dimethyl POPOP-1,4 bis- 2-(4-Methyl-5-phenyloxazolyl)-Benzene and 5 percent naphthalene in a mixed solvent of five parts dioxane (reagent grade) and one part of cellosolve (ethylene glycol monoethyl ether). The activity count was multiplied by the dilution factor to obtain the total amount of unabsorbed urea. This figure was substracted from the total applied, and the data are reported as percentages absorbed. Dioxane, cellosolve and naphthalene were obtained from the Eastman Kodak Company, Rochester, New York, and the PPO and POPOP from the Packard Instrument Company, Downers Grove, Illinois.

Results and Discussion

The results of the two experiments are presented in graph form. The detailed numerical values and analysis of variance tables are shown in the Appendix. For ease of comparison and evaluation of the effects of various factors, each figure illustrates the effect of three factors on urea absorption by apple and peach leaves. The main and interaction effects of some of the factors which were statistically significant are presented

and discussed.

Experiment 1

The overall percentages of urea foliar absorption through apple and peach foliage for the factors investigated were 26 and 14 percent, respectively. The absorption difference between the two species is highly significant (1 percent level), with apple leaves absorbing nearly double the amount of urea than peach. On the other hand, a number of interactions were significant, such as species X leaf surface and species X temperature. The interaction of species X surfactant X leaf surface approached significance. Hence, with some experimental conditions, peach leaves may absorb more than apple. The information provided from the combined effects of various factors need to be considered carefully, since the influence of one factor may modify others.

The period of absorption was highly significant. The overall means for 1 and 8 hours of absorption were 13.4 and 26.8 percent, respectively. This would indicate that uptake generally continued for more than 1 hour. Absorption in some cases ceased after 1 hour (e.g. Figure 4 vs 5). The possible cause of the cessation in uptake is discussed later.

There was a large difference between the absorption rates from the upper and lower surfaces of the leaves. Combining the results from peach and apple, the average percent absorption (1 and 8 hours) was 8.0 and 32.2 percent for the upper and lower surfaces, respectively. Surface effects with humidity and the period absorption showed two highly significant interactions. These two interactions were of a magnitude type rather than directional. Thus, with an increase of either period of

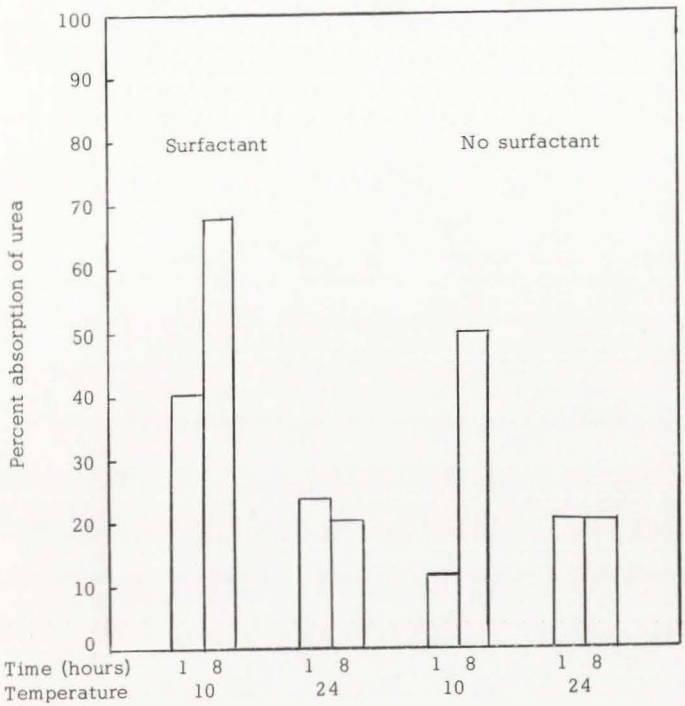


Figure 4. Absorption of ^{14}C urea by the lower surface of apple leaves at 25 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

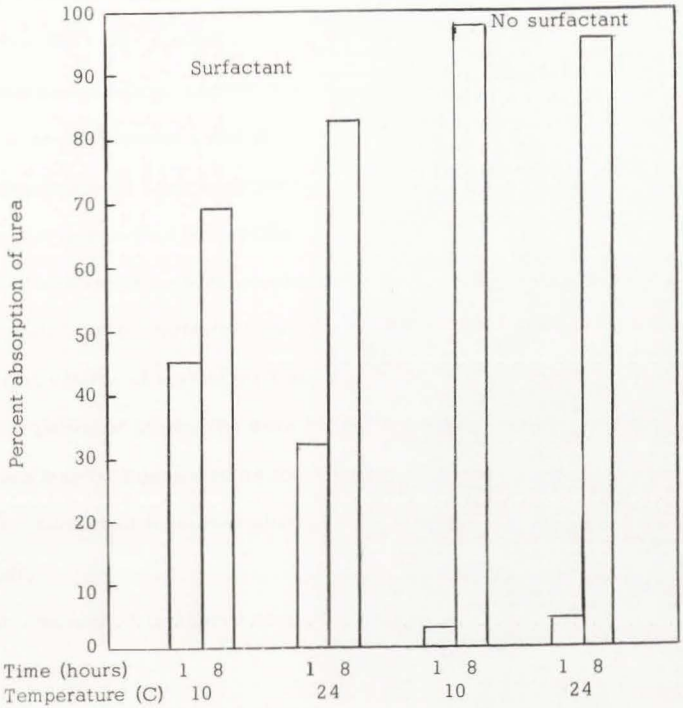


Figure 5. Absorption of ^{14}C urea by the lower surface of apple leaves at 85 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

absorption or humidity, an increase in absorption occurred. On the other hand, one factor at either a low or high level of another factor was not equally effective. Effects of humidity on increasing absorption were greater for the lower surface as compared with the upper. Surfactant under low humidity conditions seemed to enhance absorption in apple but had no effect on peach (Figures 4 and 6).

Depending on other experimental conditions, the surfactant either enhanced or suppressed absorption. During the 1-hour uptake, surfactant increased absorption. On the contrary, absorption was reduced for the 8-hour periods when surfactant was present (Figures 5 and 7). The two interaction effects of surfactant X surface X temperature and surfactant X surface X period of absorption were statistically significant. These interactions may be interpreted as follows:

1. Surfactant increased absorption more at a low temperature than at a high.

2. Surfactant increased absorption more for the lower surface than for the upper.

3. Surfactant increased absorption more during the first hour and suppressed it afterwards (Figures 4, 5, 6 and 7).

4. For the upper surface, the surfactant increased absorption at high humidity and high temperature only (Figures 10 and 11). The surfactant did not influence urea absorption with peach leaves (Figure 10) but markedly increased absorption in apple leaves (Figure 11).

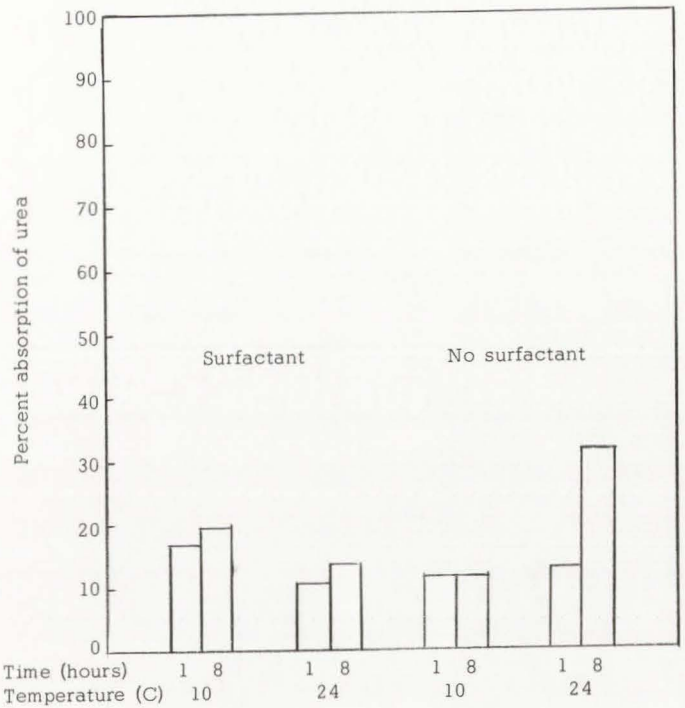


Figure 6. Absorption of ^{14}C urea by the lower surface of peach leaves at 25 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

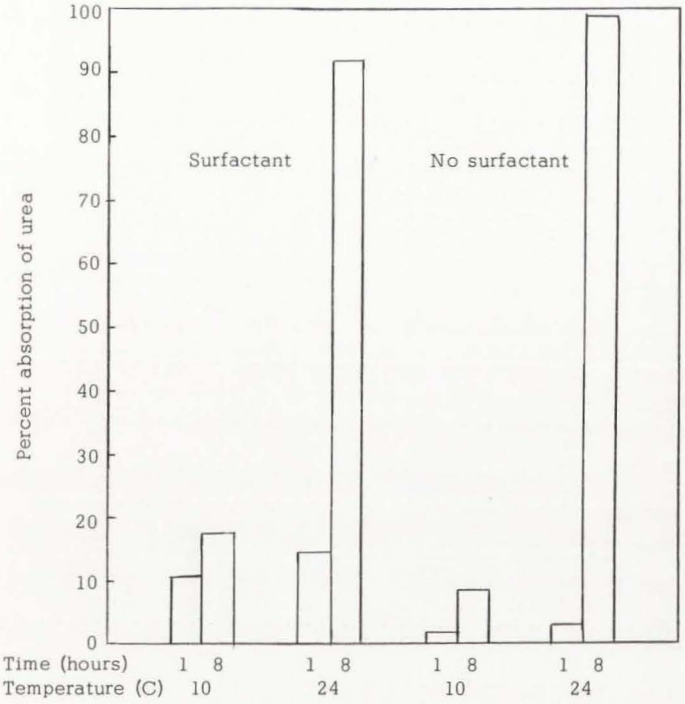


Figure 7. Absorption of ^{14}C urea by the lower surface of peach leaves at 85 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

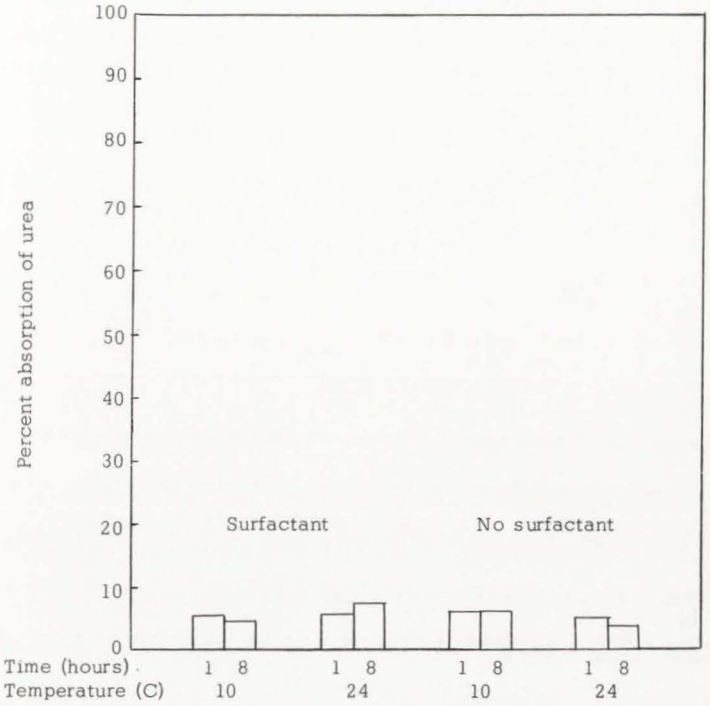


Figure 8. Absorption of ^{14}C urea by the upper surface of peach leaves at 25 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

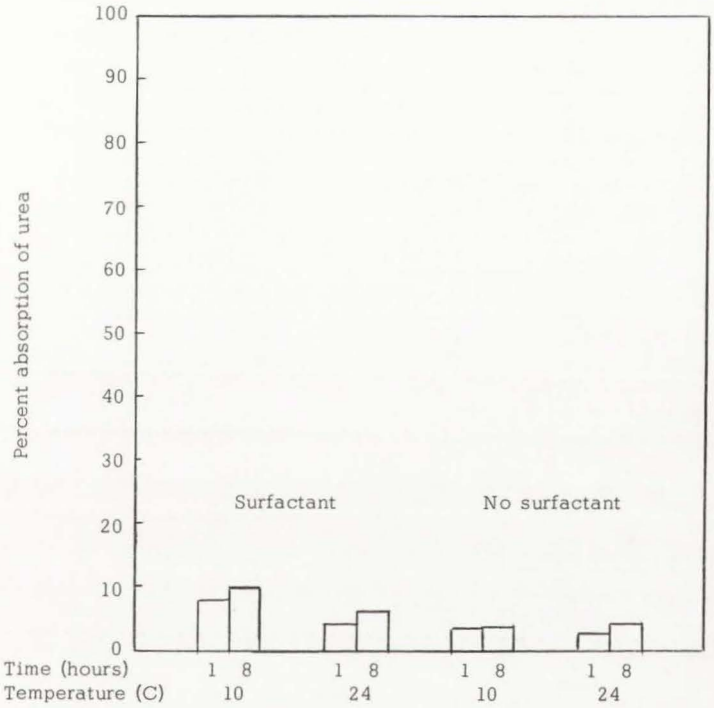


Figure 9. Absorption of ^{14}C urea by the upper surface of apple leaves at 25 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

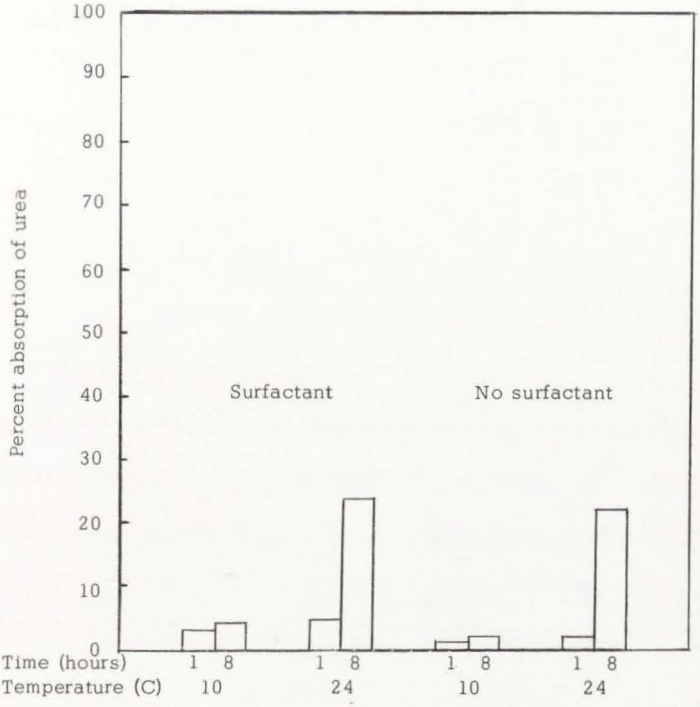


Figure 10. Absorption of ^{14}C urea by the upper surface of peach leaves at 85 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

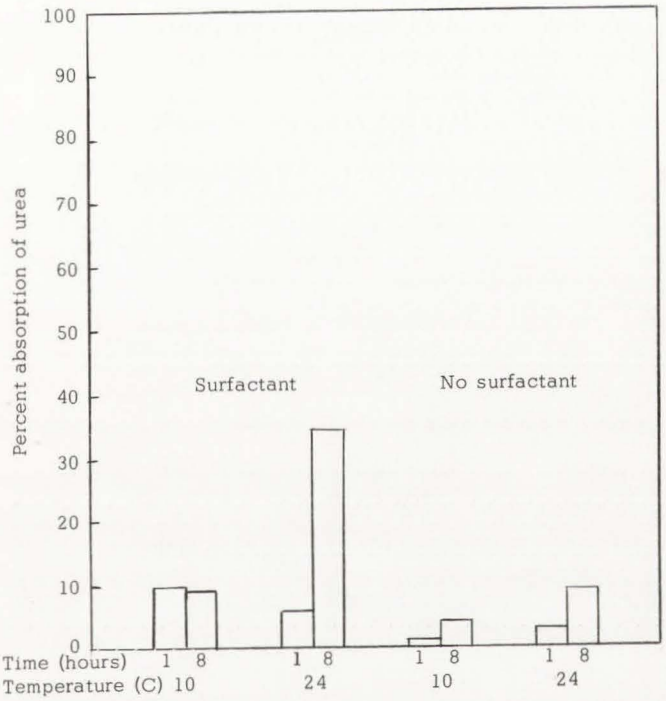


Figure 11. Absorption of ^{14}C urea by the upper surface of apple leaves at 85 percent relative humidity. The surfactant used was .1 percent Colloidal X-77.

Comparing the effects of surfactant on the two species, it was observed that apple leaves were influenced more than were peach leaves (Figures 5 and 7). Wetted cuticle and epidermal hairs of apple leaf may have permitted a rapid initial entry into the leaf tissue (overall averages of 13.9 vs 14.2 percent and 30.7 vs 21.53 percent for peach and apple leaves, respectively).

A decrease in absorption rate after 1 hour, which occurred with the surfactant-treated leaves as compared without surfactant, may be associated with the high concentration of surfactant on the leaf surface as the water evaporated. Dehydrated or almost dehydrated surfactant left a thin film of surfactant on the cuticle and may have prevented urea entry. Surfactant also may have affected absorption by its penetration into the leaf cells and somehow causing metabolic inhibition.

Considering the general influence of humidity on foliar absorption of urea, the overall means were 16.4 and 23.8 at 25 percent and 85 percent relative humidities, respectively. This difference was statistically significant at the 5 percent level. Highly significant interactions were observed between temperature and humidity and between surfactant and humidity. High temperature (24 C) and high humidity (85 percent relative) conditions were favorable for increased absorption (Figures 4 vs 5, 6 vs 7, 8 vs 10, and 9 vs 11).

Assuming the temperature of the leaf surface and surrounding atmosphere as being almost equal, the vapor pressure gradient between a drop of water and the air at 25 percent relative humidity with the temperature

at 10 or 24 C is 6.9 mmHg and 16.8 mmHg, respectively. The vapor pressure deficit at 10 C and 25 percent relative humidity is calculated as follows:

$$vpa = \frac{RH \times vps}{100}$$

vpa = vapor pressure of the water in air in mm of Hg

vps = pressure of aqueous vapor over water in mm of Hg. This value is obtained from the constant table for a particular temperature given.

$$vpa = \frac{25 \times 9.2}{100} = 2.3$$

vpd = vps-vpa=9.2-2.3=6.9

The vapor pressure differences at 85 percent relative humidity are 2.3 for 10 and 3.3 for 24 C. As shown, at a condition of high humidity, there is little difference between the evaporation rates of water at the two temperatures. Under low humidity conditions (25 percent) and at 24 C, the rate of evaporation is nearly 2.5 times higher (16.8/6.9=2.5) than at 10 C. A fast drying rate, therefore, seems to be a limiting factor in absorption at high temperature and low humidity conditions.

At either high or low humidity and 10 C conditions, peach leaves absorbed a small percentage of urea (20 percent maximum). This indicates that low temperature has markedly reduced absorption of urea by peach leaves. Visual observations showed that the droplets of urea had not dried out at the end of 1 hour under high humidity conditions. Under favorable conditions, limited uptake occurred during the first hour but

increased more than 8 fold during the 8-hour period (Figures 5 and 7). This pattern of absorption is likely to be of the diffusion type, consequently the rate increased with time as the urea solution became more concentrated on the leaf surface.

Experiment 2

Some of the findings of the first experiment were verified by the results obtained from this experiment. Effects of all factors and their interactions were statistically significant at the 1 percent level except interaction effects of species X period of absorption, which was significant at the 5 percent level and the species X surface X period of absorption interaction, which was not significant. These observations indicate that although 45-day-old leaves were used, the rates of absorption were similar to those in the previous experiment under comparable conditions.

Contrary to the first experiment, the higher temperature increased the urea uptake of apple and peach in all cases (Figures 12 and 13). Manifestation of the increase in absorption at the high temperature is presumably related to the high level of humidity. The humidity was maintained at 85 percent throughout the course of this experiment.

Urea absorption through the lower surface of peach leaves for 48 hours resulted in 25.4 and 98.9 percent absorption at 10 and 24 C, respectively. Apple leaves absorbed 75.8 and 91.8 percent at 10 and 24 C, respectively, for the same period of absorption. Thus, a higher temperature greatly increased urea foliar absorption by peach leaves; apple leaves absorbed more at the lower temperature, hence with apple

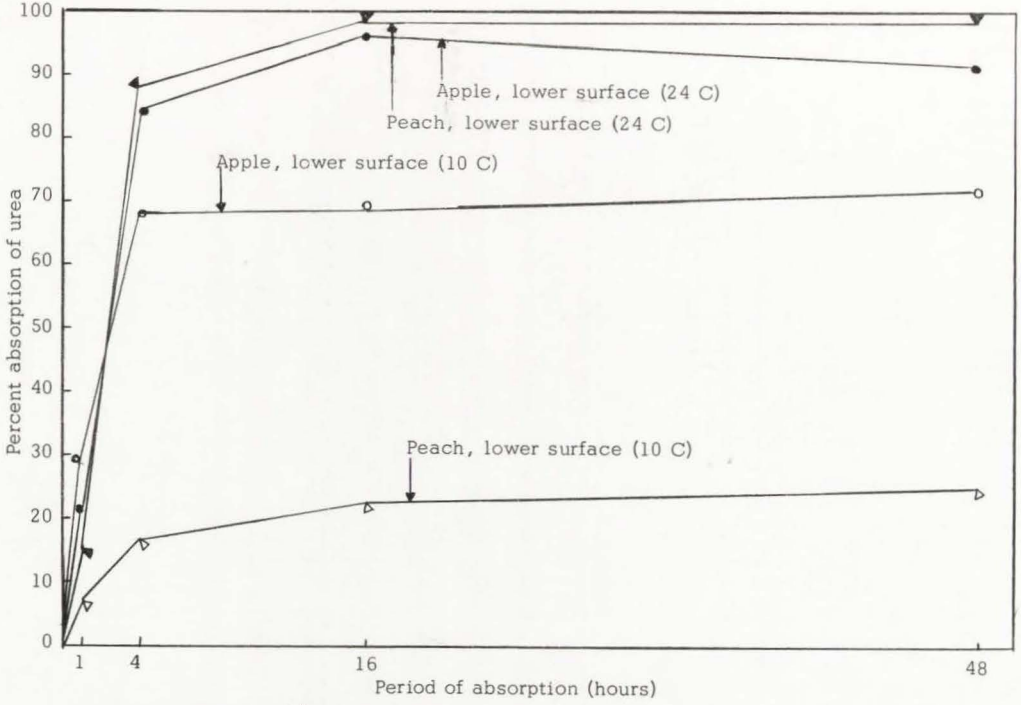


Figure 12. Absorption of ^{14}C urea by the lower surface of 45-day-old apple and peach leaves.

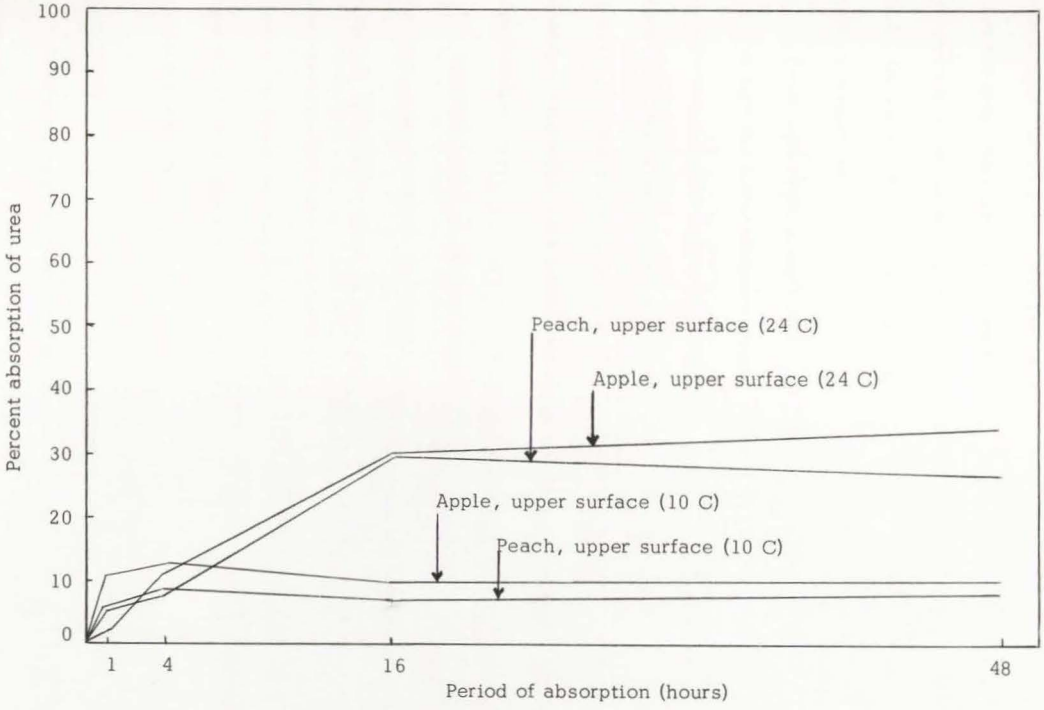


Figure 13. Absorption of ^{14}C urea by the upper surface of 45-day-old apple and peach leaves.

there was not as large a difference in the absorption percentage as with peach when absorption at the two temperatures are compared. Uptake from the upper leaf surfaces of both species substantially increased (three to four times) during the 4 to 16-hour period after application at 24 C, though at 10 C apparent absorption occurred only within the first 4 hours (Figure 13).

Peach and apple leaves absorbed 98 and 96 percent of the applied urea through the lower surfaces within 16 hours at 24 C, respectively. Since nearly all of the urea was absorbed by the leaf within 16 hours, there was little uptake from 16 to 48 hours. At the low temperature, apparently 2.7 and 4.2 percent of the urea was absorbed between 16 and 48 hours. However, this additional absorption is likely not statistically significant.

From the result of the two experiments described above, it is evident that under favorable conditions either species is able to absorb a relatively large percentage of urea within a short period of time. It is also apparent that adverse environmental conditions do not reduce absorption by apple as they do with peach. Under the conditions of these experiments, the interaction effects of temperature and humidity greatly influenced the rate of uptake.

Discussion

Epidermal hairs present on the lower surface of apple leaves increase their surface areas and are likely responsible for at least a portion

of the urea absorbed. Peach leaves do not have epidermal hairs and are more waxy in nature on their lower surfaces. This would, therefore, allow them to initially hold more liquid for possible absorption.

Some investigators believe there is metabolic acceleration of absorption following foliar application of chemicals such as 2,4-D. In these experiments, however, evidence indicates the limiting factor of absorption of urea is a physical rather metabolic phenomena. The absorption rate through the lower apple surface at 10 and 24 C was about the same as at the high humidity (Figure 5). At a low humidity, absorption was lower at 24 C than 10 C. This may be a result of a faster drying rate with the lower humidity, thus the urea solution was not in a fluid state and available for rapid absorption.

The findings are in agreement with those of Middleton and Sanderson (1965). They found that absorption of ^{137}Cs and ^{89}Sr was directly related to the external concentration of the solution. According to these investigators, absorption continued at a high rate at a relative humidity of about 50 percent. Uptake was sharply reduced as the supply diminished. Results of the experiments reported here indicate that the rate of uptake was low for the first hour, especially when a surfactant was not used (Figure 4 vs 5). Concentrated urea developing a large gradient between the outside and inside of the leaf may have been responsible for the increased rate of absorption after 1 hour.

Reduced absorption under low humidity conditions was unlikely due to the closure of stomata. Treated leaves were kept under light and

were fully turgid. Decreased absorption (3 fold or more) under low humidity conditions was also evident from the upper leaf surface (Figures 8 vs 10 and 9 vs 11). Teubner et al. (1957) reported a greater absorption of ^{32}P from the upper surface than from the lower. They used bean leaves which contained seven times more stomata on the lower surface than the upper.

Low humidity, high temperature and a combination of both induce thicker cuticle formation and higher suberization. These conditions, therefore, may reduce foliar penetration due to modification of the plant. According to Goodman and Goldberg (1960), high atmospheric humidity hydrated some of the cuticular components, such as pectin and cellulose. Hydration caused swelling of these compounds and, as a result, provided larger avenues for chemical penetration. It is likely that cuticular hydration for peach may not occur as readily at 10 C compared with 24 C, hence absorption is reduced.

Lower surfaces of both types of fruit tree leaves absorbed more urea than did upper surfaces. This was in agreement with results obtained by Cain (1956), who worked with urea on coffee and cocoa leaves. This, however, was not in agreement with Goodman and Goldberg (1960), who experimented with streptomycin, and the work of Teubner et al. (1957) with beans.

Slight suppression in absorption, which occurred with the surfactant, may have been due to the formation of a thin film of dehydrated or concentrated form of this compound over the cuticle (Figures 5 and 7).

It was noticed that for apple, surfactant slightly increased absorption at low humidity conditions (Figure 4). The data also show that with reduced humidity, a large portion of urea was not absorbed. It seemed that a relatively large amount of urea remaining in the solution mixture at the final stages of absorption may have modified the adverse effect of the surfactant. Under these conditions, the suppressing effect of a surfactant was not as evident.

Parr and Norman (1965) indicated the possibility of formation of chemical complexes with a surfactant. It appeared that the surfactant used (Colloidal X-77) did not form a complex with urea. Great inhibition in absorption would have been observed otherwise.

In order to observe any nitrogen response in peach, it seems a rapid initial entry of sufficient quantity is required. Since a higher drying rate and low temperature in the field are often limiting factors, absorption under these conditions may be improved by the use of a higher concentration of urea. Urea concentrations of 10 to 20 pounds per 100 gallons have increased nitrogen in leaves and induced more growth than in controls (Bullock, Benson and Tsai, 1952; Eckert and Childers, 1954; Norton and Childers, 1954). Similar to these results, 20 pounds of urea per 100 gallons of water gave nitrogen response under field conditions (Walker, 1952).

In reference to the findings reported in this paper and others, it could safely be stated that peach can absorb urea efficiently, but an optimum condition must be present. Field conditions are variable;

therefore, optimum absorption conditions usually can not be met. It is realized that a good nitrogen response with urea may not be obtained on commercial orchards unless penetration can be improved before the sprayed solution dries and absorption ceases.

SECTION III

CUTICULAR PENETRATION OF UREA AND AUTORADIOGRAPHY

Materials and Methods

Procedure for cuticular penetration

Healthy greenhouse-grown leaves of peach and apple about 1 month old were chosen for cuticular permeability experiments. In order to measure the permeability of the cuticle, it was decided to remove it from the leaf and work with it independently. The cuticle was separated from the rest of the leaf by enzyme action. The method used at first was similar to that of Orgell (1955), but it was observed that this procedure did not work well for removing apple cuticle. The method consisted of placing 50 1-cm plant discs punched from a leaf in 25 ml of a 2 to 3 percent pectinase enzyme solution having a pH of 4 and being maintained at 35 C $\pm$ 1. This solution also contained .1M acetate buffer and ppm merthiolate for prevention of mold and bacterial growth. The flasks were twirled gently several times a day to accelerate separation of the cuticle from the adjoining leaf epidermal cells and parenchyma.

The above enzyme solution with inclusion of other enzymes (cellulase and hemicellulase) was also tested and was preferable to pectinase alone for a clean separation of the upper cuticle of apple. The procedure of Schieferstein and Loomis (1956) containing .2 percent purified pectinase plus .2 percent partially purified hemicellulase and

.5 percent crude cellulase did not work well for apple cuticle separation; therefore, it was modified in order to separate both upper cuticles satisfactorily. Among the several combinations of the three enzymes used by these two researchers, a new mixture of .5 percent pectinase, .5 percent cellulase and .2 percent hemicellulase was developed. This was the most satisfactory mixture for the work reported here.

The enzyme solution described above was used in this study and prepared in an acetate buffer of the same strength and pH as used by Orgell (1955), but the temperature was held at 32 C. Peach cuticles, upper and lower, were separated very easily within a few days. The apple cuticles were more difficult to separate, and it was hard to get one that was clean and entirely free of attached leaf-cell particles.

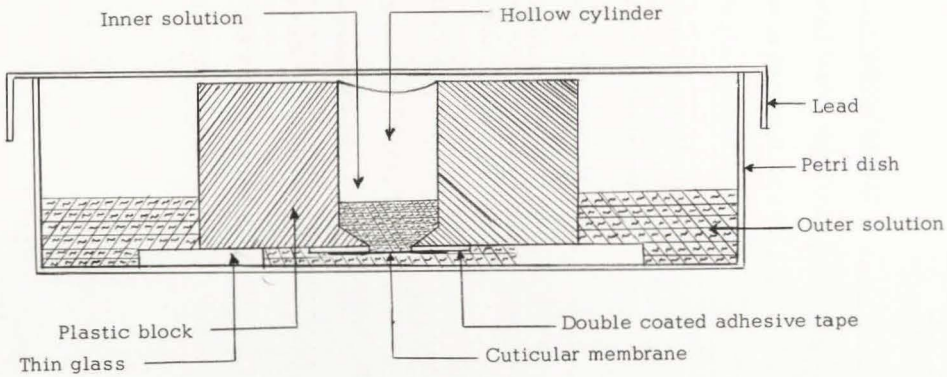
The separated cuticles were washed with intermittent changes of distilled water many times until the wash was completely clear of plant debris. The cuticles were washed by placing them on a filter paper in a suction funnel and running distilled water over them and draining the water by slight suction and gravity. The filter papers with the washed cuticles were then dried at room temperature and stored in a covered container until used. The lower cuticles of both peach and apple leaves were discarded because of their having perforations where the stomata had been over the cuticle and where the epidermal hairs on the apple had resulted in non-continuous membrane. The upper surface cuticle discs were examined under a microscope for possible rupture or other imperfections, and only undamaged specimens were used in these experiments.

A 2 by 3 cm block of clear plastic 12 mm thick having a small hollow cylinder 8 mm in diameter in its center top with an opening 3 mm in diameter on its reverse side was used for the cuticular permeability tests (Figure 14). A piece of double coated transparent adhesive tape with liner (Scotch No. 665) was placed tightly over the bottom hole. This double coated adhesive tape was used for sticking the cuticle on the permeability test apparatus.

A sharp hypodermic needle with a 90 degree point was used to puncture the scotch tape over the hole in the plastic block. The outer protective layer of thin plastic was removed from the scotch tape, and the plastic block was then centered face down over a cuticle so that the cuticle was directly beneath the hole. With gentle pressure, the cuticle was adhered to the block. This immobilized cuticle was next examined under a low power microscope to verify that it was still unruptured.

The plastic block with a cuticle disc on the lower surface was placed on two small pieces of thin glass 10 mm by 20 mm in a petri dish having a diameter of 5.5 cm. This was done so that the cuticle disc did not touch the bottom of the petri dish. Six ml of distilled water were then poured into the dish. One hundred µl of .05M urea solution having an activity of .05 µc/µl was placed inside the hole in the plastic block.

A microscope cover slip was placed over the hole in the plastic block, and the petri dish lid was replaced. The dish was then placed in a water bath of 10 or 24 C, depending on the experiment. After 4, 16, 24 and 48 hours, 100 µl of water were removed from the dish and analyzed



SCALE 1 mm = .40 mm

Figure 14. Apparatus used for measuring the permeability of a cuticular membrane to urea.

for urea radioactivity (see Section II, method of radioactivity mreasurement). The activity was converted to millimicromoles of urea penetrating the cuticle.

Procedures for radioautography

Selected leaves were treated with labeled urea similarly to the method used in Section II for absorption under controlled conditions. After washing the treated spot, the lanolin ring was removed with soft absorbent tissues, and the treated area was covered with a small piece of masking tape. The leaves were then dried between pieces of thick blotter paper under moderate pressure as described by Crafts and Yamaguchi (1964).

The dried leaves were pasted on sheets of thick paper with their treated sides facing the paper. A sheet of medical X-ray film was then placed on top of the leaves, and the two sheets were kept in contact in an X-ray exposure folder for 35 days. The exposure folders were placed alternately with thick cardboard and sheets of foam rubber. On top of this stack was placed a piece of plywood with a heavy weight. The developing of the film was carried out according to the manufacturer's directions.

Results

Cuticular penetration

There were highly significant differences in urea penetration between the two species, among the four periods of penetration and between the two levels of temperature. All of the interactions of these three factors were also significant. The average cuticular penetration for all factors investigated was 958 and 1023 millimicromoles of urea for peach and apple, respectively. Urea penetration was higher in apple than it was in peach at the lower temperature level (10 C) for all the absorption periods studied (4, 16, 24 and 48 hours). Urea penetrated peach and apple cuticle at an almost equal rate, at the higher temperature (24 C) during the first 4 hours. Urea penetrated the peach cuticle more rapidly than it did apple after the 4-hour period. The ability of urea to pass through peach cuticle increased with time, possibly as a result of temperature and humidity and/or the effect that urea may have had on the cuticular membrane.

At the end of the 24-hour absorption period, nearly equal amounts of urea had penetrated apple leaves at 10 as at 24 C (Figure 15). The ratio of peach cuticular penetration for the two temperatures (24 over 10 C) was 2.1 after the 4-hour period and 2.7 after 48 hours. The ratio for apple cuticle was 1.4 after a 4-hour period. There was a deviation in the penetration trend after 16 and 24 hours (1.5 and 1.1, respectively) for apple. After 48 hours of penetration, the ratio increased to 1.4.

Penetration of organic and inorganic chemicals, including urea,

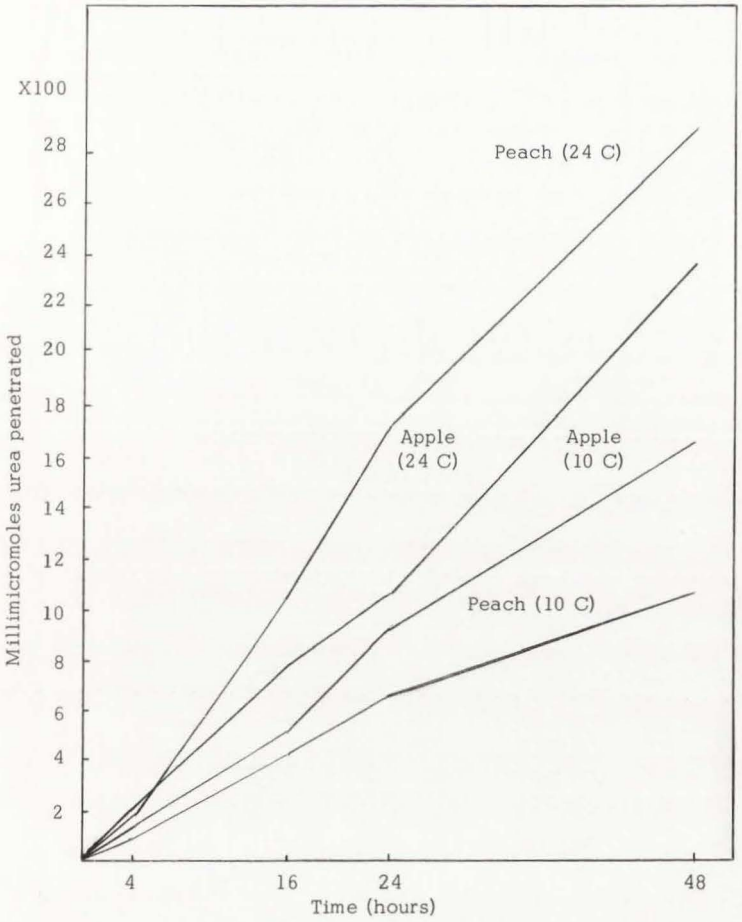


Figure 15. Penetration of ^{14}C urea through isolated cuticular membranes of apple and peach leaves.

through isolated cuticular membranes have been investigated with a number of plants (Darlington and Cirulis, 1963; Yamada, Wittwer and Bukovac, 1964). Although several factors affecting penetration of chemicals through cuticular membranes have been studied, they have seldom included the effects of temperature. Yamada, Wittwer and Bukovac (1965) indicated that urea penetrated the cuticle more readily than cations or anions. The rate of penetration of .1 mM of urea through stomaceous onion leaf cuticle increased at the end of a 25-hour test period. The authors suggested that urea was a self permeating agent in the case of onion cuticle. Urea penetration through tomato fruit cuticle occurred in a linear relationship with time. With peach cuticle held at 24 C, the rate of penetration increased after a rather short period (4 hours). This may have been due to the permeating ability of urea.

From the results of this experiment, it was concluded that:

1. Both apple and peach cuticular membranes were permeable to urea.

2. The permeability rate was greater with the increased temperature (10 vs 24 C).

3. The permeability of the peach cuticular membrane increased with time at 24 C but not at 10 C.

Radioautography

The relative humidity was maintained at 85 percent for the radioautography experiments. Colloidal X-77 (.1 percent) was used in all

experiments unless otherwise noted. The variable treatments were temperature (10 and 24 C) and absorption periods (1, 4, 8, 12, 16 and 24 hours).

After 4 hours of absorption at 10 C, urea had translocated a very limited distance in apple leaves (Figure 16, a and b). After 16 hours, however, translocation had increased 2 to 3 fold (Figure 16, c and d). Only a limited amount of urea was absorbed in peach leaves after 16 hours when held at 10 C (Figure 16, e). Translocation occurred between the veins rather than through the veins in both apple and peach when the solution was applied to the upper surface (Figure 16, a, b, c and d; Figure 17, a, d and e for apple; Figure 18, c and d for peach). Urea was absorbed and translocated through the veins of peach lower and, to a limited extent, through apple leaves when applied to the lower surface (Figure 17, c for apple; Figure 18, a and b; Figure 21, b and c for peach). The surfactant applied on the lower surface of the peach leaves did not materially influence translocation (Figure 18, a and b; Figure 21, b). More translocation occurred in apple than with peach through the upper surfaces (Figure 17, d and e for apple; Figure 18, c and d for peach).

No translocation was evident, and a limited amount of absorption occurred with peach leaves within 1 hour after treatment at 10 C (Figure 19, a, b, c and e). After 8 hours, there was limited uptake but still no indication of translocation (Figure 19, d). Translocation was limited in peach leaves also at 24 C (Figure 20, a, b, c, and d). Leaves "e" and "f" (Figure 20) exhibited some absorption and translocation after

Figure 16. The effect of ^{14}C urea applied to the upper surface of apple and peach leaves at 10 C. The reverse side of the treated leaves are illustrated in the upper portion of the photograph; ^{14}C radioactivity within the leaves is demonstrated in the lower portion. Urea was washed after 4 hours from the apple leaves "a" and "b" and after 16 hours from leaves "c" and "d." It was washed from peach leaf "e" after 16 hours. Exposure time, 35 days; treatment was 10 µl of 1 percent urea with activity of .5 µc.

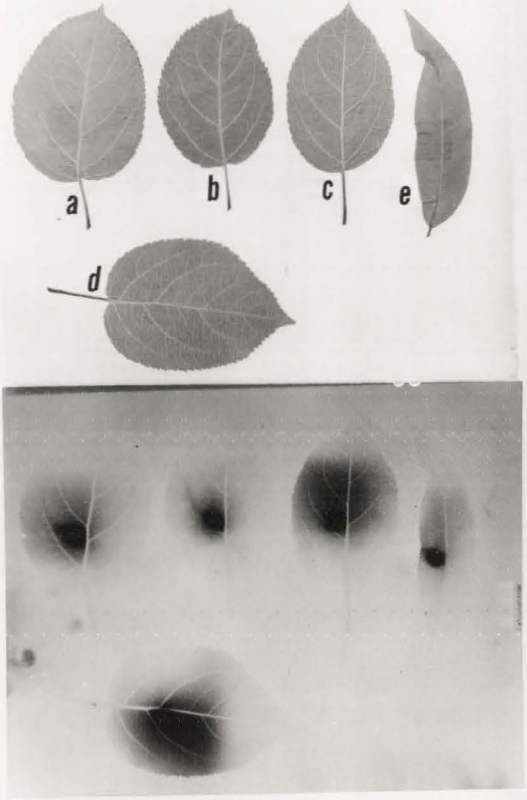

a
b
c
e
d

Figure 17. The effect of ^{14}C urea applied to the surface of apple leaves. The reverse side of the treated leaves are illustrated in the upper portion of the photograph; ^{14}C radioactivity within the leaves is demonstrated in the lower portion. Urea was applied to the upper surface of leaf "a" at 10 C and washed after 8 hours. Urea was applied to the lower surface of leaves "b" and "c" at 24 C and washed after 8 hours. Leaves "d" and "e" received same treatment as leaves "b" and "c" except treatment was applied to the upper surface. Exposure time, 35 days; treatment was 10 µl of 1 percent urea with activity of .5 µc.

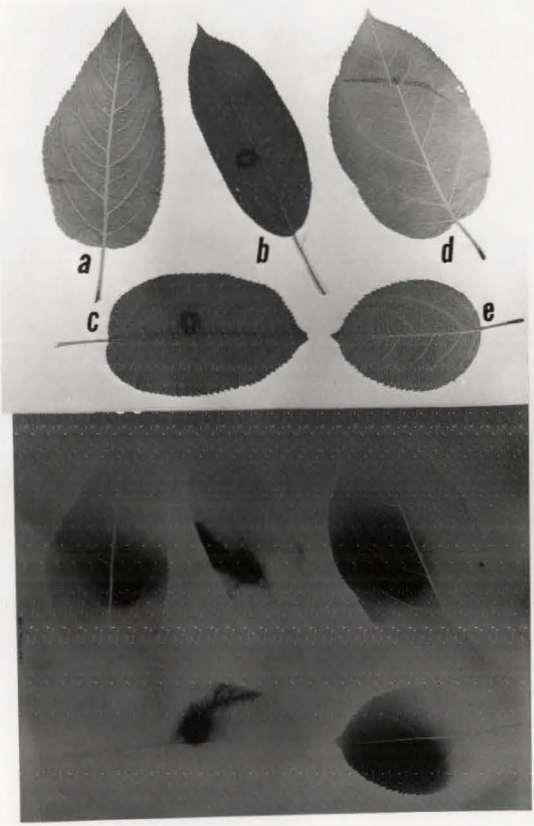

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Figure 18. The effect of ^{14}C urea applied to the surface of peach leaves at 24 C and washed after 8 hours. The reverse side of the treated leaves are illustrated in the upper portion of the photograph; ^{14}C radioactivity within the leaves is demonstrated in the lower portion. Urea without surfactant was applied to the lower surface of leaves "a" and "b," and with surfactant to the upper surface of leaves "c" and "d." Urea was washed from all leaves after 8 hours. Exposure time, 35 days; treatment was 10 µl of 1 percent urea with activity of .5 µc.

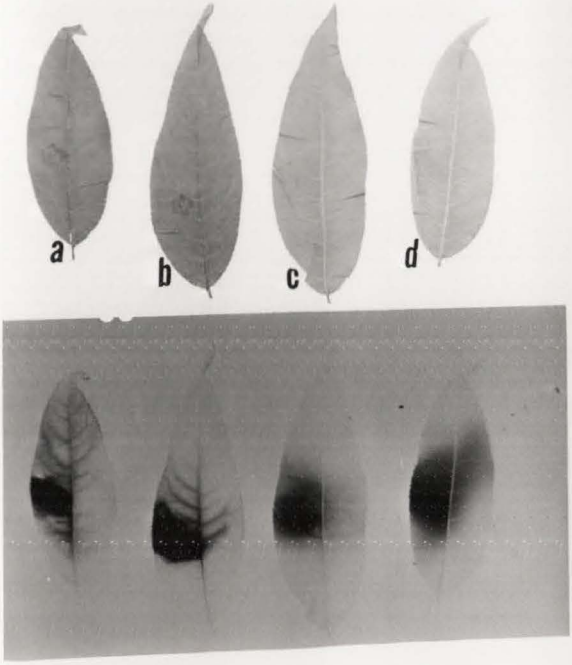
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Figure 19. The effect of ^{14}C urea applied to the surface of peach leaves. The reverse side of the treated leaves are illustrated in the upper portion of the photograph; ^{14}C radioactivity within the leaves is demonstrated in the lower portion. Urea was applied to the upper surface of leaves "a" and "b" at 10 C. They were washed after 1 hour. Similar treatments were applied to leaf "c," except to the lower surface. Leaf "d" received a similar treatment as leaf "c," except the treated spot was washed after 8 hours. The upper surface of leaves "e" and "f" were treated at 24 C and washed after 1 and 8 hours, respectively. Exposure time, 35 days; treatment was 10 µl of 1 percent urea with activity of .5 µc.

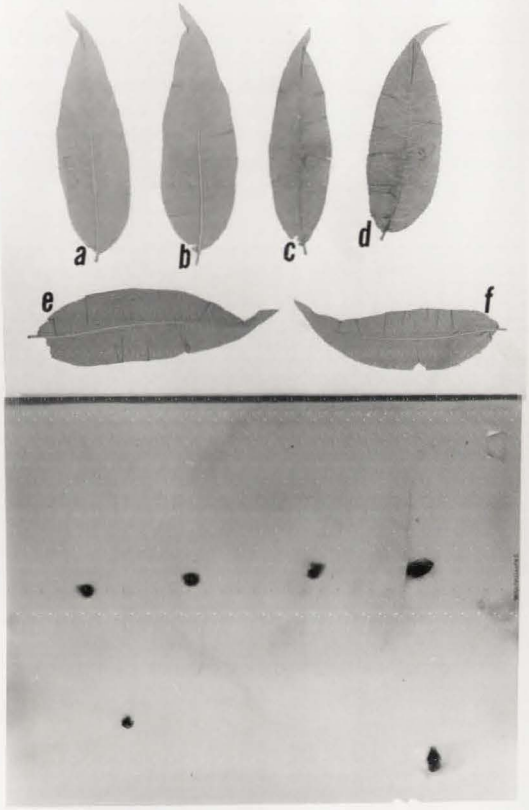
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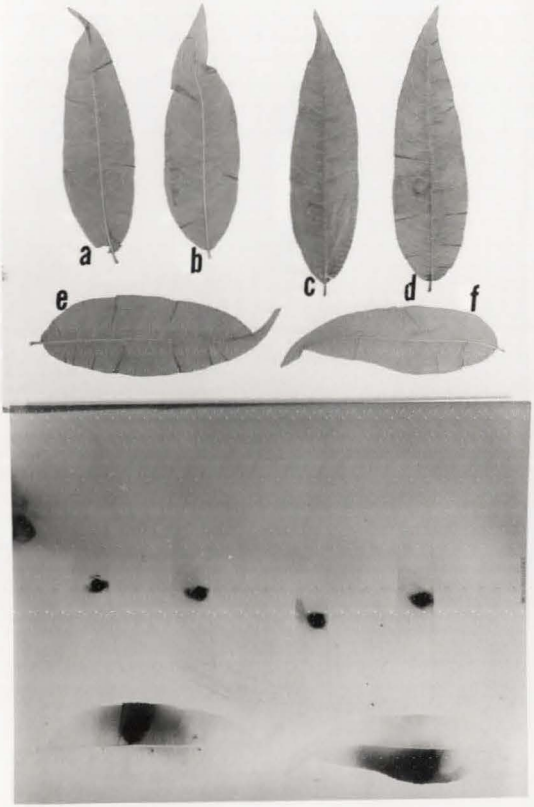
Figure 20. The effect of ^{14}C urea applied to the upper and lower surface of peach leaves at 24 C. The reverse side of the treated leaves are illustrated in the upper portion of the photograph; ^{14}C radioactivity within the leaves is demonstrated in the lower portion. Urea was applied to leaves "e" and "b" on the upper surfaces and washed after 1 hour. Leaves "c" and "d" received a similar treatment except on the lower surface. Leaves "e" and "f" were treated on the upper surface and washed after 8 hours. Exposure time, 35 days; treatment was 10 µl of 1 percent urea with activity of .5 µc.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8 hours though the extent of translocation was less than with apple leaves receiving a similar treatment. High ^{14}C activity was apparent in the veins of peach leaves after 12 and 24 hours of treatment (Figure 21, b and c).

SECTION IV

MICRORADIOAUTOGRAPHY AND HISTOCHEMICAL STUDIES

Materials and Methods

Plant materials were chosen from the greenhouse-grown trees as described earlier. One-month-old leaves were used. An area having a diameter of 10 mm on the lower surface of the leaves was treated with 25 microliters of .4 percent urea having an activity of .2 microcurie ^{14}C and containing .1 percent Colloidal X-77. Absorption was allowed to continue for 4 hours at 24 C and 85 percent relative humidity. After the termination of that period, the leaf was thoroughly washed with distilled water, and strips of leaf about 3 mm wide were cut and frozen.

Freezing was accomplished immediately after cutting. A small cone about 1 cm wide and 2 1/2 cm long made of aluminum foil was constructed, and several drops of water pre-cooled nearly to the freezing point were placed in it. The cone was then held with forcepts, and the lower half was immersed in a container of liquid nitrogen. After the drops of water had frozen, a second pair of forcepts was used to hold a strip of treated leaf inside the cone. More drops of water were added at intervals until the strip was entirely encased with ice. The tissue was quickly frozen using liquid nitrogen. One problem encountered with this type of quick freezing was that of shattering of the ice. In order to reduce this problem, shortly after the water was frozen, the cone was removed from the liquid

nitrogen. The frozen cones of ice and plant were stored at -20 C for a few days prior to sectioning.

To mount the frozen tissue on a cryostat specimen holder, a few drops of chilled water were placed on the holder, and the cone of frozen tissue was inverted over it; the two were then quick frozen together. More drops of water were added until the specimen became tightly adhered with ice to the holder. The sectioning temperature was maintained at -10 C.

Specimens were sectioned at a thickness of either 12 or 16 microns. Most of the specimens cut at 12 microns shattered, so the majority were cut at 16. The sections were picked up with a microscope slide covered with double coated scotch tape. These slides had been previously chilled in the cryostat and, before using, they were sprayed on their posterior surfaces with freon gas. This extra chilling procedure was necessary to make a section adhere to the cover glass.

These slides were stored in a plastic slide box having a capacity of 25. After 50 slides had been prepared, the boxes were transferred to a cold chamber containing dry ice to chill them lower than the cryostat temperature. Later, the boxes were transferred to a freeze dryer and positioned in such a manner that the slides were maintained horizontally with the specimens facing up. The sections were dried under high vacuum for 8 hours, after which the boxes were quickly closed and placed in a refrigerator. Later, the slides were allowed to equilibrate at room temperature. A method suggested by Jensen (1962) consisting of frozen sectioning

and freeze drying with the application of a stripping film (AR 10, Eastman Kodak Company) was tested for studying the preliminary specimens. Satisfactory resolution was not obtained in this study using this method. Another method, as developed by Pickering (1966), was adapted with two modifications. One in the freezing technique, as described above, and the other in the application of the liquid emulsion on the fixed tissue sections. Photographic emulsion (L. 4 type from Ilford Limited, England) was diluted 1:2 and applied to the plant sections which were previously fixed in formaldehyde vapor. This differs from Pickering's method, since he applied a thin layer of dried emulsion on unfixed sections.

The procedure for fixing the plant section in formaldehyde vapor was similar to the method described by Benditt, Martin and Platter (1965). The temperature used for vaporization of paraformaldehyde was reduced from 80 to 50 C because of undesirable drying and shrinking of both tissue and scotch tape. The sections were fixed for 10 hours. Later, they were removed from the vapor chamber and cooled to room temperature. A piece of teflon pressed gently for a short time against the specimens flattened them firmly to the scotch tape. For emulsion application, the dipping technique described by Caro and Van Tubergen (1962) was used. Other directions were also followed accordingly. Exposure time varried from 24 to 120 hours. The slides were developed for 5 minutes in D-19 developer at 20 to 21 C.

Fresh cryostat sectioned tissues were used for studying cutin and pectinaceous substances. Pectinaceous substances were stained with

ruthenium red, 1:5000, according to Jensen (1962). Gurr's (1965) method was used for examining the cutin. The preparations were mounted in 50 percent glycerol and examined shortly afterward.

Results and Discussion

Microradioauograms prepared from apple leaf sections indicated that ^{14}C urea adhered to the epidermal hairs (Figures 22 and 23). Microscopic examination of several hairs indicated that within a hair the ^{14}C activity was somewhat uniformly distributed, and such activity was always present on all hairs examined. However, the extent of ^{14}C activity was not uniform among the hairs. In the cross section of about 25 percent of the hairs, patterns of ectodesmata-like structures similar to those shown by Franke (1961) were observed by the ^{14}C track (Figure 24). These tracks were absent in some hairs (Figure 23). Further work is required in order to establish the nature of these observed patterns.

In numerous slides viewed, activity was not uniform throughout the tissue (Figures 25 and 26). Penetration through the lower surface of the leaves may occur through the cuticle, stomata or epidermal hairs in the case of apple. Peach leaves do not have epidermal hairs, hence penetration may occur through the cuticle and stomata. Cuticular absorption was evidenced by movement of ^{14}C urea and/or its metabolites through several layers of cells when ^{14}C urea was applied to the midrib vein of apple leaves (Figures 27 and 28). Stomata are not present on the midrib, hence absorption must have occurred through the cuticle in this particular

Figure 22. Microradioautogram of epidermal hair of apple leaf showing adsorption and absorption of ^{14}C urea. Magnification X1500.

GENERAL DISCUSSION

The factors influencing the absorption of urea by apple and peach leaves were studied. During the course of this investigation, the following areas were studied: absorption under greenhouse conditions, absorption under controlled environmental conditions, cuticular permeability, whole-leaf radioautography, microradioautography and histochemistry.

In this work, foliar absorption of urea from the lower surface of peach leaves grown under greenhouse conditions (24 C, day; 18 C, night) was relatively high. Bullock, Benson and Tsai (1952) working with peach leaves cultured in the greenhouse reported that limited absorption occurred in some experiments. Weinberger, Prince and Havis (1949) also did not obtain good response with this species; however, their experiments were done under field conditions. As a result of urea sprays, the nitrogen level in some cases increased when higher concentrations 10 pounds/ 100 gallons or more) were used (Eckert and Childers, 1954; Norton and Childers, 1954). In this study, the temperature (24 C) and the high relative humidity in the greenhouse and the high concentration of urea (4 percent) used likely resulted in a higher rate of absorption than would have occurred under field conditions.

Apple leaves absorbed most of the urea spray within the first hour following application. The possibility of involvement of epidermal hairs present on the lower surface of the apple leaf may have accounted for

this higher uptake (Franke, 1961). Considerably more urea was absorbed from the lower leaf surface compared with the upper surface for both species (Figure 2). This may have been due to a thinner cuticle. Guard cells have been reported to contain a large number of ectodesmata and have been reported by some researchers to be paths of entry (Middleton and Sanderson, 1965; Sargent and Blackman, 1965; Franke, 1967).

Continued absorption from the lower surface after the first hour may be aided by the presence of stomata since the solution appeared to have dried on the surface after that time. While it is not known definitely, vapor from the stomata may have kept the urea in a semi-fluid condition because of high transpiration. Absorption did not occur from the upper surface of peach leaves after 1 hour (Figure 2). No information concerning stomatal entry was obtained in this work, hence only speculation can be provided. There are workers who feel that stomata provide the major portal of entry of chemical into the leaf (Skoss, 1955), and there are others who believe there is a limited amount absorbed through stomata (Franke 1964, 1967; Sargent and Blackman, 1962).

High humidity (85 percent and temperature 24 C) increased urea absorption through peach leaves. Drier conditions (25 percent relative humidity) decreased absorption in peach even though the temperature was high. The interaction of temperature and humidity perhaps influenced absorption in two ways. The higher temperature increased permeability of the cuticle more in peach than in apple (Figure 15). The higher temperature, however, would increase the rate of evaporation of moisture from

the treated area, resulting in more rapid drying condition thus reduced absorption once the surface had dried. The absorption rate at high humidity during the first hour was relatively low in peach followed by an increase in absorption during the next few hours. This may have been associated with the higher concentration of urea solution on the leaf surface during the drying process. Surfactant, which increased the absorption from the lower surface especially in the first hour of foliar uptake, may have had some effect on the entry of urea through the stomata. The surfactant appeared to have a suppressing effect on urea uptake after 1 hour of absorption. While the nature of this suppression is not understood, it may have been due to the formation of a thin concentrated film of this compound over the cuticle. This may have prevented or reduced further uptake.

The studies performed on cuticular penetration with urea showed that high temperature aided penetration to a greater extent with peach than with apple. Permeability of peach cuticle increased with time when temperature was high.

Radioautograms of treated leaves indicated that 4 to 24 hours after treatment ^{14}C urea and/or its metabolites were translocated through only part of the leaf. Urea applied on the lower surface of the leaf generally moved through the veins, while application on the upper surface showed movement through interveinal spaces.

Microradioautograms of treated sections of apple leaves showed that the epidermal hairs of apple absorbed a relatively large quantity of

urea. Under favorable conditions of absorption for both apple and peach leaves (85 percent relative humidity, 24 C), absorption occurred as evidenced by the microradioautograms. Definite entry through the lower cuticle of the peach leaf was apparent.

Urea after entering the plant was presumably in a soluble form during the short period of uptake (4 hours). Most of the soluble urea and/or its metabolites could be washed out with application of the standard microtechnique method for microradioautography; therefore, the standard technique was not used. A modified method of microradioautography used in this study may provide a useful tool for further studies. The technique, however, requires some refinements in order to obtain better resolution for observing more detail.

Ectodesmata-like structures were observed in about 25 percent of the hairs of apple leaves, from the ^{14}C track. They were similar to those described by Schenpf (1958) and Franke (1961). The nature of these patterns were not studied in these experiments. Further work is needed to study the function of these structures. From the histochemical studies, it was evident that the degree of cutination in both apple and peach were apparently the same. Although pectinaceous substances were distributed similarly throughout the tissue of both species, they varied in regard to the outermost portion of the cuticle.

SUMMARY

Studies were conducted under greenhouse conditions to investigate the relative efficiency of urea absorption by 1-month-old peach and apple leaves. A 4 percent solution of urea containing .1 percent Colloidal X-77 was applied to the test leaves in the form of a fine spray. To aid in this procedure, an improved microsprayer with a 1 milliliter capacity was developed during the course of the study. With this sprayer, it was possible to measure small quantities of the applied urea with an accuracy of $\pm$ 1 percent as it was delivered to the leaf.

The greenhouse experiments indicated that the lower surface of peach leaves absorbed urea and approached the quantity absorbed by apple leaves at the end of 48 hours. Further experiments were conducted to evaluate the effect of temperature, humidity, and surfactant (Colloidal X-77) on absorption of a 1 percent ^{14}C urea solution by apple and peach leaves. Uptake was much greater from the lower surface of the leaves as compared to upper surface. Low relative humidity (25 percent) reduced absorption substantially. High temperature (24 C) under low humidity (25 percent) decreased absorption. Uptake was greatly increased under high temperature (24 C) and high relative humidity (85 percent). Peach leaves were more sensitive to temperature than apple, in regard to the amount of absorption that occurred. This was especially evident with the lower surface under high humidity conditions. In peach, a 5 to 10 fold decrease

in absorption was observed when temperature was lowered from 24 C to 10 C. Surfactant seemed to aid absorption through the lower surface within a short period after application. After 1 hour, however, less absorption occurred through leaves receiving surfactant than those not receiving surfactant.

Urea absorption through 45-day-old leaves at 85 percent relative humidity and 24 C indicated that within 48 hours over 90 percent of the urea applied to lower surfaces was absorbed by both species of leaves. The lower surface of peach leaves held at 10 C and, otherwise, comparable conditions as above absorbed only one-third as much as did apple.

Cuticular permeability tests indicated that upper cuticles from both species of leaves were permeable to urea. Generally, permeability was higher at 24 C than at 10 C; however, it seemed that permeability of peach cuticle increased with time at the higher temperature. After 48 hours, the amount of urea which penetrated through the peach cuticle at 24 C was 2.7 fold as much as at 10 C.

Translocation of urea and/or its metabolites had not taken place from the treatment spot after 1 hour. A definite absorption within 1 hour and translocation after 4 hours were observed under favorable conditions (24 C and 85 percent relative humidity). Radioautograms of ^{14}C urea treated apple and peach leaves indicated that the ^{14}C compounds had been translocated within a large portion of the leaf within 8 hours after application.

Studies were also performed on these species utilizing

microradioautography and histochemistry techniques. Microradioautograms prepared from treated leaf sections indicated that adsorption and absorption of radioactive urea occurred on the epidermal hairs of apple leaves. Urea entry occurred in both apple and peach leaves as evidenced by high activity of ^{14}C urea and/or its metabolites within the leaf tissue. Treatments of ^{14}C urea, on the apple veins only, showed that absorption had taken place into the cellular layers of the vein. Microscopic observations of freshly sectioned leaves of both apple and peach demonstrated a relatively high amount of pectinaceous substances between the cell walls and especially the bundle sheath and bundle-sheath extension cells. Pectinaceous substances were present more in apple cuticle than in peach cuticle.

LITERATURE CITED

Bald, J. G. 1952. Stomatal droplets and the penetration of leaves by plant pathogens. Amer. Jour. Bot. 39:97-99.

Behrens, R. W. 1964. The physical and chemical properties of surfactants and their effects on formulated herbicides. Weeds 12: 255-258.

Benditt, E. P., G. M. Martin and H. Platter. 1965. Application of freeze-drying and formaldehyde-vapor fixation to radioautographic localization of soluble amino acids, p. 65-75. In C. P. Leblond and Katherine B. Warren (Eds.). The use of radioautograph in investigating protein synthesis. Academic Press, New York and London.

Boynton, D. 1954. Nutrition by foliar application. Ann. Rev. Plant Physiol. 5:31-54.

Boynton, D., D. Margolis and C. R. Gross. 1953. Exploratory studies on nitrogen metabolism by McIntosh apple leaves sprayed with urea. Proc. Amer. Soc. Hort. Sci. 62:135-146.

Brants, H. D. 1965. Relation between ectodesmata and infection of leaves by C^{14} labeled tobacco mosaic virus. Virology 26:554-557.

Bruno, G. A. and J. E. Christian. 1961. Determination of carbon-14 in aqueous bicarbonate solutions by liquid scintillation counting techniques, application to biological fluids. Anal. Chem. 30: 1216-1218.

Bullock, R. M., N. R. Benson and Betty K. W. Tsai. 1952. Absorption of urea sprays on peach trees. Proc. Amer. Soc. Hort. Sci. 60:71-74.

Cain, J. C. 1956. Absorption and metabolism of urea by leaves of coffee, cacao and banana. Proc. Amer. Soc. Hort. Sci. 67:279-286.

Caro, L. G. and R. P. Van Tubergen. 1962. High resolution autoradiography. I. Methods. Jour. Cell Biol. 15:173-188.

Cook, J. A. and D. Boynton. 1952. Some factors affecting the absorption of urea by McIntosh apple leaves. Proc. Amer. Soc. Hort. Sci. 59:82-90.

Crafts, A. S. and S. Yamaguchi. 1964. The autoradiography of plant materials. Calif. Agric. Exp. Sta. Manual 35. Univ. of Calif., Davis. 143 p.

Crisp, C. E. 1965. The biopolymer cutin. PhD Dissertation. University of California. 225 p. University Microfilms, Ann Arbor, Michigan.

Darlington, W. A. and N. Cirulis. 1963. Permeability of apricot leaf cuticle. Plant Physiol. 38:462-467.

Dilley, D. R. 1960. The assimilation of isotopic carbon and nitrogen labeled urea into the amino acids of apple and peach leaves. PhD Dissertation. North Carolina State College, Raleigh. 99 p.

Dilley, D. R. and D. R. Walker. 1961a. Assimilation of C^{14}, N^{15} labeled urea by excised apple and peach leaves. Plant Physiol. 36:757-761.

Dilley, D. R. and D. R. Walker. 1961b. Urease activity of peach and apple leaves. Proc. Amer. Soc. Hort. Sci. 77:121-134.

Drost-Hansen, W. 1965a. Aqueous interfaces. Methods of study and structural properties. Part I. Indus. Eng. Chem. 57(3):38-44.

Drost-Hansen, W. 1965b. Aqueous interfaces. Methods of study and structural properties. Part II. Indus. Eng. Chem. 57(4):18-37.

Drost-Hansen, W. 1966. The puzzle of water. Inter. Sci. Tech. Reprint. October. p. 86-96.

Drost-Hansen, W. 1967. The effects of biological systems of higher-order phase transitions in water. Annals New York Acad. Sci. 125:471-501.

Drost-Hansen, W. and Anitra Thorhaug. 1967. Temperature effects in membrane phenomena. Nature 215:506-508.

Eckert, J. W. and N. F. Childers. 1954. Effect of urea sprays on leaf nitrogen and growth of Elberta peach. Proc. Amer. Soc. Hort. Sci. 63:19-22.

Esau, Katherine. 1962. Plant anatomy. John Wiley and Sons, Inc., New York and London. 735 p.

Fisher, E. G. 1952. The principles underlying foliage applications of urea for nitrogen fertilization of the McIntosh apple. Proc. Amer. Soc. Hort. Sci. 59:91-98.

Fisher, E. G., D. Boynton and K. Skodvin. 1948. Nitrogen fertilization of the McIntosh apple with leaf sprays of urea. Proc. Amer. Soc. Hort. Sci. 51:23-32.

Fisher, E. G. and J. A. Cook. 1950. Nitrogen fertilization of the McIntosh apple with leaf sprays of urea II. Proc. Amer. Soc. Hort. Sci. 55:35-40.

Fisher, E. G. and D. R. Walker. 1955. The apparent absorption of phosphorus and magnesium from sprays applied to the lower surface of McIntosh apple leaves. Proc. Amer. Soc. Hort. Sci. 65:17-24.

Franke, W. 1959. Konnen Pflanzen Nahrstoffe durch die blatter aufnehmen? I vege der stoffeinwanderung. Umscha 59:308-310.

Franke, W. 1961. Ectodesmata and foliar absorption. Amer. Jour. Bot. 48:683-691.

Franke, W. 1964. Role of guard cells in foliar absorption. Nature 202:1236-1237.

Franke, W. 1967. Mechanisms of foliar penetration of solutions. Ann. Rev. Plant Physiol. 18:281-300.

Goodman, R. N. and H. S. Goldberg. 1960. The influence of cation competition, time, and temperature on the uptake of streptomycin by foliage. Phytopathology 50:851-854.

Gurr, E. 1965. The rational use of dyes in biology and general staining methods. Williams and Wilkins Co., Baltimore. 422 p.

Gustofson, F. G. 1956. Absorption of ^{60}Co by the leaves of young plants and its translocation through the plant. Amer. Jour. Bot. 43:157-160.

Hamilton, J. M., D. H. Palmiter and L. C. Anderson. 1943. Preliminary tests with uramon in foliage sprays as a means of regulating the nitrogen supply of apple trees. Proc. Amer. Soc. Hort. Sci. 42:123-126.

Jansen, L. L., W. A. Gentneer and W. C. Shaw. 1961. Effects of surfactants on the herbicidal activity of several herbicides in aqueous systems. Weeds 9:381-405.

Jensen, W. A. 1962. Botanical histochemistry. Principles and practice. W. H. Freeman and Co., San Francisco and London. 408 p.

Jyung, W. H. and S. H. Wittwer. 1964. Foliar absorption--an active uptake process. Amer. Jour. Bot. 51:437-444.

Kamimura, S. and R. N. Goodman. 1964. Evidence of energy requirement for absorption of carbon ^{14}-labeled streptomycin and leucine by apple leaves. Phytopathology 54:1467-1474.

Klingman, Glenn C. 1966. Weed control as a science. John Wiley and Sons, Inc., New York, London, Sydney. 421 p.

Koontz, H. and O. Biddulp. 1957. Factors affecting absorption and translocation of foliar applied phosphorus. Amer. Jour. Bot. 32:463-470.

Middleton, L. J. and J. Sanderson. 1965. The uptake of inorganic ions by plant leaves. Jour. Exp. Bot. 16:197-215.

Mueller, L. E., P. H. Carr and W. E. Loomis. 1954. The submicroscopic structure of plant surfaces. Amer. Jour. Bot. 41:593-600.

Norton, R. A. and N. F. Childers. 1954. Experiments with urea sprays on the peach. Proc. Amer. Soc. Hort. Sci. 63:23-31.

Oland, K. and T. B. Opland. 1956. Uptake of magnesium by apple leaves. Plant Physiol. 9:401-411.

Orgell, W. H. 1955. The isolation of plant cuticle with pectic enzymes. Plant Physiol. 30:78-80.

Orgell, W. H. and R. L. Weintraub. 1957. Influence of some ions on the foliar absorption of 2,4-D. Botan. Gaz. 119:88-93.

Parr, J. F. and A. G. Norman. 1965. Considerations in the use of surfactants in plant systems: A review. Botan. Gaz. 126:86-96.

Pickering, E. R. 1966. Autoradiography of mobile C^{14}-labeled herbicides in sections of leaf tissue. Stain Tech. 41:131-137.

Roberts, M. F., R. F. Batt and J. T. Martin. 1959. Studies on plant cuticle. II. The cutin component of the cuticle of leaves. Ann. Appl. Biol. 47:573-582.

Roberts, E. A., M. D. Southwick and D. H. Palmiter. 1948. A microchemical examination of McIntosh apple leaves showing relationship of cell wall constituents to penetration of spray solutions. Plant Physiol. 23:557-559.

Rodney, D. R. 1952. The entrance of nitrogen compounds through the epidermis of apple leaves. Proc. Amer. Soc. Hort. Sci. 59:99-102.

Sargent, J. A. and G. E. Blackman. 1962. Studies on foliar penetration. I. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. Jour. Exp. Bot. 13:348-368.

Sargent, J. A. and G. E. Blackman. 1965. Studies on foliar penetration. II. The role of light in determining the penetration of 2,4-dichlorophenoxyacetic acid. Jour. Exp. Bot. 16:24-47.

Schenpf, E. 1959. Untersuchungen uber darstellung und bau der ekodesmen und ihre beeinflussbarkeit durch stoffliche faktorem. Planta 52:644-708.

Schieferstein, R. H. and W. E. Loomis. 1956. Wax deposits on leaf surfaces. Plant Physiol. 31:240-247.

Scott, Flora M. 1950. Internal suberization of tissues. Botan. Gaz. 111:378-394.

Scott, Flora M., M. R. Schroeder and M. T. Franklin. 1948. Development, cell shape, suberization of internal surface and abscission in the leaf of the Valencia orange, Citrus sinensis. Botan. Gaz. 109:381-411.

Sitte, P. and R. Pennier. 1963. Umtersuchunger an cuticularen zellwandschichten. Planta 60:19-40.

Skoss, Jesse D. 1955. Structure and composition of plant cuticle in relation to environmental factors and permeability. Botan. Gaz. 117:55-72.

Swanson, C. A. and J. B. Whitney, Jr. 1953. Studies on the translocation of foliar-applied P^{32} and other radio isotopes in bean plants. Amer. Jour. Bot. 40:816-823.

Teubner, F. G., S. H. Wittwer, W. G. Long and H. B. Tukey. 1957. Some factors affecting absorption and transport of foliar-applied nutrients as revealed by radioactive isotopes. Mich. Agr. Exp. Sta. Quart. Bull. 39:398-415.

Thorne, G. N. 1958. Factors affecting uptake of radioactive phosphorus by the leaves and its translocation to the other parts of the plant. Amer. Jour. Bot. 22:381-398.

Utah State University Soils Laboratory. 1961. Determination of total nitrogen in soil or plant material. Mimeographed Procedure.

Van Overbeek, J. and R. Blondeau. 1954. Mode of action of phytotoxicals. Weeds 3:55-65.

Volk, R. and C. McAuliffe. 1954. Factors affecting the foliar absorption of N^{15} labeled urea by tobacco. Proc. Soil. Sci. Soc. Amer. 18:308-312.

Walker, D. R. 1952. Foliar versus soil application of nitrogen to peach trees. Unpublished MS thesis. Utah State University Library, Logan, Utah. 22 p.

Walker, D. R. 1955. Foliar and soil applications of nitrogen to sour cherry and magnesium to apple trees. PhD Dissertation. Cornell University, Itacha, New York. 204 p.

Walker, D. R. and E. G. Fisher. 1955. Foliar sprays of urea on sour cherry trees. Proc. Amer. Soc. Hort. Sci. 66:21-27.

Weinberger, J. H., V. E. Prince and L. Havis. 1949. Tests on foliar fertilization of peach trees with urea. Proc. Amer. Soc. Hort. Sci. 53:26-28.

Wittwer, S. H. 1957. Nutrient uptake with special reference to foliar absorption, p. 139-164. In Atomic Energy and Agriculture, AAAS, Washington, D. C.

Wittwer, S. H. and F. G. Teubner. 1959. Foliar absorption of mineral nutrients. Ann. Rev. Plant Physiol. 10:13-32.

Yamada, Y., S. W. Wittwer and M. G. Bukovac. 1964. Penetration of ions through isolated cuticles. Plant Physiol. 39:28-32.

Yamada, Y., S. W. Wittwer and M. G. Bukovac. 1965. Penetration of organic compounds through isolated cuticular membranes with special reference to ^{14}C urea. Plant Physiol. 40:170-175.

APPEN DIX

Table 1. Effect of time on absorption of 4 percent urea sprays through the upper and lower surfaces of 1-month-old apple and peach leaves. Data are expressed as percent urea absorbed.

Leaf surface	Period of absorption (hours)					
	Apple			Peach		
	1	6	48	1	6	48
Upper	32.7[a]	44.9	42.9	24.5	18.4	14.5
	28.6	51.1	36.8	16.6	16.4	16.4
	32.7	44.9	42.9	24.5	16.4	16.4
	32.7	49.0	44.9	22.5	14.5	14.5
Average	31.6	47.4	42.1	24.5	16.4	15.4
Lower	61.3	81.7	91.9	42.9	63.3	78.6
	65.4	85.8	91.9	49.0	53.5	81.7
	65.4	79.6	89.8	40.9	53.5	89.8
	63.3	83.7	91.9	44.1	55.6	89.8
Average	63.8	82.7	91.3	44.2	57.4	84.9

[a]One leaf from each of five trees was combined for each replicate. Each value given is one replication.

Table 2. Analysis of variance of the data in Table 1

Source	DF	MS	F
Species	1	4649.18	505.85**
Surface	1	20000.05	2369.67**
Period of absorption	2	1254.37	148.62**
SXSu	1	41.50	4.91*
SXP	2	246.36	29.18**
SuXP	2	1199.31	142.09**
SXSuXP	2	285.23	33.79**
Error	36	8.44	
Total	47	658.83	

[a]S = Species; Su = Surface; P = Period of absorption.

Table 3. Effect of temperature, time, relative humidity and surfactant on absorption of urea by 1-month-old peach and apple leaves applied to upper and lower surfaces. Data expressed as percent urea absorbed.

Treatment	Replication	Temperature: 10 C, Absorption period (hours) 1	10 C, 8	24 C, 1	24 C, 8
Relative humidity 25%					
No surfactant					
Peach					
Upper	1	4.8	4.2	6.6	3.8
	2	7.5	8.1	3.6	4.2
		6.1	6.1	5.1	4.0
Lower	1	12.7	10.6	10.8	32.6
	2	10.7	11.5	14.8	30.9
		11.7	11.5	12.8	31.7
Apple					
Upper	1	2.7	4.2	3.9	3.5
	2	4.5	3.2	2.6	5.3
		3.6	3.7	3.2	4.4
Lower	1	13.6	48.3	18.0	18.0
	2	9.9	51.7	23.3	23.0
		11.7	50.0	20.6	20.5
Surfactant					
Peach					
Upper	1	4.2	3.9	6.9	6.3
	2	6.7	4.8	4.4	9.0
		5.4	4.3	5.6	7.6
Lower	1	14.3	22.2	9.0	14.1
	2	19.7	17.5	11.1	13.6
		17.0	19.8	10.5	13.8
Apple					
Upper	1	8.7	8.6	3.5	7.5
	2	7.8	12.0	5.1	5.5
		8.2	10.3	4.3	6.5
Lower	1	43.9	70.5	22.0	20.9
	2	37.2	65.4	26.0	19.3
		40.5	67.9	24.0	20.1

Table 3. Continued

Treatment	Replication	Temperature: 10 C, Absorption period (hours) 1	10 C, 8	24 C, 1	24 C, 8
Relative humidity 85%					
No surfactant					
Peach					
Upper	1	0.7	1.8	2.1	24.8
	2	1.5	1.9	1.5	20.0
		1.1	1.8	1.8	22.4
Lower	1	1.5	8.4	2.7	98.9
	2	2.4	8.1	3.3	98.9
		1.9	8.2	3.0	98.9
Apple					
Upper	1	1.3	3.7	3.9	7.1
	2	1.0	4.5	1.8	10.8
		1.1	4.1	2.8	8.9
Lower	1	1.9	96.5	4.2	96.2
	2	3.4	97.6	4.7	95.3
		2.6	97.5	4.4	95.7
Surfactant					
Peach					
Upper	1	3.3	3.1	3.6	23.2
	2	2.4	4.9	5.4	25.8
		2.8	4.0	4.5	24.5
Lower	1	9.3	21.4	15.8	90.5
	2	12.4	14.1	12.7	92.9
		10.8	17.7	14.2	91.7
Apple					
Upper	1	9.0	9.9	6.3	31.8
	2	9.9	8.4	5.1	36.3
		9.4	9.1	5.7	34.5
Lower	1	48.9	66.7	30.0	83.0
	2	41.6	71.7	34.2	83.0
		45.2	69.2	32.1	83.0

Table 4. Analysis of variance of the data in Table 3

Source[a]	DF	MS	F
Species	1	4640.45	18.07**
Surface	1	18810.67	73.27**
Humidity	1	1766.42	6.88*
Surfactant	1	627.91	2.44 NS
Period of absorption	1	5744.57	22.37**
Temperature	1	641.27	2.49 NS
SXSu	1	2894.68	11.27**
SXH	1	21.87	NS
SXSr	1	722.47	NS
SXP	1	7.97	NS
SXT	1	1316.49	5.12*
SuXH	1	2060.04	8.02**
SuXSr	1	431.84	NS
SuXP	1	5053.91	19.68**
SuXT	1	407.93	NS
HXSr	1	454.92	NS
HXP	1	3647.65	14.20**
HXT	1	1968.00	7.66**
SrXP	1	296.18	NS
SrXT	1	263.67	NS
PXT	1	3831.39	14.92**

Table 4. Continued

Source[a]	DF	MS	F
SXSuXH	1	445.87	NS
SXSuXSr	1	897.31	NS
SXSuXP	1	501.27	NS
SXSuXT	1	110.47	NS
SXHXSu	1	388.86	NS
SXHXP	1	0.52	NS
SXHXT	1	116.10	NS
SXSrXP	1	13.73	NS
SXSrXT	1	230.32	NS
SXPXT	1	0.15	NS
SuXHXSr	1	6.79	NS
SuXHXP	1	694.27	NS
SuXHXT	1	93.69	NS
SuXSrXP	1	1419.79	5.53*
SuXSrXT	1	1442.51	5.61*
SuXPXT	1	581.00	NS
HXSrXP	1	899.38	3.50 NS
HXSrXT	1	545.70	NS
HXPXT	1	1150.24	4.48*
SrXPXT	1	505.22	NS
SXSuXHXSr	1	0.48	NS
SXSuXHXP	1	20.84	NS

Table 4. Continued

Source[a]	DF	MS	F
SXSuXHXT	1	22.44	NS
SXSuXSrXP	1	239.94	NS
SXSuXSrXT	1	1.30	NS
SXSuXPXT	1	74.80	NS
SXHXSrXP	1	252.75	NS
SXHXSrXT	1	138.74	NS
SXHXPXT	1	136.21	NS
SXSuXPXT	1	41.64	NS
SuXHXSrXP	1	203.18	NS
SUXHXSrXT	1	70.57	NS
SuXHXPXT	1	981.67	3.82 NS
SuXSrXPXT	1	179.21	NS
HXSuXPXT	1	0.21	NS
SXSuXHXSrXT	1	3.13	NS
SXSuXHXSrXT	1	1.06	NS
SXSuXHXPXT	1	136.89	NS
SXSuXSrXPXT	1	317.44	NS
SXHXSrXPXT	1	370.36	NS
SuXHXSrXPXT	1	231.46	NS
Error	64	256.73	

[a]S = Species; Su = Surface; H = Humidity; Sr = Surfactant; P = Period of absorption; T = Temperature.

Table 5. Effect of time and temperature on absorption of urea by upper and lower surfaces of 45-day-old peach and apple leaves. Data are expressed as percent urea absorbed.

	Replication	Absorption period (hours) 1	4	16	48
Temperature 10 C					
Peach					
Upper	1	6.1	8.9	7.7	8.3
	2	4.9	7.2	5.8	8.3
		5.5	8.5	6.7	8.3
Lower	1	7.6	18.0	20.0	24.3
	2	5.7	14.8	25.4	26.6
		6.6	16.4	22.7	25.4
Apple					
Upper	1	10.1	11.8	9.5	9.2
	2	11.3	12.6	10.4	11.3
		10.7	12.2	9.9	10.2
Lower	1	28.4	71.7	70.5	69.4
	2	30.8	65.0	66.7	76.3
		29.6	68.3	68.6	72.8
Temperature 24 C					
Peach					
Upper	1	4.2	5.8	31.6	28.0
	2	5.1	8.6	27.0	25.5
		4.6	7.2	29.3	26.7
Lower	1	13.5	91.2	97.7	99.1
	2	16.8	84.8	98.8	98.8
		15.1	88.0	98.2	98.9
Apple					
Upper	1	1.3	9.5	28.8	34.5
	2	2.2	12.5	30.3	33.8
		1.7	11.0	29.5	34.1
Lower	1	23.0	83.3	95.2	90.0
	2	20.6	85.7	97.0	93.6
		21.8	84.5	96.1	91.8

Table 6. Analysis of variance of the data in Table 5

Source[a]	DF	MS	F
Species	1	2141.36	447.98**
Surface	1	29678.55	6208.90**
Temperature	1	7947.66	1662.69**
Period of absorption	3	4050.10	847.30**
SXSu	1	1207.63	252.64**
SXT	1	2031.78	425.05**
SXP	3	26.76	5.59**
SuXT	1	2790.54	583.79**
SuXP	3	1649.47	345.07**
TXP	3	1240.89	259.60**
SXSuXT	1	1772.36	370.78**
SXSuXP	3	11.14	2.33 NS
SXTXP	3	49.44	10.34**
SuXTXP	3	269.22	56.32**
SXSuXTXP	3	137.30	28.72**
Error	32	4.78	
Total	63	1111.52	

[a]S = Species; Su = Surface; T = Temperature; P = Period of absorption.

Table 7. Effect of time and temperature on penetration of urea through isolated upper cuticles of peach and apple leaves. Data are expressed as millimicromoles of urea which penetrated the cuticular membrane.

	Temperature	Replication	Period of penetration (hours) 4	16	24	48
Peach		1	96	451	672	1083
	10 C	2	89	408	687	1141
		3	104	427	630	975
			96	428	663	1066
		1	181	985	1894	3054
	24 C	2	206	1121	1751	2847
		3	179	1063	1723	2792
			188	1056	1789	2897
Apple		1	131	522	987	1712
	10 C	2	147	476	891	1663
		3	129	558	898	1620
			135	518	925	1665
		1	197	848	1152	2217
	24 C	2	174	732	1063	2462
		3	212	765	971	2476
			194	781	1062	2385

Table 8. Analysis of variance of the data presented in Table 7

Source[a]	DF	MS	F
Species	1	50432.00	9.96**
Period of penetration	3	7308453.00	1444.35**
Temperature	1	4421392.00	873.79**
SXP	3	48112.00	9.50**
SXT	1	1171216.00	231.46**
PXT	3	756330.60	149.47**
SXPXT	3	196773.30	38.88**
Error	32	5060.00	
Total	47	653914.2	

[a]S = Species; P = Period of Penetration; T = Temperature.

VITA

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