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Studies on the Absorption of Nitrogen by Citrus
From Foliar Applications of Urea

By
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I. INTRODUCTION

Foliar applications of minor elements have been used successfully to correct deficiencies in some fruit trees. Recent reports (54) indicate that a satisfactory level of phosphorus nutrition can be maintained in certain vegetable crops by foliar application of o-phosphoric acid. The current widespread applications of insecticides, fungicides, and growth regulators to crops have caused considerable interest in the possibilities of applying major nutrient elements to the leaves of plants.

Nitrogen, being one of the major elements or "energy elements", has long been used in agricultural practices. However, its use as a nutrient applied to the foliage of plants was not known until recent years. Apples, tomatoes, and some ornamental plants are found to be responsive to nitrogen sprays, while peach trees and other stone fruits were not benefited by foliar application of nitrogen. The factors which control the absorption of materials sprayed on the foliage are not yet fully understood, but it seems that the efficiency of absorption of a spray application varies among different kinds of plants, probably because of morphological differences in the leaves.

Due to the waxy nature of their leaves, several questions arise concerning citrus and the use of foliar application of nitrogen. First is the question of whether citrus trees are responsive to nitrogen sprays; second, what concentration may be used; third, how rapidly is nitrogen absorbed; and fourth, does urea affect the plant in ways other than those related to the supply of nitrogen?

Experiments of Jones and Parker (36), and Haas (30) partially answered one of these questions since they found that both Washington navel orange trees and lemon cuttings were responsive to foliar application of nitrogen in the form of urea.

Since little is known about using foliar application of nitrogen on citrus, the work included in this thesis was designed to study the following phases:

- A. The Effect of the concentration of urea spray on citrus.
- B. The use of sucrose in eliminating leaf injury caused by high concentrations of urea sprays.
- C. The effects of stickers and a wetting agent on nitrogen absorption by the leaves.
- D. The effect of the acidity of the spray solution on nitrogen absorption.
- E. Comparative studies of foliar and soil applications of nitrogen.
- F. The rapidity of nitrogen absorption by the leaves.

II. REVIEW OF LITERATURE

A. The use of urea as a source of nitrogen in plants

Urea is found in higher plants only in small quantities (25) and is present in maximum amounts in seedlings, in young buds and leaves, and other actively growing tissues. Urea in young plant tissues is to a large extent combined with aldehydes (38). In terms of percentage of total amide, it amounted to 28-50% of the total amides in the seedlings of Dolichos biflorus and Phaseolus mungo according to Damodaran and Venketesan (16).

The formation of urea within the plant body has been fairly well demonstrated by different investigators. A general agreement is established that urea in plants is not derived wholly from a single source, but from two or more different substances. In a series of studies (15, 16), it was found that a part of the urea amide in the seedlings of D. biflorus arose from the hydrolysis of arginine and arginine-like compounds by the action of arginase. Another study by Fosse (25), showed that urea is derived from uric acid in the following steps:

The urea thus formed is not the result of a synthesis but is set free from uric acid and the nucleins. Metabolism of nitrogen is continued by hydration of urea to ammonia. These studies demonstrate the fact that the formation of urea is one of the important biochemical changes taking place in the course of protein regeneration in plants. As Gilbert (26)

stated, "The nitrogenous compounds eliminated by animals are chiefly urea, uric acid, and ammonia, which, along with other compounds, result from the breaking down of proteins and protoplasm. The same compounds are apparently formed in the life processes of the plant cell, but are again directly utilized in the formation of new protein compounds and are not eliminated as in animals".

It has not been demonstrated that molecular urea can be absorbed and utilized directly by plants. However, Bitcover and Wander (6) reported that the urea form of nitrogen can be absorbed by citrus trees. In their experiment, a known quantity of urea nitrogen in a known volume of nutrient solution was passed through the pot in which the tree was growing, and the solution was collected in a flask for quantitative determination of nitrogen. The process took about 10 minutes to be completed. The amount of nitrogen lost was calculated by difference from the concentration in the original nutrient solution. Results indicated that this value of nitrogen loss ranged from 10.6 to 26.5%. Based on an assumption that in such a short time (about 10 minutes) the changes or transformation of urea caused by microorganisms would be minimum, they considered that the changes in concentration of nitrogen in the solution would be solely due to absorption by the tree.

In the soil, urea is rapidly transformed into ammonia, and subsequently into nitrates. The process includes both ammonification and nitrification, and it goes through the following reactions:

The ammonium carbonate in solution partially reacts with the base ex-

change complex, and the pH of the medium goes up. The ammonium carbonate is nitrified according to the following reaction:

As the ammonium carbonate is oxidized to nitric acid, the hydrogen of the acid is dissociated as H^+ ions and gives the lower pH value at this point. The NO_3^- ion is associated with other bases and the nitrification process is completed. However, under certain circumstances, when the oxygen supply is insufficient to complete the oxidation of the nitrogen to the nitrate form, the following reactions will occur:

In this case nitrite accumulation may be considerable. Bitcover and Wender (6) reported that relatively high temperature is a factor in nitrite accumulation, and it seems that this high temperature may depress the activity of the nitrite-oxidizing microorganisms which ordinarily would convert the nitrite to nitrate.

Pasteur, according to Waksman (64), was the first to recognize that the transformation of urea to ammonia is brought about by a living organism, Torula ammoniacale. However, it was later discovered that organisms capable of decomposing urea are found in most families of bacteria, actinomycetes, and fungi. Miquel (64) found that the urea organisms of the surface soil were 1-2% of the total bacteria, and that manure and urine contain 10% of their flora as urea bacteria.

The rate of urea decomposition to ammonia is dependent on the soil type, soil moisture and temperature. In general, this process is very rapid in comparison with other sources of nitrogenous materials.

Kleberger (37) stated, "The speed of decomposition of urea varies with the physical texture of the soil; the lighter the soil, the quicker the decomposition". While Borders (7) found that certain soils possess only a slight ability to transform urea into ammonia, he also found that the addition of an ammonification factor, such as fresh manure or soy-bean urease, favors this transformation. Turchin (62) claimed that urea is quickly transformed in most soils. This was especially true for podzols and the slightly degraded chernozems, but very little transformation was noted in sandy soils and in carbonate soils. Rotini (52) had experimental evidence that the decomposition of urea in arable soils into ammonia takes place only in certain soils; he noted that practically no decomposition occurred in soils sterilized by heat or in artificial soil. Prince and Winsor (48) investigated the rate of decomposition of urea in cultures containing different percentage of soil and sand, about 10% moisture being used in sand cultures and 15% in half sand and half soil. The cultures were maintained at room temperature. The index for determining the rate of decomposition of urea was the amount of ammonia present in the cultures at different periods of time. After 5 days they found that only 3% of urea was converted to ammonia in the sand cultures, 67% in half sand and half soil, and 90% in soil alone.

A correlation was found to exist between the soil moisture content and the rate of transformation of urea to ammonia and subsequently to nitrate in Norfolk sand (35). The rate of ammonia accumulation from urea decreased with an increase in the soil moisture in the early period of incubation. Later, when nitrification began, the quantity of ammonia accumulated from urea decreased with an increase in soil moisture up to

13.95% of the dry weight of the soil. At 15.85% and 17.95% moisture the ammonia accumulation again increased. At 20.55% moisture, which represents an anerobic condition of the soil, very little ammonia accumulated from urea. Littauer (41) observed that although drought retarded the rate of urea transformation, increasing the soil moisture above 50% of its maximum water-holding capacity did not cause any significant change in the rate of decomposition. Smith (56) reported that the optimum soil moisture content for nitrate production from various nitrogenous materials was between 50 and 60% of the maximum water-holding capacity of Norfolk sandy loam soil. However, he obtained a larger nitrate accumulation from urea at 70% of the water-holding capacity of the soil than from ammonium sulfate, dry ground fish meal, or slaughter-house tankage.

Many investigators have observed that temperature is a factor which controls, in a great measure, the quantity of nitrate produced in unit time. Schlosing's observation, cited by Greaves (27), showed that nitrification is very slow at 7.5° C., quite marked at 11° C., reaches its maximum at 37° C., and inhibited entirely at 55° C. In Jones' study, he found that over an average daily temperature range varying between 10° and 30° C., the fluctuations in the production of nitrates from urea seemed to vary directly with temperature. Other findings (46) reported that the optimum temperature for nitrification in soil cultures is about 35° C. or slightly higher, although the process can take place between 15° and 40° C.

Because of its rapidity of transformation to ammonia and nitrates, which are generally considered to be the forms of nitrogen absorbed and utilized by plants, urea is one of the important nitrogen ferti-

lizers for plants. Various experiments in comparing the efficiency of urea and other soluble nitrogen-containing materials, such as ammonium and nitrate compounds, have been reported in the literature. Higher yields of sugar cane (17, 8), rice (61), soy-bean (2), mustard (40), and cotton (5) have been obtained by the application of urea. Appreciable growth produced by the urea-injected branches of pear (58) and apple seedlings (53) has been recorded. The immersion of iris bulbs up to 24 hours in 1% urea induced longer stem, deeper green foliage, and earlier and more abundant bloom (44). In a "crop-producing capacity" test, Anderson (1) found that 175 lbs. nitrogen as urea applied per acre of tobacco is approximately equivalent to 200 lbs. of nitrogen as cottonseed meal. However, Lewis (40) found no significant difference between the effects of ammonium and urea salts in pot cultures of barley plants.

Detrimental effects of urea also have been reported. Urea at a concentration of 1,000 p.p.m. showed 75% inhibition of growth on cress seedlings (4). Haas (29) stated "Urea-containing compounds when used in excess on citrus in soil sometimes cause yellowing of the leaf tip, or a mottling near the apex of the leaves". Wilting of young citrus trees was noted during the warmest period of the year when the source of nitrogen in the nutrient solutions was in the form of urea (6). There is also evidence that when urea was applied in direct contact with the seeds of cotton and beet, germination was considerably decreased (3). This injurious action of urea seems to be closely related to its conversion into ammonium carbonate, which dissociates with the liberation of free ammonia.

An experiment has been reported by Skok (55), who found that the appearance of calcium deficiency symptoms in the bean plant was noticeably delayed in plants that received urea as a source of nitrogen as compared with those that received nitrate nitrogen. He interpreted these results to be in support of Eckerson's findings (19) that calcium is essential for the assimilation of nitrates, by being necessary for the successful catalysis of the reduction of nitrates to nitrites by the so-called reductase enzyme. His plants receiving urea were thus supplied with a reduced form of nitrogen and the necessity for nitrate reduction was eliminated. He suggested that since other elements—potassium, phosphorus, and sulphur—were also found to be necessary for normal reductase activity by Eckerson (19), their deficiency symptoms may also be lessened in severity by the use of urea.

Similar experiments have been reported by Breen, Cillam, and Tendam (10). They found that tomato plants utilizing urea as a source of nitrogen, when deprived of their normal supply of phosphorus, did not exhibit the usual signs of phosphorus deficiency as soon as did deficient plants growing in a medium containing nitrate nitrogen. Chemical analyses of the plant tissue showed that the plants utilizing urea contained much more phosphorus per unit of dry weight than did the plants receiving nitrate nitrogen. Furthermore, during phosphorus starvation it took the plants growing in a medium containing urea longer to deplete this reserve store of phosphorus. They claimed that the delay in the appearance of deficiency symptoms by the plants furnished urea might be due to a greater accumulation of phosphorus and not to the removal of the necessity for nitrate reduction by the substitution of

urea for nitrate in the nutrient solution.

B. Foliar application of nitrogenous compounds

In 1939, while Hamilton, Palmiter and Weaver (32) of New York State Agricultural Experiment Station were working on the evaluation of fermate, ferric-dimethyl-dithio-carbamate ($\text{FeC}_7\text{H}_{18}\text{N}_3\text{S}_6$), for the control of apple scab and cedar-apple rust fungi, they found that the compound caused considerable increases in the green coloring of the leaves, and they thought it was possible that the greening of the leaves was due, at least partly, to the nitrogen which is contained in fermate. This possibility that nitrogen may be assimilated through the leaves in appreciable quantities suggested its application in foliar sprays as a means of controlling the supply of nitrogen to fruit trees.

In 1942, Hamilton, Palmiter and Anderson (31) commenced experiments to determine the effects of various nitrogenous compounds applied with the regular sulfur scab sprays on apple trees. They found that urea sprays at a concentration of 5 lbs. to 100 gallons of water caused a very rapid increase in the green color and chlorophyll content of the leaves without apparent injury to the foliage. Their experiments have suggested the usefulness of urea sprays in solving special problems of nitrogen fertilization where a control of amount and timing may be of paramount importance.

Because one of the major problems in the production of apples in the northeastern United States was the regulation of the nitrogen level to obtain satisfactory yields of well colored and high quality fruit, and because of Hamilton and his co-workers' discovery that

nitrogen sprays might possibly control the nitrogen level of fruit trees, much work along this line has been done by various investigators. However, this work was limited only to apple trees until 1948, when Weinberger, Prince and Havis (65) started to use urea sprays on the foliage of stone fruits. Even though they found that application of urea on the foliage of peach trees was not effective, other workers followed them in trying foliar application of nitrogen on many other fruit trees, field crops, and ornamental plants. Grapes (24), apricots, Japanese plums, almonds, and other stone fruits (50) receiving up to three applications of 5 lbs. of urea to 100 gallons of water were not benefited by the nitrogen sprays, while figs, olives, walnuts (50), cotton (59), roses, and chrysanthemums (47) seemed to assimilate the nitrogen applied as urea sprays.

Work done by Cook and Boynton (13) has confirmed earlier evidence that the lower surface of the leaf takes in urea much more readily than the upper surface and that young leaves absorbed it more efficiently than old ones in the case of young apple trees. On the other hand, Rodney (51) found that the amount of nitrogen entering through the upper surface was similar to that entering through the lower surface when sprays were applied in July. However, he believed it may be that a slightly greater amount of material entered through the lower surface from sprays applied in September. This was evidence that the nitrogen compounds entered directly through the leaf cuticle, since the upper surface of apple leaves contains no stomates. Cook and Boynton also found that there was a direct relationship between high urea absorption and high initial nitrogen level in the leaves when the values were

expressed as the percent of the applied urea which was absorbed by the leaves. Also high absorption seemed to be associated with low temperature, while the inclusion of a wetting agent in the spray solution more than doubled the percent absorption. Under all conditions there was a continuing movement of urea from the surface to the inside of the leaf over a period of three or more days following spray applications, and the urea was converted to protein nitrogen or moved out of the absorbing leaf over more than three days.

The experience with apples has stimulated interest among citrus growers in the use of nitrogen sprays in California. This work was started in 1947 and is still being continued at the Citrus Experiment Station in Riverside. Jones and Parker (36), spraying urea on Washington navel orange trees, found that the sprayed trees showed a marked increase in green color, a slight increase in vegetative growth, and an increase in fruit production over the unsprayed trees. Haas (30), in a study with lemon cuttings, carried out under greenhouse conditions to avoid possible effects of rain, found that sprayed leaves showed a marked improvement in the green color and some new growth in a few days. Tip and marginal burn accompanied the increased color in the older leaves, but this was eliminated by the addition of 2.5 lbs. of hydrated lime in 100 gallons of the spray solution. He suggested that with citrus trees, urea may be included with the various types of nutritional and insecticidal sprays which are being applied throughout the year.

During the period from 1939 to 1948, various organic and inorganic nitrogen-containing materials were tested as foliar sprays.

Results indicated that among all kinds of nitrogen carriers, materials which contain nitrogen in the form of urea (for example, Uramon) seemed to be the best for foliar applications (22, 23, 33). Based on the successfulness of a great volume of work with urea sprays on apple trees in New York State and in the northwestern United States, DuPont Chemical Company developed in 1949 a concentrated nitrogen fertilizer especially for application of foliar sprays, and named it "NuGreen". It is a light green-colored, semi-granular material containing 44% nitrogen in the form of urea along with conditioning agents which prevent caking and assure free flow and yet maintain high solubility.

Since The value of foliar application of urea results from the rapid absorption and utilization of the urea nitrogen by the leaves. Tests with apple leaves showed that a large proportion of the spray was absorbed during the first few hours and further absorption occurred later (12). This nitrogen which is absorbed by the leaves starts to be assimilated in a short period of time, and this enables growers to regulate the supply of nitrogen for optimum effects on early growth and bloom, fruit set, fruit development, and fruit coloration at maturity. Nitrogen applied to the soil may require considerable time to reach the leaves where it is used. It may take only a few days under good conditions, or many weeks if cold, wet soil conditions or dry weather prevail. In some cases the nitrogen applied to soil is carried below the root zone by heavy rains, or it may remain above the principal root zone for prolonged periods because of lack of rain or irrigation. Competition from cover crops or deficiency of functional roots may limit nitrogen absorption. Thus, under good conditions the nitrogen

may reach the leaves at the proper time, but under adverse conditions sufficient nitrogen may not become available when the nitrogen requirements of the tree are great.

Foliar applications of urea have also benefited pest control. In one experiment (45), the amount of fruit infected by apple scab increased from 17 to 30% as the rate of soil nitrogen was increased, while the urea-sprayed trees showed little increase in fruit scab over that on unfertilized trees. Palmiter (45) thought that the better scab control in the urea-sprayed trees may have been due partly to a better nitrogen balance in the trees, but the results also indicated a direct increase in fungicidal effectiveness when the urea was used in combination with sulfur and arsenate of lead. As the amount of urea added to the spray was increased, the amount of fruit scab was decreased from 17 to 13%. Stoddard (60) stated, "The fungicidal activity of sulfur plus urea is greater than that of sulfur alone. Against Sclerotinia and apple-scab conidia on glass slides, a mixture of sulfur and urea was approximately twice as effective as sulfur alone". He explained that the improved growth and color of the sprayed foliage indicated that nitrogen is absorbed. Normally an increase in foliage nitrogen increases susceptibility to scab. The improved control in these fungi is evidence of fungicidal synergism between sulfur and urea.

Urea sprays at a concentration of 5 lbs. per 100 gallons of water caused a visible deepening in the green color of the apple leaves (50). Hamilton (31) reported that Uramon sprays applied three times in early spring seemed to increase foliage color early in the season, but the effect gradually disappeared, while trees receiving an additional

application 30 days after bloom showed better color of the foliage throughout the growing season than the trees receiving three applications in the early spring. Fisher (21) reported the similar effects of late application of urea on apples, stating, "The later a spray is applied the greater is the nitrogen effect following that spray, within the limits from pre-blossom period to the time of the second cover spray".

Other evidence (23) showed that spray treatments with urea resulted in greater nitrogen and chlorophyll levels than for a given amount of nitrogen applied on the soil. Leaves from vigorous trees with a heavy crop of fruit which were sprayed with Uramon at a concentration of 2 lbs. per 100 gallons did not have a nitrogen content appreciably above that of the tree receiving no fertilizer, but leaves from the less vigorous trees with a light crop of fruit did have a significant increase (31).

The effects of urea sprays have been tested on a variety of vegetable crops. Cucumbers, celery, carrots, radishes, and tomatoes were found to be responsive to urea sprays, whereas snap beans and spinach were not affected by the sprays (49).

In testing roses in the greenhouse (47), beginning with the third weekly application of NuGreen at the concentration of 5 lbs. per 100 gallons, it became obvious that the sprayed bushes looked darker green than the controls. By the time the fifth application was made, the results were even more striking. In addition to better foliage, plants treated with NuGreen averaged 10 buds whereas untreated plants averaged only 6 buds per plant. A comparable increase developed in the number

and length of stems on the treated as compared with untreated plants. In chrysanthemums (47), the results were more striking, perhaps because the rougher leaves on the plants wetted more evenly and thus enabled more of the material to enter. As with the roses, the difference in foliage size and color soon became obvious.

Due to the striking effects of urea spray on plants, the consumption of this material has been increasing. About 500 tons of commercial urea were sold in New York State for the purpose of foliar applications on apple trees in 1950 (9). In 188 groves in the Cornell Cost of Spraying Survey, 43 used this as a main source of nitrogen.

III. METHODS AND PROCEDURES

A. Dipping trials with individual branches of mature trees

Dipping trials were made with individual shoots of mature trees. With the exception of the preliminary trials in using sucrose as a protectant against leaf burn caused by urea sprays and the tests conducted to study the response of three species of Citrus to urea spray in leaf injury, the plants used were Duncan grapefruit trees budded on sour orange rootstocks. The choice of Duncan grapefruit trees was made because of the ready availability and the relatively larger leaves of these trees.

The effect of the treatments upon the tip and marginal burn of the leaves, and on the total nitrogen content after treatment, received major attention in these studies.

Shoots uniform in age and location on the tree were selected and tagged with numbers. Different treatments were tested by dipping each shoot in the prepared solution of C.P. urea for about ten seconds. Each treatment was replicated from 3 to 5 times.

The first leaf samples were taken to measure the initial nitrogen content immediately before the treatments were applied. Later samples were taken from these same leaves, in order to eliminate the variation of the nitrogen content due to the thickness of the individual leaves. However, severe dropping of leaves resulted in some cases due to the treatments with high concentration of urea; consequently, it was sometimes impossible to take samples from the same leaves. In these cases, sample disks were taken from other similarly treated leaves.

B. Field experiments

A portion of the trees used for field experiments were 4-year-old sour orange seedlings located in a nursery of the Florida Citrus Experiment Station. The soil on which the trees were growing was classified as Lakeland fine sand with a low degree of fertility. Since no fertilizer had been applied during the past two years, most of the trees showed a considerable degree of yellowing due to deficiencies of nitrogen, magnesium, zinc, iron, and perhaps other plant nutrients. In February, 1952, when the experiments were started, fertilizer containing all necessary elements except nitrogen was applied to each tree at a rate of one-half pound per tree of 0-6-8-4-1- $\frac{1}{2}$ analysis. A second application of fertilizer of the same amount and analysis was made a month later.

In some experiments, 2-year-old Ruby grapefruit on sour orange rootstocks in the same location were used. Fertilizer treatments before experiments were the same as those on the 4-year-old seedlings.

In March, 1952, deficiency symptoms of magnesium, zinc, and iron had disappeared; however, yellowing of the leaves due to nitrogen deficiency was still present, and the trees showed stunted growth with a small amount of foliage. Treatments on these trees were started in March, and the urea was supplied in the form of NuGreen.

The nursery was rectangular in shape, approximately 40 x 600 feet, and leaf nitrogen of the sour orange seedlings varied due to location within the area. A preliminary survey showed that these values ranged from 0.84 to 3.23 milligrams nitrogen per 10 cm² of leaf area. This gave a good opportunity to study the relationship of nitrogen

absorption to the initial nitrogen content of the leaves.

Applications of NuGreen spray were made on the grapefruit trees by using a 3-gallon hand sprayer. The rate of applications for each treatment was 1.0 gallon spray solution per tree. On the sour orange seedlings, spray solutions were applied by means of a power sprayer. Approximately 1.0 gallon of spray solution was applied to each tree at each application.

C. Greenhouse pot cultures

Experiments were set up in the greenhouse under controlled conditions primarily for studies of nitrogen absorption. Ten-inch clay pots and glazed porcelain jars of 5-gallon size were washed and sterilized in 3% formaldehyde solution for 30 minutes. Each pot was filled with the well-washed sand to about 1 inch from the top. Two-year-old Valencia orange trees budded on rough lemon rootstocks and 2-year-old sour orange seedlings were planted individually on September 26, 1951. The budded trees were cut back to about 24 inches in height. The nutrient solution used is shown in Table 1.

In addition to the nutrients listed in Table 1, 1 ml. of the supplementary solution was added to each liter of nutrient solution. The supplementary solution was made up with the salts listed in Table 2. At the same time iron was added in the form of 0.5% iron tartrate solution, at the rate of 1 ml. per liter, once or twice a week or as indicated by the appearance of the trees.

The nutrient solution was applied to the trees twice a week at the rate of 1,000 ml. per tree. Each pot was watered with tap water a

Table 1. Nutrient Solution used for Pot Cultures in the Greenhouse

Salts	Molarity of Stock Solution	ml. per liter	p.p.m. of Nutrients				
			P	K	Mg	Ca	S
K_2SO_4	0.50	5		195.5			80.0
$MgSO_4 \cdot 7H_2O$	1.00	2			48.6		64.2
$Ca(H_2PO_4)_2 \cdot H_2O$	0.05	10	31			20	
$CaSO_4 \cdot 2H_2O$	0.01	200				80	64.2

Table 2. The Composition of Supplementary Solution used along with the Nutrient Solution in Greenhouse Pot Cultures

Salts	gm./liter	Amt. Element (p.p.m.)
H_3BO_3	2.36	0.50 B
$MnSO_4 \cdot 2H_2O$	1.70	0.50 Mn
$ZnSO_4 \cdot 7H_2O$	0.22	0.05 Zn
$CuSO_4 \cdot 5H_2O$	0.08	0.02 Cu
$H_2MoO_4 \cdot H_2O$ (85% MoO_3)	0.02	0.01 Mo

day or so before the nutrient applications in order to prevent the accumulation of the unused salts.

The purpose of the studies made in the greenhouse was to give better control to those factors such as rain and insect damage, as well as to the amount of urea spray solution put on the leaf surfaces. The technique in applying the solution to the leaves was similar to the one

which Cook and Boynton (13) and Montelaro (43) have used. The solution was applied on the leaf surfaces by using a camel-hair brush which was attached to a 2-ml. pipette. The amount of actual nitrogen applied to the leaves by this technique can be calculated with fairly high accuracy. Urea of C.P. grade was used.

Each treatment was applied to ten leaves which were grouped in age as closely as possible. These ten leaves for each treatment were selected from five different trees, using two leaves on each tree. At the end of each absorption period, which was assigned specifically for different treatments, the leaves were washed with 2 portions of 300 ml. of deionized water. The washing was done by means of a paint brush which was then thoroughly cleaned with another portion of 300 ml. of deionized water. The total volume of the washings amounted to one liter, and the aliquots of this on which urea nitrogen was determined varied, generally, from 2 to 5 ml.

From the differences between the amount of nitrogen applied on the leaves and the amount of nitrogen present in the washing after a certain length of time of application, the nitrogen loss was calculated as percent of applied nitrogen which was absorbed during that particular period. Since no appreciable loss of urea through the action of urease on leaf surfaces was found on apples (13), it was assumed that this loss of nitrogen after a certain period of time was solely due to the absorption by the leaves.

For comparative study of soil and foliar application of nitrogen, potted 2-year-old sour orange seedlings were used. Each pot was covered tightly with a metal cover to prevent any spray material from going down

into the pot and being absorbed by the trees through their roots.

D. Analytical methods

The analytical method used for total nitrogen determination was mainly the one which is described by Cotton (14). The leaf sample was digested in 2 ml. of concentrated sulfuric acid, and then oxidized with 30% hydrogen peroxide. The mixture was diluted to 100 ml., and a 5-ml. aliquot was transferred to a 50-ml. volumetric flask. One ml. of 2.5 N sodium hydroxide and 1 ml. of 10% sodium silicate were added and the solution was made up to volume with deionized water. The color of a 5-ml. portion of this diluted solution was developed by means of Nessler's reagent, prepared according to Vanselow (63). The color intensity was compared with a series of standards in a photoelectric colorimeter using a 425-B light filter.

For determination of urea nitrogen, the method described by Jamieson (34) was employed. A 2- to 5-ml. aliquot was transferred to a 12-ml. centrifuge tube. Five ml. of glacial acetic acid was added and stirred. Then 1 ml. of 5% xanthydrol in methyl alcohol was added and stirred again. After standing overnight in a cold room (approximately 50° F.), 1 ml. of methyl alcohol saturated with dioxanthydrol-urea was added to the surface, and after the mixture was centrifuged for 15 minutes, the supernatant liquid was drained off. The precipitate was washed with 4 ml. of 3:1 methyl alcohol-water solution saturated with dioxanthydrol-urea. The mixture was stirred, centrifuged again for 15 minutes, and the supernatant liquid was drained off. The precipitate was then dissolved in 11 ml. of 50% sulfuric acid. The color intensity was

read in an electrophotometer, using a 425-B light filter, and compared with a series of standards prepared simultaneously.

For total chlorophyll determination of leaves, the method reported by Compton and Boynton (12) was used. Fifty disks of 1 cm² area were removed from fresh leaves and placed immediately in a 30-ml. portion of 95% ethyl alcohol. The tissue was left to stand in the solvent for 24 hours and then mixed in a Waring blender for 5 minutes. The solution was then filtered into a 100-ml. volumetric flask using a Whatman 42 filter paper, and made up to volume by washing the pulp with portions of solvent. The extract was read in a photoelectric colorimeter, using a light filter which transmitted above 610 millimicrons. The value was reported as milligrams of chlorophyll per 100 cm² leaf area for two sides.

The water-soluble portion of nitrogen in the leaves was extracted according to the procedures given by Cook and Boynton (13). The urea fraction of the water-soluble nitrogen was determined by means of xanthidrol reagent (34). A group of untreated leaves served as a blank. After the extraction of the water-soluble nitrogen, the residue was dried and analyzed for insoluble nitrogen. The results of total nitrogen content were adjusted for variations in leaf area and reported as mg. N per 10 cm².

E. Sampling procedure and expression of results

When a large number of leaf samples has to be taken at the same time for chemical analyses, the usual procedure which involves cleaning, drying, grinding and weighing is too time consuming. Furthermore, most of the trees used in these studies were quite small, with only limited

amounts of foliage, and this made it impossible to follow the usual sampling procedure of taking several leaves from each quarter of the treetop. Therefore, the following modified procedure for sampling leaves was used:

Ten comparable leaves on a young tree or on a single shoot of a bearing tree were chosen. A single disk of 1 cm^2 in area was removed from the lamina of each leaf by means of leaf punch. These 10 disks of leaf tissue from 10 different leaves were collected as a composite sample. The sample thus obtained was cleaned with deionized water in order to remove the foreign matter and the spray materials retained on the surfaces of the leaves, which might interfere with the results of the chemical analysis. The sample was put into a 50-ml. Erlenmeyer flask for the determination of total nitrogen. The results were reported as milligrams of nitrogen per sample of 10 cm^2 fresh leaf tissue.

On several occasions, when the trees used were comparatively large in size, whole leaves were collected at random in the usual way. After the leaves were cleaned, 1 cm^2 of fresh tissue was removed from each leaf for determination of dry matter content, and the rest of the tissues were then dried, ground, and mixed. Portions of this dry sample corresponding to 10 cm^2 of fresh leaf were weighed out for determination of nitrogen. The results were expressed as milligrams of nitrogen per 10 cm^2 fresh leaf. The procedure was used in order to give uniformity of presentation of data.

IV. PRESENTATION OF DATA AND RESULTS

A. The effect of the concentration of urea spray on citrus

Since urea sprays sometimes have injurious effects on foliage, a study of the effect of different concentrations of urea solution sprayed on citrus leaves was made as the first step in these investigations.

Thirty-five similar shoots of a 28-year-old bearing Duncan grapefruit tree budded on sour orange rootstock were selected for uniformity. Seven different concentrations of urea solution were tested by means of dipping each shoot in the prepared solution. Each treatment was replicated five times and the treatments were arranged in a randomized block design. The different concentrations were: 0, 5, 10, 15, 20, 30, and 40 lbs. of urea per 100 gallons of water. Four applications of each treatment were made at weekly intervals, beginning October 30, 1951.

Leaf samples were taken twice. The first set of samples was taken on October 30, just before the treatments were begun. The second set of samples was taken on November 28, eight days after the last application of the urea solutions.

The treated leaves were carefully examined from time to time for marginal burn. Tip and marginal burns of the leaves in the treatments of 30 lbs., and even more so of 40 lbs., of urea per 100 gallons of water were noticeable 3 days after the second application. This burning became more and more severe, and by the time eight days had elapsed after the fourth application, when the leaves were sampled for nitrogen determination on November 28, 1951, some of the leaves had a burned area

Table 3. Total Nitrogen Content of Dunean Grapefruit Leaves before and after Four Applications of Urea of various Concentrations. (mg. N/ 10 cm²)

Shoot No.	Urea Treatments lbs./100 gallons	Total Nitrogen		Nitrogen Increase mg./10 cm ²	Mean Increase (Increase)
		Before Treatments	After Treatments		
1	0	2.34	2.68	0.34	0.26
4	0	2.34	2.68	0.34	
12	0	2.34	2.56	0.22	
18	0	2.50	2.60	0.10	
23	0	2.40	2.68	0.28	0.36
2	5	2.34	2.96	0.62	
7	5	2.24	2.40	0.16	
15	5	2.46	2.96	0.50	
27	5	2.13	2.40	0.27	0.56
35	5	2.22	2.48	0.26	
5	10	2.49	3.04	0.55	
9	10	2.46	3.12	0.66	
10	10	2.46	2.92	0.46	0.63
24	10	2.46	2.96	0.50	
31	10	2.34	2.96	0.62	
3	15	2.46	2.84	0.38	
13	15	2.34	3.10	0.76	0.58
25	15	2.49	2.84	0.35	
29	15	2.34	2.92	0.58	
34	15	2.22	3.28	1.06	

Table 3. Total Nitrogen Content of Duncan Grapefruit Leaves before and after Four Applications of Urea of various Concentrations - Continued.

Shoot No.	Urea Treatments lbs./100 gallons	Total Nitrogen Before Treatments	Total Nitrogen After Treatments	Nitrogen Increase mg./10 cm ²	Mean Increase (Increase)
6	20	2.22	3.24	1.02	1.05
11	20	2.46	3.44	0.98	
14	20	2.22	3.24	1.02	
20	20	2.34	3.28	0.94	
30	20	1.83	3.12	1.29	
8	30	2.40	3.24	0.84	0.89
16	30	2.49	3.32	0.83	
21	30	2.49	3.20	0.71	
23	30	2.13	3.40	1.27	
32	30	2.25	3.04	0.79	
17	40	1.92	3.28	1.36	1.38
19	40	2.40	3.76	1.36	
22	40	2.16	3.96	1.80	
26	40	2.25	3.76	1.51	
28	40	2.34	3.20	0.86	

Least Significant Difference Required for Treatment Means:

1% level : 0.41 mg./10 cm², 5% level : 0.31 mg./10 cm².

covering as much as one-third of the entire leaf. Leaves treated with lower concentrations, below 20 lbs. per 100 gallons, showed no burning at any time.

The total nitrogen contents of the treated leaves are given in Table 3. The results of analysis of variance of the data are presented in Table 3a, Appendix.

It is apparent that grapefruit leaves are able to absorb nitrogen in the form of urea applied to the foliage; however, variations in the nitrogen absorption existed between individual shoots.

Treatments with 40 lbs. of urea per 100 gallons gave considerable greater increase of nitrogen in the leaves than did any other treatments; however, the effects of leaf injury showed that the use of this concentration is not practical.

There was little difference between treatment means of 30 and 20, and between treatment means of 15 and 10 lbs. of urea per 100 gallons of water. Statistically, differences between treatment means required 0.31 mg. nitrogen per 10 cm² leaf tissue at the 5% level in order to be significant.

Treatments with 5 lbs. of urea per 100 gallons of water did not increase the nitrogen content as compared with the checks. It was thought that the amount of actual nitrogen in this concentration was probably not sufficient to cause a measurable increase in the nitrogen content of the leaves. If 10 lbs. of urea per 100 gallons of water caused a noticeable nitrogen increase when the solution was applied 4 times, then naturally the question arises as to whether 5 lbs. of urea per 100 gallons of water will give similar results when 8 applications

of this concentration are made. Other experimental trials were conducted to answer this question.

Triplicate shoots of Duncan grapefruit tree were tested with the solution of 5 lbs. of urea per 100 gallons of water. Treatments consisted of from one to eight applications of this solution were applied at 2-day intervals from September 7 through September 22, 1952. Leaf samples were taken from each shoot for nitrogen determination on September 7, just before the first applications of treatments were made and two days after applications of the respective treatments. The checks were sampled on September 7 and 24. The total nitrogen content before and after treatments, together with the values of nitrogen increase, are given in Table 4, while the results of analysis of variance of the data are presented in Table 4a, Appendix.

Significant differences of nitrogen increase for treatments were obtained. The total nitrogen content of the leaves increased as the number of applications increased. This regression relationship of the two is shown in Figure 1. The increase was markedly noticeable after the third application. The difference was significant at the 5% level after 3 applications and it was significant at the 1% level after 5 applications of the 5-pound concentration of urea. In the previous experiment where 4 applications of the same concentration did not increase the nitrogen content, it was apparently not solely due to the low concentration itself. Since one of the characteristics of nitrogen in plants is its translocation from one part of the plant to another, with the previous experiment where the leaf samples were taken eight days after the last application, the comparatively small amount of

Table 4. Effect of Successive Applications of Urea (5 lbs. per 100 gallons) on the Nitrogen Content of Grapefruit Leaves (mg. N per 10 cm² leaf area)

Treatment	Total Nitrogen		Nitrogen Increase	Mean
	Before Treatments	After Treatments		
Checks	2.64	2.72	0.08	0.15
	2.40	2.64	0.24	
	2.44	2.58	0.14	
One Application	2.64	2.80	0.16	0.15
	2.49	2.60	0.11	
	2.49	2.68	0.19	
Two Applications	2.61	2.68	0.07	0.20
	2.40	2.72	0.32	
	2.40	2.60	0.20	
Three Applications	2.46	3.04	0.58	0.46
	2.55	2.84	0.29	
	2.70	3.20	0.50	
Four Applications	2.49	3.04	0.55	0.49
	2.64	3.04	0.40	
	2.25	2.76	0.51	
Five Applications	2.64	2.80	0.16	0.50
	2.49	3.20	0.71	
	2.58	3.20	0.62	
Six Applications	2.88	3.20	0.32	0.58
	2.70	3.32	0.62	
	2.52	3.32	0.80	
Seven Applications	2.40	3.08	0.68	0.71
	2.01	2.68	0.67	
	2.13	2.92	0.79	
Eight Applications	2.31	3.12	0.81	0.86
	2.49	3.48	0.99	
	2.70	3.48	0.78	

L.S.D. — 1% : 0.345 mg./10 cm², 5% : 0.252 mg./10 cm².

Figure 1. Regression of Number of Applications of Urea at a Concentration of 5 lbs. per 100 Gallons of Water on Nitrogen Increase of Duncan Grapefruit Leaves.

nitrogen absorbed by the leaves from 4 applications of the lower concentration was probably translocated from the absorbing leaves to other parts of the tree before the 8-day period was up. In this experiment, however, leaf samples were taken only 2 days after the application of each treatment, and it appeared that the absorbed nitrogen was still present in the absorbing leaves when they were sampled.

A similar experiment was set up with 4-year-old sour orange seedlings under field conditions. Treatments consisted of from one up to eight applications of urea spray at a concentration of 10 lbs. per 100 gallons which were applied to duplicate trees at weekly intervals starting April 21, 1952. Total nitrogen and chlorophyll contents of the leaves before the first and 72 hours after the last applications of each treatment were measured as the index of nitrogen absorption by the leaves. The results are summarized in Table 5.

As in the results previously obtained, there was a direct relationship between the number of applications and the total nitrogen increase in the leaves. One or two applications of urea at a concentration of 10 lbs. per 100 gallons did not increase the nitrogen content in sour orange seedling leaves. However, the nitrogen increase was marked 72 hours after the third application, and the total nitrogen increase was progressively greater as the number of applications was increased.

There was also a significant correlation between the number of applications and the amount of chlorophyll increase (Figure 2). The increase of chlorophyll was not as marked as the increase of nitrogen when less than 4 applications of urea were made. However, after the

Table 5. Total Nitrogen and Chlorophyll Contents of Leaves of Sour Orange Seedlings sprayed with Urea at a Concentration of 10 lbs. per 100 Gallons of Water

Treatment	Nitrogen, mg./10 cm ²			Chlorophyll, mg./100 cm ²		
	Before Treatments	After Treatments	Difference	Before Treatments	After Treatments	Difference
Checks	1.43 1.41	1.41 1.38	-0.02 -0.03	0.82 0.78	0.77 0.71	-0.05 -0.07
One Application	1.36 1.40	1.32 1.40	-0.04 0.00	0.78 0.80	0.78 0.80	0.00 0.00
Two Applications	1.47 1.53	1.48 1.56	0.01 0.03	0.87 0.97	0.87 0.97	0.00 0.00
Three Applications	1.14 1.20	1.37 1.45	0.23 0.25	0.75 0.81	0.77 0.83	0.02 0.02
Four Applications	1.28 1.30	1.47 1.57	0.19 0.27	0.83 0.97	0.92 1.04	0.09 0.08
Five Applications	1.43 1.33	1.94 1.46	0.51 0.13	0.97 0.91	1.30 1.18	0.33 0.27

Table 5. Total Nitrogen and Chlorophyll Contents of Leaves of Sour Orange Seedlings sprayed with Urea at a Concentration of 10 lbs. per 100 Gallons of Water - Continued

Treatment	Nitrogen, mg./10 cm ²		Chlorophyll, mg./100 cm ²	
	Before	After	Before	After
	Treatments	Treatments	Treatments	Treatments
	Difference	Difference	Difference	Difference
Six Applications	1.22	1.60	0.38	0.84
	1.34	1.80	0.46	0.92
Seven Applications	1.50	2.31	0.81	0.90
	1.38	2.11	0.73	0.82
Eight Applications	1.18	1.94	0.76	0.81
	1.22	2.10	0.82	0.97

L.S.D. for Nitrogen Means:

1% = 0.33 mg./10 cm²

5% = 0.22 mg./10 cm²

L.S.D. for Chlorophyll Means:

1% = 0.28 mg./100 cm²

5% = 0.19 mg./100 cm²

Figure 2. Regression of Number of Applications of Urea at a Concentration of 10 lbs. per 100 Gallons of Water on Chlorophyll Increase of Leaves of Sour Orange Seedlings.

fifth application of urea the rate of increase in chlorophyll content was similar to the rate of nitrogen increase.

B. Effect of the concentration of urea sprays on leaf injury of three commercially important species of Citrus

Since leaf injury was noted on leaves which have been treated with high concentrations of urea in the preceding experiments, similar trials were also conducted in an attempt to determine this resultant effect of urea on different species of the genus Citrus. Three trees of each commercially important species were used, Valencia orange for Citrus sinensis, Duncan grapefruit for Citrus paradisi, and Dancy tangerine for Citrus reticulata. One shoot on each tree of the three species was dipped in each of the solutions of 0, 5, 10, 15, 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. A single application was made on August 14, 1952.

Observations of leaf injury at 24-hour intervals indicated that the first symptoms of leaf burn were noticeable in two days with some treatments. The severity of this leaf burn increased as the days passed by, until 7 days after the applications of urea treatment the severity of leaf burn stopped changing; however, some of the leaves which had been treated with high concentrations of urea dropped before the end of the 7-day period.

Figure 3 shows the effects of urea concentrations on leaf burn of Duncan grapefruit leaves. Figure 4 shows the same effects on Duncan grapefruit leaves, but here the leaves have been cut by means of a leaf punch which gave mechanical injuries of the leaf tissue. In comparing these two pictures, it should be noted that leaf burn showed up on

Figure 3. Leaf Injury as a Result of Urea Treatments on Uninjured Grapefruit Leaves.

From left to right in the upper row are leaves treated with 0, 5, 10, and 15 lbs. of urea per 100 gallons of water. In the bottom row are leaves treated with 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. Note that the injury starts to appear at a 30-lb. urea concentration.

Figure 4. Leaf Injury as a Result of Urea Treatments on Injured Grapefruit Leaves.

From left to right in the upper row are leaves treated with 0, 5, 10, and 15 lbs. of urea per 100 gallons of water. In the bottom row are leaves treated with 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. Note that the injury starts to appear at a 15-lb. urea concentration.

Figure 5. Leaf Injury as a Result of Urea Treatments on Uninjured Orange Leaves.

From left to right in the upper row are leaves treated with 0, 5, 10, and 15 lbs. of urea per 100 gallons of water. In the bottom row are leaves treated with 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. Note that the injury starts to appear at a 30-lb. urea concentration, and compare the degree of burning with Figure 3.

Figure 6. Leaf Injury as a Result of Urea Treatments on Injured Orange Leaves.

From left to right in the upper row are leaves treated with 0, 5, 10, and 15 lbs. of urea per 100 gallons of water. In the bottom row are leaves treated with 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. Note that the injury starts to appear at a 15-lb. urea concentration.

Figure 7. Leaf Injury as a Result of Urea Treatments on Uninjured Tangerine Leaves.

From left to right in the upper row are leaves treated with 0, 5, 10, and 15 lbs. of urea per 100 gallons of water. In the bottom row are leaves treated with 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. Note that the injury starts to appear at a 15-lb. urea concentration.

Figure 8. Leaf Injury as a Result of Urea Treatments on Injured Tangerine Leaves.

From left to right in the upper row are leaves treated with 0, 5, 10, and 15 lbs. of urea per 100 gallons of water. In the bottom row are leaves treated with 20, 30, 40, and 50 lbs. of urea per 100 gallons of water. Note that the injury starts to appear at a 15-lb. urea concentration.

uninjured leaves at a concentration of 30 lbs. of urea per 100 gallons of water, whereas on injured leaves this effect showed up at the lower concentration of 15 lbs. Similar results were obtained from orange and tangerine leaves as illustrated by Figures 5, 6, 7, and 8.

In comparing Figures 3, 5, and 7, leaf injuries showed up only slightly at a concentration of 30 lbs. of urea per 100 gallons of water on grapefruit leaves, whereas on orange leaves the degree of burning was considerably higher for this concentration. Tangerine leaves showed leaf injury at a concentration of 15 lbs.

Under the conditions of this experiment, it seemed that the tolerance of the tree to high concentrations of urea spray varied with different species. The difference between grapefruit and oranges was not great, but both seemed able to withstand a higher concentration of urea spray than the tangerine.

Another interesting point must be mentioned here, that grapefruit leaves treated with a given concentration, for example, 30 lbs. of urea per 100 gallons of water, did not show leaf injury in the first week in the experiment reported in the preceding section when the urea solution was applied on October 30, 1951, whereas this leaf injury showed up in only 2 days in the experiment reported here, when the urea solution was applied on August 14, 1952.

C. The use of sucrose in eliminating leaf injury caused by high concentration of urea spray

Leaf injury caused by urea sprays can be eliminated by the addition of certain materials in the spray solution. Haas (30) found that the leaves of lemon cuttings were injured by urea sprays at a

concentration of 44.8 lbs. per 100 gallons of water, but that when 2.5 lbs. of hydrated lime were added to this concentration of urea spray, the leaf injury was eliminated. Mack and Shaulis (42) reported that when 3-3-100 Bordeaux mixture was added to a 4-100 urea spray solution, which had been reported to damage the plant, the leaf injury of Concord grape was prevented. Other reports (43) indicated that magnesium sulfate added to the solution reduced leaf injury resulting from applications of urea spray to tomatoes.

According to Emmert and Klinkers' results (20), equal molar solutions of sucrose mixed with urea solutions stopped urea burning and enabled ten times as much urea to be used on tomatoes without burning as when no sucrose was used. This has stimulated interest in the use of sucrose on citrus for this purpose. In the first trials the equivalent of 120 lbs. of sucrose and 20 lbs. of urea per 100 gallons of water was used. The shoots of a mature Temple orange tree treated with this mixture showed no burning at any time. Then a treatment of the equivalent of 180 lbs. of sucrose and 30 lbs. of urea per 100 gallons of water was used after which no burning of the shoots resulted. Urea alone at the 20-lb. rate caused slight burning and at the 30-lb. rate burned so severely that most of the leaves dropped within a few days after the application had been made.

Since these preliminary trials looked promising, a more detailed study of the use of sucrose as a protectant against urea burning was made. Twenty-two treatments of urea-sucrose combinations were applied on Duncan grapefruit leaves. Each treatment consisted of two shoots, A and B. Another set of 22 shoots of similar uniformity was also

selected as controls. The first application of urea-sucrose mixtures was made on both shoots A and B on January 22, 1952, while the second application on January 29, the third on February 5, and the fourth on February 13, 1952, were made on shoots A only.

Shoots B, which were treated with the mixed solution only once on January 22, at no time showed any burning except when the urea concentration was high, 40 lbs. per 100 gallons of water, in which case burning of leaf tips was noticed 3 days after the applications were made. The degrees of burning on shoots A are given in Table 6.

It is apparent that sucrose, when added to the urea solution applied to citrus foliage, prevented urea burning to some extent. The highest concentration of urea used, 40 lbs. of urea plus no sucrose per 100 gallons of water, caused moderate burning of the leaves after only one application. This burning was noticed 4 days after the first application, and from then on moderate and severe burning showed up until 16 days after the fourth application, when most of the leaves had dropped off the shoots. When 120 or 180 lbs. of sucrose was added to this concentration of urea, leaf injury did not appear until 5 days after the second application. When 40 lbs. of urea and 240 lbs. of sucrose per 100 gallons of water were applied to the shoots, no burning of any type was noticed at any time.

With 30 lbs. of urea per 100 gallons of water, when 240 and 180 lbs. of sucrose were added, no burning resulted, but when 120 and 90 lbs. of sucrose were added, burning showed up slightly and moderately, respectively, after the fourth application of these sucrose concentrations; and when no sucrose was added in the solution, burning of leaves

Table 6. The Effect of Sucrose on the Reduction of Leaf Injury caused by Urea Sprays on Grapefruit Foliage

Treatment, lbs. of Urea to lbs. of Sucrose per 100 gal.	Degree of Burning on Date Examined*					
	Jan. 29	Feb. 4	Feb. 5	Feb. 7	Feb. 15	Feb. 29
40-240	0	0	0	0	0	0
40-180	0	x	x	xx	xxx	xxx
40-120	0	xx	xx	xx	xxx	xxx
40- 0	xx	xx	xx	xx	xxx	xxx
30-240	0	0	0	0	0	0
30-180	0	0	0	0	0	0
30-120	0	0	0	0	x	x
30- 90	0	0	0	0	xx	xx
30- 0	0	xx	xx	xx	xxx	xxx
20-240	0	0	0	0	0	0
20-180	0	0	0	0	0	0
20-120	0	0	x	x	x	x
20- 90	0	x	x	x	x	x
20- 60	0	x	x	x	xx	xx
20- 0	0	x	x	x	xx	xx
15-240	0	0	0	0	0	0
15-180	0	0	0	0	0	0
15-120	0	0	0	0	0	0
15- 90	0	0	0	0	0	0
15- 60	0	0	0	0	0	0
15- 30	0	0	0	0	0	0
15- 0	0	0	0	0	0	0

* Degree of Burning - 0: None, x: Slight, xx: Moderate, xxx: Severe dropping of leaves

appeared severely after the third application.

With 20 lbs. of urea per 100 gallons of water, when 240 and 180 lbs. of sucrose were added, no burning was noticed, but when 120, 90, and 60 lbs. of sucrose were added, slight burning was noticed after the second and third applications. When no sucrose was added to this concentration of urea, slight burning resulted after the second application and by the fourth application had become moderately severe.

With 15 lbs. of urea per 100 gallons of water, no leaf injury was observed at any time.

In general, when urea solutions were mixed with six times as much sucrose as urea, no leaf injury to Duncan grapefruit was observed under the conditions of this experiment.

D. The effects of sucrose, magnesium sulfate and lime on the absorption of nitrogen by citrus leaves

Sucrose, magnesium sulfate and lime have been found to reduce leaf injury which is caused by high concentrations of urea sprays on plants. An attempt was made to determine the effects of these so-called protectants against urea spray injury on the absorption of nitrogen by the leaves. Treatments consisted of : (1) 30 lbs. of urea alone; (2) 30 lbs. of urea plus 180 lbs. of sucrose; (3) 30 lbs. of urea plus 34.2 lbs. of magnesium sulfate (0.05 M solution); and (4) 30 lbs. of urea plus 3 lbs. of hydrated lime. These amounts per 100 gallons of water were applied to the leaves of 2-year-old Valencia trees which were growing in pots in the greenhouse.

The percent of applied nitrogen which was lost during the absorption period was termed as "percent absorption". The results of seven

runs are shown in Table 7. The results of analysis of variance are presented in Table 7a, Appendix.

The differences between treatment means were all significant at the 1% level. Apparently, sucrose and magnesium sulfate depressed the nitrogen absorption considerably. Lime did not depress the absorption of nitrogen from foliar applications of urea; on the contrary, it increased the average nitrogen absorption significantly. No leaf injury was observed in any case.

Table 7. The Effects of Sucrose, Magnesium Sulfate and Lime when added to Urea Sprays on the Absorption of Nitrogen by Valencia Orange Leaves

Absorption Period (hours)	Percentage of Absorption from			
	Urea Alone	+ Sucrose	+ $MgSO_4$	+ Lime
4	38.8	29.9	32.2	44.4
3½	26.7	11.8	16.2	30.1
5	46.5	32.2	40.0	46.3
2	34.1	25.8	29.4	36.5
2	30.7	21.6	28.9	34.2
3	28.3	14.3	18.3	28.3
3	26.4	14.3	21.8	28.3
Average	33.1	21.4	26.7	35.4

L.S.D. — 1% = 1.18%
5% = 0.88%

E. The effects of stickers and a wetting agent on nitrogen absorption

The ability of stickers to hold deposits through weathering and the decrease in surface tension, which gives a more even distribution of the spray droplets on the leaf surfaces, caused by the

addition of a wetting agent to the spray solution are well recognized. Various stickers and wetting agents are being included in spray applications for citrus in commercial practice. It was hoped that the use of these two materials also improve the efficiency of urea sprays on citrus.

In a preliminary study, the effect of sticker and wetting agent on the retention of spray material on the leaf surfaces was tested. Treatments were as follows: (1) 15 lbs. of urea alone; (2) 15 lbs. of urea plus 1.5 lbs. of Armour sticker; (3) 15 lbs. of urea plus 4 pints of Linck W-A wetting agent; and (4) 15 lbs. of urea plus both wetting agent and sticker. One hundred leaves for each treatment were dipped in the solution in a 100-ml. graduate cylinder. The amount of spray solution retained per unit area was measured for the four treatments. The results are given in Table 8.

Table 8. The Effects of Sticker and Wetting Agent when added to Urea Sprays on the Retention of Spray Solution on Leaf Surfaces

Treatment	ml. of Spray Solution Retained on 1,000 cm ²	Mean
Urea Alone	2.86 2.98	2.92
Urea + Sticker	3.02 3.06	3.04
Urea + Wetting Agent	3.25 3.27	3.26
Urea + Both	3.31 3.31	3.31

L.S.D. — 1% = 0.12 ml./1000 cm² 5% = 0.08 ml./1000 cm²

From this preliminary study it was learned that the addition of either a sticker or a wetting agent to urea sprays retained more spray material per unit area than did the urea solution alone; and when both of these two were added, the amount of spray material which can be retained on leaf surfaces was even more than when only one of the two was used.

Further studies on the effect of these two materials on the absorption of nitrogen by the leaves were made under greenhouse conditions. Treatments applied were the same as in the preliminary study. After various periods of time following applications (absorption periods), the leaves were washed and the washings were analyzed for urea nitrogen. The results from this experiment are presented in Table 9.

Table 9. The Effects of Sticker and Wetting Agent when added to Urea Sprays on the Absorption of Nitrogen by Valencia Orange Leaves

Absorption Period (hours)	Percentage of Absorption from			
	Urea Alone	+ Sticker	+ W.A.	+ Both
3	38.1	48.4	53.4	66.5
3	40.4	46.6	53.4	66.5
2	41.9	48.9	50.8	53.7
2	41.9	46.9	55.3	56.0
5	51.9	53.4	63.7	70.2
Average	42.8	48.8	55.3	62.6

L.S.D. — 1% = 9.82%
5% = 7.12%

Significant differences were found between some of the treatment means (Table 9a, Appendix). Highest absorption of urea by the leaves was obtained from the treatment in which both sticker and wetting

agent were added, the percent absorption for this treatment being almost 50% higher than in the treatment with urea alone. According to the data obtained here, the addition of a wetting agent to urea sprays gave a higher value of nitrogen absorption than did the addition of a sticker only.

Various commercial grades of stickers and Linck W-A wetting agent were added to urea spray solutions to compare the effects of these materials on the absorption of nitrogen. Dipping of the Duncan grapefruit shoots in the solutions were made on August 22, September 1, and September 8, 1952. Leaf samples were taken immediately before and 48 hours after the third application. The nitrogen increase due to the treatments were measured and the results are summarized in Table 10.

It is apparent that stickers and a wetting agent when added to urea sprays increased the total nitrogen content of the leaves in a measurable amount as compared with leaves treated with solutions containing urea alone. Little difference was found between six stickers tested except in the case of oil emulsion, which gave a comparatively low value. Here again Linck W-A wetting agent gave a higher value of nitrogen increase than all of the stickers. It was apparent that the amount of spray solution retained on the leaf surface has a close correlation to the nitrogen absorption.

In order to obtain values which are comparable with Table 9, the amount of nitrogen applied was estimated for treatments with urea alone, urea plus Armour sticker, and urea plus Linck W-A wetting agent. Based on the preliminary data shown in Table 8, the amounts of nitrogen applied to the leaves for these three treatments could be calculated.

Table 10. Effect of Three Applications of Urea at a Concentration of 15 lbs. per 100 Gallons of Water, plus Various Commercial Stickers and Linck W-A Wetting Agent on the Nitrogen Increase of Duncan Grapefruit Leaves
(Each figure represents the average of 3 samples in mg. N/10 cm²)

	1	2	3	Treatment Number			7	8	9
				4	5	6			
Initial N	2.00	1.97	2.00	2.12	2.04	2.10	2.05	2.08	2.11
N after 3 applications	2.10	2.31	2.41	2.59	2.48	2.52	2.44	2.43	2.67
N increase	0.10	0.34	0.41	0.47	0.44	0.42	0.39	0.35	0.56
N applied** (mg./10 cm ²)		0.735					0.765		0.819
% increase		32.4					37.9		56.2

Treatment: 1, Checks. 2, Urea Alone. 3, Good-rite Latex V.L. 600 sticker.
4, Good-rite p.e.p.s. sticker. 5, Dowax 222 sticker.
6, Colloidal Z-1 sticker. 7, Armour sticker. 8, Oil emulsion sticker.
9, Linck W-A wetting agent.

* For amount of stickers and wetting agent used and the original data see Tables 10a and 10c.

** The amount of nitrogen applied was estimated to obtain values comparable with Table 9. For procedures in calculation see Text.

For example, in the case of treatment with 15 lbs. of urea alone, the average increase of nitrogen in the leaves after three applications was 0.34 mg./10 cm² leaf area. Subtracting the value 0.10 mg./10 cm² of the checks from this, the remaining 0.24 mg./10 cm² was considered as the nitrogen increase due to the treatment itself. Since 1,000 cm² leaf area retained 2.92 ml. of the spray solution at a concentration of 15 lbs. of urea per 100 gallons, then 10 cm² leaf area retained 0.245 mg. nitrogen. Thus 0.245×3 or 0.735 mg. nitrogen was applied to 10 cm² leaf area in three applications. The percent absorption was then estimated by dividing the net increase of nitrogen, 0.24, by the amount applied, 0.735, and multiplying by 100. Similar procedures were applied to treatments containing Armour sticker and Linck W-A wetting agent.

As compared with the results shown in Table 9, the lower values obtained here were thought to be accounted for by the translocation of nitrogen out of the absorbing leaves during the 48-hour absorption period. Nevertheless there was evidence that wetting agent and stickers increased nitrogen absorption when these materials were added to urea solution applied to the foliage of citrus trees.

F. The effects of wetting agent-sucrose combinations in urea spray on the absorption of nitrogen by 4-year-old sour orange seedlings

From the results of previous experiments, it is evident that wetting agent when added to urea spray solutions enables a more even distribution and a greater amount of urea to be retained on the leaf surfaces. As a result of this situation, a higher value of nitrogen absorption was obtained from urea spray in which the wetting agent had been added. On the other hand, nitrogen absorption by citrus leaves was

depressed by the addition of sucrose to the urea sprays although sucrose had been found to be protective against leaf injury caused by high concentration of urea spray on citrus. An attempt to determine the effect of combining these two materials in urea spray on nitrogen absorption was made on 4-year-old sour orange seedlings under field conditions.

Various treatments are shown together with the results of leaf analyses for total nitrogen and chlorophyll in Tables 11 and 12. Four applications of treatments were made, with the first application on March 16, the second on March 31, the third on June 16, and the fourth on June 30, 1952. Leaf samples were taken on the 15th of each month for total nitrogen and chlorophyll determinations starting in March and ending in October, 1952. The circumference of the tree trunks, at a position six inches above the ground, was also measured on March 15 and October 15 as an index of growth response of the trees to various treatments.

Sour orange seedlings treated with 4 applications of urea spray maintained a satisfactory nitrogen level throughout the growing season (Table 11). It is noted from Figure 9, that when 2 applications of urea sprays were made in March, the nitrogen content of the leaves had increased markedly by April 15 when the leaves were sampled. The trees then maintained a rather stable nitrogen level until after May 15, when the nitrogen content decreased considerably. If no further nitrogen was applied, the nitrogen level of the leaves would have been decreased continuously at the expense of the new growth which was produced by the trees during that period. After two additional spray of urea had been applied in June, the nitrogen content of the leaves increased again,

and from then on the trees maintained a fairly stable nitrogen level until October 15 when the last set of samples was taken.

Regarding the effects of various combinations of wetting agent and sucrose on the nitrogen content of the leaves, the highest values were found from treatments with urea plus wetting agent, the lowest values were obtained from treatments with urea plus sucrose, and intermediate values from treatments with urea plus wetting agent and sucrose and from treatments with urea alone. It is apparent that the addition of a wetting agent to urea sprays increased nitrogen absorption and the addition of sucrose to urea sprays decreased nitrogen absorption by citrus leaves, and when both of these materials are added, it seems that the effects of the two are being neutralized by each other as compared with the results obtained from the treatments where urea was used alone.

Similar results were obtained for chlorophyll content of the leaves (Table 12 and Figure 10). The increase of chlorophyll content was marked in the first few months of the season after applications of NuGreen spray were made, but this remained at a rather stable level, with little increase or decrease after July. This would suggest that trees with low nitrogen and chlorophyll content are highly responsive to urea sprays; however, this response was decreased as the nitrogen content of the leaves was increased to a certain point, and above this point, the increase in nitrogen content has little effect on the chlorophyll content of the leaves.

The increase of circumference of the tree trunks, measured at the beginning and at the end of the growing season, is recorded in

Table 11. The Effect of Wetting Agent-Sucrose Additions to Urea Sprays on the Absorption of Nitrogen by Sour Orange Leaves. (Mg. of N per 10 cm² of leaf surface)

Treatment	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Ave. (seasonal)
15 lbs. Urea/100 gallons									
+ Wetting Agent - Sucrose	1.30	2.35	2.46	1.85	2.40	2.45	2.55	2.75	2.26
- Wetting Agent + Sucrose	1.23	1.50	1.65	1.55	1.70	1.90	2.01	2.04	1.70
+ Wetting Agent + Sucrose	1.40	1.72	1.79	1.66	2.04	2.08	1.97	2.10	1.85
- Wetting Agent - Sucrose	1.40	2.00	2.05	1.71	2.19	2.15	2.16	2.40	2.01
10 lbs. Urea/100 gallons									
+ Wetting Agent - Sucrose	1.21	2.22	2.34	2.15	2.59	2.56	2.81	2.83	2.35
- Wetting Agent + Sucrose	1.34	1.60	1.57	1.40	1.67	1.70	2.00	1.95	1.65
+ Wetting Agent + Sucrose	1.25	1.40	1.50	1.34	1.70	1.95	2.05	2.07	1.66
- Wetting Agent - Sucrose	1.15	1.85	2.03	1.64	2.05	2.00	2.12	2.34	1.89
Checks	1.34	1.46	1.40	1.20	1.08	1.07	1.13	1.10	1.22

Table 12. The Effect of Wetting Agent-Sucrose Additions to Urea Sprays on the Chlorophyll Content of Sour Orange Leaves. (Wt. of Chlorophyll per 100 cm² leaf surface)

Treatment	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Ave. (seasonal)
15 lbs. Urea/100 gallons									
+ Wetting Agent - Sucrose	0.80	0.92	0.99	1.37	1.98	1.98	2.24	2.18	1.56
- Wetting Agent + Sucrose	0.84	0.83	0.88	1.00	1.71	1.70	1.64	1.72	1.30
+ Wetting Agent + Sucrose	0.85	0.90	0.86	0.98	1.52	1.76	1.80	1.80	1.31
- Wetting Agent - Sucrose	0.76	0.90	0.98	1.24	1.49	1.82	1.96	1.99	1.39
10 lbs. Urea/100 gallons									
+ Wetting Agent - Sucrose	0.79	0.98	1.28	1.56	2.11	2.23	2.22	2.24	1.68
- Wetting Agent + Sucrose	0.82	0.86	0.89	1.00	1.48	1.61	1.74	1.72	1.27
+ Wetting Agent + Sucrose	0.84	0.90	0.89	1.02	1.60	1.82	1.86	1.85	1.35
- Wetting Agent - Sucrose	0.79	0.92	1.10	1.23	1.70	1.93	2.00	1.98	1.46
Checks	0.81	0.82	0.79	0.82	0.77	0.73	0.78	0.78	0.79

Figure 9. Seasonal Trend of Nitrogen Content of Leaves of Sour Orange Seedlings Showing Effect of Wetting Agent-Sucrose Additions on Absorption of Nitrogen from Urea Sprays.

Figure 10. Seasonal Trend of Chlorophyll Content of Leaves of Sour Orange Seedlings Showing Effect of Wetting Agent-Sucrose Additions on Absorption of Nitrogen from Urea Sprays.

Table. 13. Effect of Wetting Agent-Sucrose Additions to Urea Solution on Absorption of Nitrogen by Sour Orange Leaves from Foliar Sprays, as Indicated by the Increase in Trunk Circumference of Young Seedlings. (Increase in cm.)

Treatment	Replications			Ave.
	1	2	3	
15 lbs. Urea/100 gal.				
+ Wetting Agent - Sucrose	2.4	2.4	2.7	2.5
- Wetting Agent + Sucrose	2.6	2.8	2.6	2.7
+ Wetting Agent + Sucrose	3.5	3.4	3.6	3.5
- Wetting Agent - Sucrose	2.4	2.8	2.3	2.5
10 lbs. Urea/100 gal.				
+ Wetting Agent - Sucrose	3.1	2.7	3.2	3.0
- Wetting Agent + Sucrose	2.2	3.0	2.3	2.5
+ Wetting Agent + Sucrose	3.3	2.8	2.9	3.0
- Wetting Agent - Sucrose	3.0	2.0	2.5	2.5
Checks	1.6	2.4	2.0	2.0

L.S.D. -- 1% = 0.31 cm.

5% = 0.22 cm.

Table 13. No difference was found between treatments of 15 lbs. and 10 lbs. of urea per 100 gallons of water (Table 13a, Appendix). The addition of both sucrose and wetting agent gave a highly significant increase in trunk circumference over sprays containing urea only at both concentrations of urea. Addition of sucrose only to urea sprays gave no significant difference, and addition of wetting agent alone gave highly significant increase in trunk circumference with 10 lbs. but not with 15 lbs. of urea.

G. The efficiency of nitrogen absorption by 4-year-old sour orange seedlings in relation to the initial nitrogen content of the leaves

In considering the data presented in Tables 3 and 4, it was noticed in many cases that under the same treatment, when the results are expressed as percentage nitrogen increase relative to the initial nitrogen content of the leaves, higher values were found in leaves which had a lower initial nitrogen content. For example (Table 3), the initial nitrogen contents of leaves given the 15-pound urea treatment were 2.46, 2.34, 2.49, 2.34, and 2.22; the nitrogen increases for the respective values were 0.38, 0.76, 0.35, 0.58, and 1.06 mg./10 cm². The percent nitrogen increases were calculated as 15.4, 32.5, 14.1, 24.8, and 47.7, respectively.

It was not possible to develop a relationship between these two variables due to the limited size of samples in the experiment, which was not designed for this purpose; however, further observation was made on sour orange seedlings to study this matter.

Forty-two trees of 4-year-old sour orange seedlings which had different leaf-nitrogen levels due to variation in locations and

different fertilizer practices served as good materials for the study. Applications of urea spray at a concentration of 10 lbs. per 100 gallons of water were made at weekly intervals starting April 4 and ending April 25, 1952. Leaf samples were taken on April 4, just before the treatments were made, and on May 2, 1952, 7 days after the fourth application of urea spray.

The results of chemical determination of nitrogen before and after four applications of urea spray are given in Table 14. The results in testing for significance of regression coefficient and correlation coefficient are given together in Figure 11.

A highly significant correlation ($r = -0.945$) was found between the initial nitrogen content of the leaves and the common logarithm values of the percent nitrogen increase which was caused by urea treatments. The regression coefficient was also significant at the 1% level ($t = 17.68$). The relationship of the two is shown graphically in Figure 11. From these data, an increase in the initial nitrogen content of the leaves of 0.1 mg./10 cm^2 was approximately equivalent to a decrease of 0.04 common logarithm value in the relative percentage of nitrogen increase; in other words, the efficiency of nitrogen absorption of the leaves from urea spray, as measured by relative percentage of nitrogen increase, was decreasing exponentially as initial nitrogen content increased, the logarithm of the relative percent increase decreasing at the estimated uniform rate of 0.442 per milligram of the initial nitrogen content of the leaves.

Table 14. Effect of Initial Nitrogen Content of Sour Orange Leaves on the Increase in Nitrogen Content Resulting from Foliar Sprays of Urea.

Initial N	Nitrogen, mg./10 cm ²		Percent Increase	Common Logarithms of % Inc.
	After Treatment	Nitrogen Increase		
0.84	1.44	0.60	71.42	1.854
0.92	1.57	0.65	70.65	1.849
0.94	1.64	0.70	74.46	1.872
0.94	1.69	0.75	79.78	1.902
0.97	1.73	0.76	78.35	1.894
0.97	1.71	0.74	76.28	1.882
0.97	1.65	0.68	70.10	1.846
0.98	1.64	0.66	67.34	1.828
0.99	1.66	0.67	67.67	1.820
0.99	1.50	0.51	51.51	1.712
1.00	1.50	0.50	50.00	1.699
1.01	1.50	0.49	48.51	1.686
1.16	1.74	0.58	50.00	1.699
1.20	1.77	0.57	47.50	1.677
1.24	1.84	0.60	48.38	1.685
1.48	1.93	0.45	30.40	1.483
1.60	2.03	0.43	26.87	1.429
1.66	2.03	0.37	22.28	1.348
1.66	2.19	0.53	31.92	1.504
1.66	2.18	0.52	31.32	1.496
1.66	2.10	0.44	26.50	1.423
1.70	2.06	0.36	21.17	1.326
1.80	2.16	0.36	20.00	1.301
1.81	2.16	0.35	19.33	1.286
1.92	2.25	0.33	17.18	1.235
1.98	2.31	0.33	16.66	1.222
2.04	2.37	0.33	16.17	1.209
2.16	2.33	0.17	7.87	0.896
2.23	2.41	0.18	8.07	0.907
2.24	2.42	0.18	8.03	0.905
2.45	2.70	0.25	10.20	1.009
2.50	2.75	0.25	10.00	1.000
2.55	2.80	0.25	9.80	0.991
2.62	2.90	0.28	10.68	1.029
2.88	3.14	0.26	9.02	0.955

Table 14. Effect of Initial Nitrogen Content of Sour Orange Leaves on the Increase in Nitrogen Content Resulting from Foliar Sprays of Urea - Continued.

Initial N	Nitrogen, mg./10 cm ²		Percent Increase	Common Logarithms of % Inc.
	After Treatment	Nitrogen Increase		
2.90	3.20	0.30	10.34	1.015
3.00	3.25	0.25	8.33	0.921
3.02	3.30	0.28	9.27	0.967
3.10	3.35	0.25	8.06	0.906
3.20	3.48	0.28	8.75	0.942
3.20	3.48	0.28	8.75	0.942
3.23	3.50	0.27	8.35	0.922

Figure 11. Regression of Initial Nitrogen Content on Relative Percent Increase of Leaf Nitrogen Caused by Urea Sprays in Testing the Efficiency of Nitrogen Absorption of Leaves of Sour Orange Seedlings.

H. The effect of the acidity of the spray solution on the absorption of nitrogen by citrus leaves

In studying iron deficiency in citrus, Guest and Chapman (28) found that a given iron spray was more effective if the solution was acid. Cook and Boynton (13) found on apples that the pH of a urea spray had a marked effect on absorption under some conditions. They reported the percent of applied urea which was absorbed as being 61.1, 63.2, 24.9, and 50.5% for pH 5.4, 6.6, 7.3, and 8.0 respectively. As the result of these studies, an experiment was set up to determine whether the pH of the urea spray affects the absorption of nitrogen by citrus leaves.

Triplicate shoots for each treatment from a mature Duncan grapefruit tree were dipped in 10-lb. urea solutions of various degrees of acidity. Three applications were made at weekly intervals starting February 12, 1952. Immediately before and 7 days after each application, leaf samples were taken for total nitrogen determinations. The urea solutions were buffered and the desired pH values were obtained by mixtures of varying proportions of sodium borate and hydrochloric acid. The initial nitrogen and total nitrogen content after each of the three applications, together with the nitrogen differences after treatments, are given in Table 15.

It can be seen from the data that the total nitrogen content of the check leaves decreased markedly during this period. This situation was associated with the appearance of new growth on the shoots. Since the experiment was conducting in the early spring during the growing season, a decrease in nitrogen content of the older leaves was to be

Table 15. Effect of pH of Urea Spray Solution on Nitrogen Absorption by Duncan Grapefruit Leaves. (mg. N/10 cm²)

Sampling Date	pH Value					7.0	8.0	9.0	Check
	3.3	4.0	5.0	6.0	7.0				
Feb. 12	2.08	2.16	2.24	2.24	2.36	2.36	2.16	2.08	2.08
	2.16	2.08	1.88	2.24	2.08	2.24	2.24	1.96	2.08
	2.36	2.08	2.16	2.08	2.00	2.24	2.24	1.96	2.12
Ave.	2.20	2.11	2.09	2.19	2.15	2.21	2.00	2.09	
Feb. 19	2.24	2.16	2.25	2.32	2.40	2.24	2.19	2.04	2.04
	2.16	1.96	2.00	2.32	2.16	2.40	2.24	2.04	2.04
	2.36	1.96	2.20	2.12	2.16	2.32	2.04	2.12	2.12
Ave.	2.25	2.03	2.15	2.25	2.24	2.32	2.16	2.07	
Feb. 26	1.92	1.80	2.20	1.92	2.13	1.80	1.77	1.86	1.86
	1.86	2.04	1.50	1.76	1.86	1.86	1.74	1.86	1.86
	2.04	1.77	1.70	1.86	2.13	2.13	1.68	1.89	1.89
Ave.	1.94	1.87	1.80	1.85	2.04	1.93	1.73	1.87	
Mar. 5	2.22	2.04	2.22	2.31	2.22	1.68	1.86	2.00	2.00
	2.40	2.40	2.00	2.13	2.13	1.71	2.04	1.71	1.71
	2.40	1.95	2.32	2.07	2.00	2.19	2.37	1.53	1.53
Ave.	2.34	2.13	2.18	2.17	2.12	1.86	2.09	1.75	
Ave. Increase	0.14	0.02	0.09	-0.02	-0.03	-0.35	0.09	-0.34	

L.S.D. — 1% = 0.50 mg/10 cm² 5% = 0.36 mg./10 cm².

expected. The shoots which had received urea applications showed an increase in the nitrogen content of the leaves in all but one case, as compared with the untreated checks.

The average increase in nitrogen content of leaves after three applications, compared to the controls, was significant at the 5% level for urea solutions at pH 3.3, 4.0, 5.0 and 9.0. At other pH values the differences were not significant (Table 15a, Appendix).

I. Comparative studies of foliar and soil applications of nitrogen

Duplicate trees of 2-year-old sour orange seedlings growing in pots in the greenhouse were given the following treatments: (1) Urea spray; (2) Nitrogen fertilization with sodium nitrate to the roots; (3) Spray plus soil applications of nitrogen; and (4) Checks.

For Treatment 1, 200 ml. of urea at a concentration of 10 lbs. per 100 gallons were sprayed on the leaves. For Treatment 2, an equal amount of nitrogen in the form of sodium nitrate was dissolved in a liter of water and applied to the roots. For Treatment 3, 100 ml. of urea at a 10-100 concentration were sprayed on the leaves and an equivalent amount of nitrogen in the form of sodium nitrate was applied to the roots. The treatments were made at weekly intervals starting January 20 and ending April 8, 1952, so that the trees received 10 applications throughout the experiment. The amount of nitrogen which the trees received was identical in all cases (11.2 grams of actual nitrogen per tree from 10 applications). Leaf nitrogen, chlorophyll content, and dry weight of the trees (Table 16) were measured as the response of the trees to the treatments.

Table 16. Effect of Soil and Foliar Applications of Nitrogen on the Nitrogen and Chlorophyll Contents of the Leaves and the Dry Weight of the Trees of Sour Orange Seedlings.

Treatment	Nitrogen, mg./10 cm ²		Chlorophyll, mg./100 cm ²		Dry Wt. in gm. (Apr. 15)
	Jan. 20	Apr. 15 Increase	Jan. 20	Apr. 15 Increase	
Foliar Applications	1.00 1.32	2.21 2.22	1.21 0.90	1.62 1.66	689 740
Ave.		1.06		0.76	715
Soil Applications	1.41 1.22	2.41 2.34	1.66 1.12	1.75 2.04	760 720
Ave.		1.06		0.92	740
Foliar - Soil Applications	1.52 1.31	2.84 2.59	1.32 1.28	2.02 1.95	842 890
Ave.		1.30		0.99	866
Checks	1.36 1.40	1.16 1.10	-0.20 -0.30	1.11 0.96	360 280
Ave.		-0.25		-0.09	320

L.S.D. for nitrogen means: 1% = 0.92 5% = 0.56 mg./10 cm²
 L.S.D. for chlorophyll means: 1% = 0.32 5% = 0.19 mg./100 cm²
 L.S.D. for dry weight means: 1% = 115 5% = 69 grams

Figure 12. Growth Response of Foliar and Soil Applications of Nitrogen of 2-year-old Sour Orange Seedlings in Pot Culture Experiment in the Greenhouse. (Photo taken April 14, 1952)

Pot A: Tree received 10 applications of urea spray at a concentration of 10 lbs. per 100 gallons of water.

Pot B: Tree received equal amount of nitrogen in the form of sodium nitrate from soil applications.

Figure 13. Comparison of Growth Response of Foliar Sprays and Foliar Sprays plus Soil Applications of Nitrogen of 2-year-old Sour Orange Seedlings in Pot Culture Experiment in the Greenhouse. (Photo taken April 14, 1952)

- Pot A:** Tree received 10 applications of urea spray at a concentration of 10 lbs. per 100 gallons of water.
- Pot B:** Tree received equal amount of nitrogen as Pot A but half of the nitrogen was applied as urea spray and half was applied to the roots in the form of sodium nitrate.

Marked differences in nitrogen and chlorophyll increase, and in the dry weight of the trees between treated and untreated trees were observed. The nitrogen increases were higher in Treatment 3 where the nitrogen had been split into soil and foliar applications, but this was not significantly different from the other treatments where the trees received nitrogen from soil or foliar applications alone. No significant difference was found in chlorophyll increase between foliar and soil applications, but trees receiving combination applications had a higher chlorophyll increase than trees receiving only spray applications of nitrogen, and this difference was significant at the 5% level. As in the case of chlorophyll increase, trees receiving nitrogen from the combination applications had produced more dry matter by April 15, 1952, and the higher values for this treatment were statistically significant at the 1% level. Trees given soil application of nitrogen produced slightly more dry matter than trees given foliar applications of an equal amount of nitrogen, but the differences were not significant. Figures 12 and 13 illustrate the growth response of the trees to foliar and soil applications of nitrogen.

Studies comparing the availability of nitrogen from foliar and soil applications of urea were further carried out under field conditions. Two-year-old Ruby grapefruit trees budded on sour orange rootstocks were used for this purpose. Triplicate trees received 0.185 lb. nitrogen in the form of urea applied to the roots on April 29, 1952. In order to eliminate the time effect, four spray applications of urea at a concentration of 10 lbs. per 100 gallons were made to triplicate trees at 2-day intervals starting April 29. One gallon of urea solution

Table 17. Effect of Soil and Foliar Applications of Urea on Nitrogen Content of Ruby Grapefruit Leaves. (Mg./10 cm²)

Treatment	Apr. 29	May 6	Sampling Date			May 27	June 3	June 10
			May 13	May 20	May 27			
Spray	1.44	2.31	2.46	2.50	2.47	2.50	2.50	
	1.38	2.20	2.34	2.44	2.48	2.52	2.50	
	1.44	2.33	2.40	2.44	2.43	2.48	2.41	
	Ave.	1.42	2.28	2.40	2.46	2.46	2.50	2.47
Soil	1.32	1.32	1.41	2.42	2.56	2.58	2.43	
	1.40	1.38	1.41	2.49	2.62	2.66	2.64	
	1.36	1.41	1.44	2.53	2.62	2.62	2.60	
	Ave.	1.36	1.37	1.42	2.48	2.60	2.62	2.56
Checks	1.30	1.30	1.29	1.29	1.26	1.27	1.27	
	1.30	1.30	1.27	1.21	1.25	1.25	1.26	
	1.24	1.27	1.22	1.22	1.24	1.23	1.25	
	Ave.	1.28	1.29	1.26	1.24	1.25	1.25	1.26

L.S.D. for treatment means: 1% = 0.029 mg./10 cm²

5% = 0.014 mg./10 cm²

L.S.D. for sampling date: 1% = 0.056 mg./10 cm²

5% = 0.042 mg./10 cm²

was applied to each tree at each application, and so each tree had a total of 0.186 lb. of nitrogen applied. Another 3 trees served as untreated checks. Leaf samples were taken at weekly intervals. The results of chemical determinations of nitrogen are shown in Table 17.

Differences in level of leaf nitrogen between trees receiving spray and trees receiving soil applications of urea were significant at the 1% level within 6 days after applications had been made, trees receiving spray applications having the higher nitrogen level. The leaf-nitrogen level of trees receiving the same amount of nitrogen applied to the soil did not increase until 3 weeks after the application had been made. This would indicate that nitrogen applied to the soil will not reach the leaves and be utilized by the tree until 2 weeks after application; however, once the soil nitrogen reaches the leaves they will maintain a more satisfactory nitrogen level than from spray applications. As can be seen from Table 17, trees receiving soil application of nitrogen had a considerable higher nitrogen content after May 20 than trees receiving spray applications.

Additional trials with 2-year-old budded Ruby grapefruit trees were also made under field conditions. Different treatments are summarized as follows:

Treatment 1: Four spray applications of urea at a concentration of 10 lbs. per 100 gallons of water. Each tree received approximately one gallon of spray on April 29, May 6, May 13, and May 20, 1952. A total amount of 0.186 lb. nitrogen was applied in 4 applications.

Treatment 2: One soil application of urea at a rate of 0.4 lb. or 0.186 lb. actual nitrogen, was made on April 29, 1952.

Treatment 3: One soil application on April 29, and two sprays on April 29 and May 6, 1952. A total amount of 0.279 lb. actual nitrogen was applied.

Treatment 4: One soil application on April 29, and four sprays on April 29, May 6, May 13, and May 20. A total amount of 0.372 lb. actual nitrogen was applied.

Treatment 5: Two soil applications with a total nitrogen of 0.372 lb. were made on April 29 and May 13, 1952.

Treatment 6: Untreated checks.

For soil applications, the material was broadcast evenly around the tree trunk within a radius of 1.5 feet. For spray applications, one gallon of the prepared solution was applied to the foliage by means of a hand sprayer and the ground around the tree trunk was covered with a piece of plastic cloth which had a diameter of 5 feet to reduce the spray material reaching the ground to a minimum. Leaf samples were taken periodically for nitrogen determinations. The results are presented in Table 18. The data are also shown graphically in Figure 14 for convenience.

Leaf-nitrogen level was significantly higher in treated trees than in untreated checks. With equal amount of nitrogen fertilization, trees receiving soil applications had a slightly higher leaf-nitrogen level than trees receiving foliar applications throughout the experiment. Also on a basis of equal amount of nitrogen, four sprays plus one soil application were just as effective as two soil applications of urea.

In almost every case, leaf-nitrogen level of trees receiving spray applications dropped considerably after July 15. The nitrogen

Table 18. Effect of Foliar and Soil Applications of Urea on the Nitrogen Content of Leaves of Ruby Grapefruit Trees. (Mg. N per 10 cm² leaf area)

Treatment	Sampling Date				
	Apr. 29	June 15	July 15	Aug. 15	Sept. 1
Four sprays	1.71	2.80	2.72	2.28	2.12
	1.71	2.50	2.38	2.18	2.00
	1.48	2.20	2.10	2.14	1.88
	Ave.	1.63	2.50	2.40	2.20
One Soil Application	1.41	2.80	2.78	2.70	2.60
	1.41	2.71	2.69	2.60	2.60
	1.29	2.35	2.33	2.14	2.15
	Ave.	1.37	2.62	2.60	2.45
Two sprays plus one soil application	1.20	2.70	2.36	2.30	2.00
	1.92	3.00	2.99	2.90	2.70
	1.21	2.70	3.11	2.90	2.80
	Ave.	1.44	2.80	2.82	2.70
Four sprays plus one soil application	1.26	3.10	2.70	2.62	2.22
	1.71	3.29	3.30	3.00	2.88
	1.54	3.12	3.00	3.08	2.70
	Ave.	1.50	3.17	3.00	2.90
Two soil applications	1.62	3.42	3.30	3.30	3.10
	1.34	3.05	3.00	3.00	3.00
	1.26	2.68	2.70	2.70	2.75
	Ave.	1.41	3.05	3.00	2.95
Checks	1.20	1.24	1.18	1.10	1.10
	1.44	1.40	1.42	1.21	1.15
	1.26	1.00	1.11	1.08	1.05
	Ave.	1.30	1.21	1.24	1.10

Figure 14. Nitrogen Content of Leaves of Ruby Grapefruit Trees Receiving Foliar and Soil Applications of Urea.

content of leaves from trees receiving soil application also decreased, but this decrease was less in extent than with the spray applications. It is apparent that soil application of nitrogen seemed to maintain a satisfactory nitrogen level of the trees for a longer period of time than spray applications of the same amount of nitrogen.

J. The rapidity of nitrogen absorption by citrus leaves

It has been found that McIntosh apple leaves absorb nitrogen most rapidly in the first few hours after foliar applications of urea (13). Experimental trials carried under greenhouse conditions to see whether the same is true for citrus leaves. In the first test, urea solution at a concentration of 10 lbs. per 100 gallons of water was sprayed on the leaves of 2-year-old Valencia orange trees in the greenhouse. Leaf samples were taken immediately before and 1, 3, 5, 7 hours and 1, 3, 5, and 7 days after application. No significant difference in nitrogen increase was found even after the 7-day period (Table 19). This finding has confirmed the results obtained in previous experiments, that one application of urea spray did not increase the nitrogen content of the leaves by a measurable amount.

In the second test, known amounts of nitrogen in the form of urea were painted on the leaves. The leaves were then thoroughly washed with deionized water at various intervals. Chemical determinations of urea nitrogen were made on the washings. The percent of applied nitrogen which was absorbed by the leaves is presented in Table 20.

Immediate absorption of nitrogen by the leaves can be seen from this table. Even by the end of the first hour of application, as

much as 14.6% of the applied nitrogen had been absorbed. Marked increase in the rate of absorption was found 3 hours after application. The absorption rate was progressively higher as the absorption period was increased with the exception of Treatment 6, where the leaves were washed 3 days after the application. The nitrogen applied to the leaves was continuously absorbed until 7 days after the application, at which time 26% of the applied nitrogen was still found on the leaf surfaces.

Table 19. Nitrogen Content of Leaves of Valencia Orange Trees at Various Intervals after the Application of Urea Spray at a Concentration of 10 lbs. per 100 Gallons of Water. (Mg. N per 10 cm² leaf area)

Interval	Dec. 25, 1951		Jan. 9, 1952		Mar. 3, 1952	
	Initial Nitrogen	N After Treat.	Initial Nitrogen	N After Treat.	Initial Nitrogen	N After Treat.
1 hour	1.53	1.52	2.22	2.22	2.52	2.50
3 hours	1.41	1.41	2.13	2.12	2.40	2.41
5 hours	1.54	1.55	2.04	2.05	2.61	2.64
7 hours	1.59	1.60	2.31	2.31	2.34	2.34
1 day	1.53	1.55	2.16	2.18	2.37	2.35
3 days	1.60	1.60	2.30	2.30	2.52	2.54
5 days	1.26	1.25	2.29	2.30	2.70	2.73
7 days	1.29	1.31	2.40	2.37	2.54	2.55

No significant difference between treatments.

Table 20. Percent of Applied Nitrogen Absorbed by Valencia Orange Leaves at Various Intervals under Greenhouse Conditions.

Interval	July 3, 1952	Aug. 5, 1952	Sept. 27, 1952	Ave.
1 hour	17.5	14.9	11.3	14.6
3 hours	42.7	40.2	40.5	41.1
5 hours	45.6	54.1	42.8	47.5
7 hours	46.5	64.2	50.2	53.6
1 day	64.1	73.1	58.4	65.2
3 days	86.3	86.4	67.6	80.1
5 days	75.5	72.5	69.7	72.6
7 days	76.8	77.1	68.2	74.0

L.S.D. for treatment means:

1% = 1.26%

5% = 0.91%

Along with the absorption rate studies, an attempt was made to study in some detail how rapidly the absorbed urea would be translocated to the other parts of the tree from the absorbing leaves. Sets of leaf samples were taken for initial nitrogen determinations. Known amounts of urea nitrogen were brushed on the leaves, and the amounts of nitrogen absorbed were determined for various absorption periods. At the end of each absorption period the leaves were sampled, and the water-soluble and the insoluble portions of nitrogen in the leaves were determined. The data of this experiment are presented in Table 21.

A consistent trend of nitrogen absorption as a function of time was observed, i.e. higher absorption rates were found with longer absorption periods. On the other hand, the percent of absorbed urea nitrogen which was still in water-soluble form in the leaves decreased

as the length of time after application increased. The total nitrogen content showed little increase after urea applications. This would suggest that the major portion of the absorbed urea nitrogen is promptly translocated out of the absorbing leaves, and that this process is continuously taking place until at least 120 hours or 5 days after application.

Table 21. Translocation Studies of the Absorbed Urea in Valencia Orange Leaves.

Interval (hours)	Applied N (mg.)	Absorbed N (mg.)	Absorption (%)	Sol. N (mg.)	Sol. N (%) ^a	Mg. N/10 cm ²	
						Before Application	After Application
1	5.0326 5.2124	0.7549 0.7402	15.0 14.2	0.5971 0.6099	79.1 82.4	1.97 2.04	2.00 2.02
3	5.0204 5.1302	1.9881 1.9700	39.6 38.4	1.6780 1.6016	84.4 81.3	1.87 1.88	1.88 1.84
6	5.1234 5.4220	2.3926 2.2881	46.7 42.2	1.8309 1.8328	76.5 80.1	1.89 1.99	1.88 2.01
24	5.0401 5.6280	3.1299 3.0729	62.1 54.6	2.2128 2.2279	70.7 72.5	2.02 2.00	2.00 1.92
48	5.6210 5.3454	3.6087 3.2607	64.2 61.0	2.2843 1.7999	63.3 55.2	1.95 1.79	2.01 1.76
72	5.0094 5.7243	3.4164 3.6693	68.2 64.1	1.0967 1.0714	32.1 29.2	1.98 2.06	1.97 2.06
120	5.6326 5.1240	3.8527 3.4716	68.4 67.8	0.7590 0.2743	19.7 7.9	2.08 1.98	2.07 2.00

^a Percent of applied nitrogen which was still in water-soluble form.

V. DISCUSSION

Possible errors in sampling procedure might exist on a few instances where high urea concentrations were applied. With the resultant effect of leaf drop from high concentration of urea sprays, it was not always possible to take the second set of samples from the same leaves from which the first set of samples had been taken. In this case, a small variation in nitrogen content due to the variable thickness of individual leaves is to be expected. However, since the magnitude of leaf thickness is so small in ordinary citrus leaves (average less than 1 mm.), the variation in total nitrogen content of the leaves is considered to be unimportant.

Foliar applications of nitrogen in the form of urea have been reported to be successful on some plants. The results reported herein have indicated that citrus trees are also responsive to nitrogen sprays. The ability of plants to withstand a given concentration of urea spray varies slightly even within the genus Citrus. Grapefruit and oranges are tolerant to somewhat higher concentrations of urea than tangerines. The factors which control the resistance of leaves to urea injury are not known.

The response of citrus to urea spray was measured by the nitrogen level and chlorophyll content, as well as by the dry matter produced by the trees. In one case where grapefruit shoots were treated with urea at a concentration of 5 lbs. per 100 gallons, the nitrogen content of the leaves did not increase significantly when the leaves were sampled 8 days after the fourth application (Table 3). In another case where the grapefruit shoots were treated with urea at the same

concentration, the nitrogen content of the leaves increased by a significant amount when the leaves were sampled 2 days after the third application (Table 4). Since one of the characteristics of nitrogen metabolism in plants is its translocation from one part of the plant to another, the lack of an increase of nitrogen in the leaves to a measurable amount in the first case was thought to be due to the prolonged time before sampling. Later studies demonstrated that only 7.9 - 19.7% of the absorbed urea still remained in a water-soluble form 5 days after application, and the sprayed leaves failed to show any measurable increase in total nitrogen level (Table 21). These findings suggest that a major portion of the urea taken into the leaves and thought by Cook and Boynton (13) to be converted into non-soluble protein might have been translocated out of the leaves during the longer absorption periods.

Two types of experiments have been used in studying the absorption of nitrogen by the leaves. In the first type, a known amount of nitrogen was applied to the leaves, and the leaves were then washed with portions of deionized water at certain intervals. The difference between the amount of nitrogen applied and the amount present in the washings was considered to be the amount of nitrogen absorbed by the leaves in that particular period, and the results were reported as percent of applied nitrogen which was absorbed by the leaves, or percent absorption. As Cook and Boynton (13) stated, "The disappearance of nitrogen from the leaf surfaces was attributed to (a) absorption by the leaves, (b) adsorption on the leaf surfaces by a force great enough to resist the washing, or (c) release from the leaf surfaces as ammonia

vapor through the action of urease". It was assumed that surface adsorption without subsequent absorption by the leaf was not important. Also no measurable amounts of ammonia released by the action of urease were found over a ten-day period by Cook and Boynton. Thus the loss of nitrogen on the leaf surfaces after the application was considered to be the amount which was absorbed by the leaves. Results from experiments of this type indicated that leaves absorbed nitrogen from a single application of urea (Tables 7, 9, and 20). In the second type of experiment, the initial nitrogen of the leaves was determined before any treatments were made, and the nitrogen content of the leaves after treatment was also determined. Results of this type of experiment showed that the nitrogen increase of leaves due to foliar applications of urea was not a measurable amount until two or three applications had been made (Tables 4 and 5). This would lead, therefore, to the conclusion that absorption of nitrogen by citrus leaves occurs when a single application of urea spray is made, but that the amount absorbed from a single application at concentrations of 10 lbs. or less per 100 gallons is not great enough to cause a significant difference in the total nitrogen content of the leaves even in the first day or two following application.

Injury of citrus leaves from urea spray can be reduced to some extent by the addition of sucrose in the spray solution (Table 6). Two possibilities might help to explain this situation. The first is of a physiological nature, while the second is purely a physical phenomenon. Emmert and Klinker (20) thought that higher concentrations of carbohydrates must be present in the leaf tissues to combine with the urea

as it enters the tissue if burning is to be prevented. Results of their experiments with tomatoes showed that leaves absorbed sucrose from spray applications and that addition of equimolar quantities of sucrose in urea sprays permitted ten times as high concentrations of urea to be applied without leaf injury as could be done with sprays containing urea only. It is possible that the reduction of urea injury by the addition of sucrose to the sprays is due to a better utilization of urea nitrogen in the leaf tissues. Furthermore, the possible metabolism of urea in plants is likely to consist of the following biochemical changes:

In these biochemical reactions, the required α -ketoglutaric acid, fumaric acid, or possibly still other plant acids are intermediate products of carbohydrate metabolism. Accordingly, if there is an insufficient amount of these required plant acids which would combine with the NH_4^+ from the hydrolysis of urea to form subsequent amino acids, the excess NH_4^+ dissociates with the liberation of free ammonia, and the injurious effect of free ammonia to leaf tissue is easily

recognized. Thus, the addition of sucrose or other carbohydrates to urea sprays to furnish sufficient amounts of the materials which are required by the metabolism of urea in plants seems to be a logical means of preventing urea injury.

Table 7 shows that sucrose depressed nitrogen absorption by the leaves to a certain extent. Similar results have been reported on apples and tomatoes (13, 43). It seems that the reduction of leaf injury caused by the addition of sucrose to sprays might be due in part to the direct reduction of absorption of urea by the leaves.

In studying the effects of sucrose, magnesium sulfate and lime on the absorption of nitrogen, it was found that sucrose and magnesium sulfate depressed the rate of nitrogen absorption to various degrees (Table 7). Kreuz (39) in studying the cells of Tradescantia elongata and Taraxacum officinale found that dextrose definitely decreased the permeability to urea, the decrease being greater as the proportion of dextrose increased. He also found that the permeability of highly permeable tissues was more reduced by dextrose than was that of less permeable tissues. Apparently, magnesium sulfate has a similar effect on the permeability of cells. It has been demonstrated by Brooks (11), that certain elements are capable of preventing or reducing the penetration of dyes into the cell; potassium, calcium, sodium, and magnesium chlorides all checked the penetration of the dye dahlia into the living cells of Nitella. On the other hand, hydrated lime when added to urea spray did not reduce the rate of absorption by the leaves. In five of the seven runs, lime increased the percent absorption of nitrogen significantly. This situation might be explained by the

results shown in Table 15. The nitrogen absorption was found to be much higher at pH 9.0 than at 8.0. A water solution of urea has pH 8.4, and addition of lime in this case raised the pH to 10.5. Presumably the higher absorption at pH 9.0 continues to be effective also at higher pH values.

The addition of stickers and a wetting agent to the spray solution has been found to increase nitrogen absorption by the leaves from urea sprays (Tables 9 and 10). This is probably due to the ability of the sticker to hold deposits through weathering and the decrease in surface tension, which gives a more even distribution of the spray droplets on the leaf surfaces, caused by the addition of a wetting agent. Since most of the urea nitrogen applied can be absorbed by citrus leaves in the first few hours after application, as shown in Table 20, weathering of the spray residue is not an important factor; and it appears that the use of a wetting agent to furnish a more even distribution of spray material on the leaf surface is of beneficial in this type of spray applications.

The efficiency of nitrogen absorption was found to be higher in trees which had a lower initial nitrogen content (Table 14). The term "efficiency of absorption" is used in order to distinguish from "percent absorption" which was used by Cook and Boynton (13). In their work with apple trees, they found that there was a direct relationship between high urea absorption and high initial nitrogen level when their results were expressed as percent of applied nitrogen which was absorbed by the leaves. In the experiment reported here, the actual amount of nitrogen applied to the leaves was not known, but the initial

nitrogen content of the leaves before application was determined. From the total nitrogen content after applications of treatment and the initial nitrogen content of the leaves, the net increase of nitrogen could be calculated. The percent nitrogen increase relative to the initial nitrogen content of the leaves was then estimated by dividing the net increase with the initial nitrogen content of the leaves. Since different approaches were used, the results from these two types of experiment are not comparable.

The data shown in Table 15 indicate that the pH of the spray solution has a definite effect on the absorption of urea by the leaves. The treatment at pH 8.0 represents closely the value of an unbuffered urea solution. Change in pH in either direction seemed to increase absorption by the leaves, with increases significant at the 5% level for pH 3.3, 4.0, 5.0 and 9.0. While Cook and Boynton (13) found on apples that there was relatively high absorption at pH 5.4 and 6.6 with little difference between them, a low value at pH 7.3, and an intermediate value at pH 8.0. Drawert (18) in experimenting with the epidermal cells of Sedum praealtum, Triticum vulgare, Allium cepa, and Elodea canadensis immersed in urea solution. He found that at a given pH of the immersion solution, urea is taken up more rapidly as the acidity of cell sap increases. Cells with sap of a given acidity take up urea more rapidly as the alkalinity of the immersion solution increases. The effects or interactions between the acidity of the cell sap and the acidity of the spray solution are obvious. Results obtained here where the value of nitrogen increase is higher at pH 9.0 than at 8.0 are in accordance with Drawert's findings; however, the increase

of nitrogen at lower pH 3.3 and 5.0 must be attributed to some other factors which are not yet fully understood. These values are more in agreement with the work of Cook and Boynton, cited above, but no minimum at pH 7.0 was observed.

An attempt to compare the effects of foliar and soil applications of urea in sand cultures of citrus in the greenhouse failed. Three pots of 2-year-old sour orange seedlings had received 210 p.p.m. nitrogen in the form of urea from the nutrient solution applied at weekly intervals starting March 12, 1952. The growth of these 3 trees was excellent during the first 3 months of the experiment, but about the middle of July, 1952, all three trees wilted and they died within one month. Examination of the roots showed that symptoms similar to those caused by oxygen deficiency were present. Chemical analyses indicated that the concentration of nitrite nitrogen of the sand from these 3 pots which had received urea from the nutrient solution ranged from 14 to 25 p.p.m. As compared with the concentration of nitrite which ranged from 2 to 4 p.p.m. in pots receiving no urea from soil application, the accumulation of nitrite in the first case was considered to be rather high. Bitcover and Wander (6) thought that the toxic effect on the urea-supplied plants was probably due to a lack of sufficient oxygen about the roots, but there is also the possibility of a direct injurious effect by the high concentration of the nitrite present.

In the soil, urea is transformed into ammonia and subsequently into nitrates. The process includes both ammonification and nitrification, and various microorganisms are involved. If the microorganisms

which are required for the completion of nitrification are absent or conditions are unfavorable, as was thought to be the case in the well-washed sand used in the pot cultures, the process would not be completed and an accumulation of nitrite would result.

VI. SUMMARY

1. Citrus leaves absorb and assimilate nitrogen from sprays of urea.

2. Concentrations of spray solution higher than 20 lbs. of urea per 100 gallons of water cause injury to citrus leaves. Tangerine leaves are somewhat more easily injured than orange and grapefruit leaves.

3. Addition of sucrose to the urea spray decreases leaf injury; forty lbs. of urea plus 240 lbs. of sucrose in 100 gallons of water caused no injury when sprayed on leaves.

4. The use of higher concentrations of urea spray made possible by the protective action of sucrose resulted in greater increases in leaf nitrogen, although at any given concentration of urea the addition of sucrose reduced somewhat the absorption of nitrogen from urea.

5. Magnesium sulfate and hydrated lime both reduced injury to leaves from urea sprays, the former depressing nitrogen absorption somewhat and the latter increasing it slightly.

6. One and two applications of urea at rates of 10 lbs. or less per 100 gallons produced no measurable responses, but three or more applications even when used at a rate of 5 lbs. per 100 gallons caused increases in nitrogen and chlorophyll content of leaves. These increases were progressively greater as the number of spray applications increased.

7. Addition of a wetting agent to the spray solution markedly increased the absorption of urea nitrogen by leaves. This effect was further increased by addition of a sticker. In sprays combining wetting agent and sucrose the former modified the depressing effect

of the latter.

8. Leaves low in nitrogen showed a greater efficiency of nitrogen absorption from urea sprays than leaves high in nitrogen, when efficiency is measured by the percent of nitrogen increase relative to the initial nitrogen level of the leaves.

9. Absorption from urea sprays is significantly greater below pH 5.0 and above pH 9.0 than at pH 8.0, which is close to the reaction of an unbuffered urea solution.

10. For equivalent amounts of nitrogen applied, little difference was found in the total increases in leaf nitrogen and chlorophyll contents between applications to the soil and to the leaves. However, during the first two weeks after application the response was more rapid to foliar applications, while soil applications produced a more lasting effect and gave higher leaf values after the third week.

11. Absorption of nitrogen from urea sprays was readily measured within an hour after application, and continued for at least 7 days at a steadily decreasing rate.

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VIII. APPENDIX

Table 3a. Analysis of Variance: Nitrogen Increase of Duncan Grapefruit Leaves after Four Applications of Urea of Various Concentrations.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	34	6.032		
Treatment	6	4.740	0.7900	14.62**
Replication	4	0.002	0.0005	0.09
Error	24	1.290	0.0540	

Least Difference Required for Significance Between Treatment Means:
 1% = 0.414 mg./10 cm²
 5% = 0.305 mg./10 cm²

Table 4a. Analysis of Variance: Nitrogen Increase of Duncan Grapefruit Leaves treated with Urea Solution at a Concentration of 5 lbs. per 100 gallons of Water.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	26	1.9245		
Treatment	8	1.4936	0.1867	8.52**
Replication	2	0.0804	0.0402	1.83
Error	16	0.3505	0.2190	

Least Difference Required for Significance Between Treatment Means:
 1% = 0.345 mg./10 cm²
 5% = 0.252 mg./10 cm²

Table 5a. Analysis of Variance: Nitrogen Increase of 4-year-old Sour Orange Seedlings Sprayed with Urea at a Concentration of 10 lbs. per 100 Gallons of Water.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	17	1.626		
Treatment	8	1.541	0.193	21.4**
Error	9	0.085	0.009	

Least Difference Required for Significance Between Treatment

Means: $1\% = 0.33 \text{ mg./10 cm}^2$
 $5\% = 0.22 \text{ mg./10 cm}^2$

Table 5b. Analysis of Variance: Chlorophyll Increase of 4-year-old Sour Orange Seedlings Sprayed with Urea at a Concentration of 10 lbs. per 100 Gallons of Water.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	17	2.043		
Treatment	8	1.953	0.244	27.1**
Error	9	0.081	0.009	

Least Difference Required for Significance Between Treatment

Means: $1\% = 0.28 \text{ mg./100 cm}^2$
 $5\% = 0.19 \text{ mg./100 cm}^2$

Table 7a. Analysis of Variance: The Effects of Sucrose, Magnesium Sulfate and Lime when Added to Urea Sprays on the Absorption of Nitrogen by Valencia Orange Leaves.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	27	23.25		
Treatment	3	8.44	2.81	4.53**
Error	24	14.80	0.62	

Least Difference Required for Significance Between Treatment

Means: 1% = 1.183 percent

5% = 0.875 percent

Table 8a. Analysis of Variance: The Effects of Sticker and Wetting Agent when Added to Urea Sprays on the Retention of Spray Solution on Leaf Surfaces.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	7	0.212		
Treatment	3	0.203	0.068	34.0**
Error	4	0.009	0.002	

Least Difference Required For Significance Between Treatment

Means: 1% = 0.12 ml./1000 cm²

5% = 0.08 ml./1000 cm²

Table 9a. Analysis of Variance: The Effects of Sticker and Wetting Agent when Added to Urea Sprays on the Absorption of Nitrogen by Valencia Orange Leaves.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	19	1532.149		
Treatment	3	1081.129	360.376	12.8**
Error	16	451.020	28.190	

L.S.D.-- 1% = 9.82 percent, 5% = 7.12 percent

Table 10a. Treatments of Different Stickers on Grapefruit Leaves.

Sticker or Wetting Agent	Amt. Material/100 gal.
Good-rite Latex V.L. 600	1 pint
Good-rite p.e.p.e.	$\frac{1}{2}$ pint
Dowax 222	8 pints
Colloidal Z-1 sticker	$\frac{1}{4}$ pound
Armour sticker	$1\frac{1}{2}$ pounds
Oil emulsion	4 pints
Linck W-A wetting agent	4 pints

Table 10b. Analysis of Variance for Table 10a.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	26	0.3910		
Treatment	8	0.3821	0.0478	95.6**
Error	18	0.0089	0.0005	

L.S.D. -- 1% = 0.052 mg./10 cm² 5% = 0.038 mg./10 cm².

Table 10c. Nitrogen Content before and after Three Applications of Urea at a Concentration of 15 lbs. per 100 Gallons of Water plus Various Commercial Stickers and Linck W-A Wetting Agent. (Mg. N/10 cm²)

Treatment	Initial Nitrogen	Nitrogen after Applications	Nitrogen Increase	Mesh
Checks	2.08	2.20	0.12	0.10
	1.92	2.00	0.08	
	2.00	2.10	0.10	
Urea alone	1.96	2.31	0.35	0.34
	2.00	2.32	0.32	
	1.95	2.30	0.35	
Good-rite Latex V.L. 600	1.98	2.39	0.41	0.41
	2.00	2.40	0.40	
	2.02	2.45	0.43	
Good-rite p.e.p.s.	2.10	2.55	0.45	0.47
	2.14	2.60	0.46	
	2.16	2.66	0.50	
Dowax 222	2.04	2.47	0.43	0.44
	2.00	2.42	0.42	
	2.08	2.55	0.47	
Colloidal Z-1	2.00	2.41	0.41	0.42
	2.15	2.56	0.41	
	2.15	2.59	0.44	
Armour sticker	2.05	2.45	0.40	0.39
	2.00	2.36	0.36	
	2.10	2.51	0.41	
Oil emulsion	2.10	2.46	0.36	0.35
	2.04	2.36	0.32	
	2.10	2.47	0.37	
Linck W-A Wetting agent	2.10	2.66	0.56	0.56
	2.08	2.62	0.54	
	2.15	2.73	0.58	

Table 11a. Nitrogen Content of 4-year-old Sour Orange Seedlings for Studying the Effects of Wetting Agent-Sucrose Combinations in Urea Sprays on the Absorption of Nitrogen by the Leaves
 —Original Data. (Mg. N/10 cm²)

A. 15 lbs. Urea per 100 gallons

Sampling Date	+ W. Agent - Sucrose	- W. Agent + Sucrose	+ W. Agent + Sucrose	- W. Agent - Sucrose
Mar. 15, 1952.	1.28 1.36 1.26	1.21 1.20 1.28	1.42 1.46 1.32	1.45 1.37 1.38
Ave.	1.30	1.23	1.40	1.40
Apr. 15	2.34 2.40 2.31	1.46 1.52 1.52	1.76 1.80 1.60	2.08 1.92 2.00
Ave.	2.35	1.50	1.72	2.00
May 15	2.47 2.49 2.42	1.67 1.60 1.68	1.80 1.81 1.76	2.10 1.97 2.08
Ave.	2.46	1.65	1.79	2.05
June 15	1.65 2.30 1.60	1.53 1.52 1.60	1.68 1.70 1.80	1.76 1.67 1.70
Ave.	1.85	1.55	1.66	1.71
July 15	2.42 2.46 2.32	1.68 1.68 1.74	2.04 2.06 2.02	2.20 2.18 2.19
Ave.	2.40	1.70	2.04	2.19
Aug. 15	2.45 2.50 2.40	1.86 1.84 2.00	2.07 2.10 2.07	2.22 2.11 2.14
Ave.	2.45	1.90	2.08	2.15
Sept. 15	2.53 2.60 2.52	2.00 1.97 2.06	1.98 2.00 1.93	2.20 2.12 2.16
Ave.	2.55	2.01	1.97	2.16
Oct. 15	2.80 2.82 2.63	2.05 2.02 2.05	2.09 2.12 2.09	2.45 2.30 2.45
Ave.	2.75	2.04	2.10	2.40

Table 11a. Nitrogen Content of 4-year-old Sour Orange Seedlings for Studying the Effects of Wetting Agent-Sucrose Combinations in Urea Sprays on the Absorption of Nitrogen by the Leaves
 —Original Data. (Mg. N/10 cm²) — Continued.

B. 10 lbs. Urea per 100 gallons

Sampling Date	+ W. Agent - Sucrose	- W. Agent + Sucrose	+ W. Agent + Sucrose	- W. Agent - Sucrose	Checks
Mar. 15	1.20	1.32	1.28	1.09	1.33
	1.24	1.32	1.24	1.16	1.35
	1.19	1.38	1.23	1.20	1.34
Ave.	1.21	1.34	1.25	1.15	1.35
Apr. 15	2.28	1.58	1.44	1.75	1.42
	2.30	1.58	1.38	1.80	1.50
	2.26	1.64	1.38	2.00	1.46
Ave.	2.28	1.60	1.40	1.85	1.46
May 15	2.34	1.56	1.60	1.90	1.40
	2.38	1.55	1.50	2.09	1.42
	2.30	1.60	1.40	2.10	1.38
Ave.	2.34	1.57	1.50	2.03	1.40
June 15	2.14	1.39	1.40	1.60	1.08
	2.20	1.39	1.32	1.62	1.30
	2.11	1.42	1.30	1.70	1.22
Ave.	2.15	1.40	1.34	1.64	1.20
July 15	2.60	1.66	1.90	2.01	1.04
	2.62	1.65	1.68	2.04	1.10
	2.55	1.70	1.52	2.10	1.10
Ave.	2.59	1.67	1.70	2.05	1.08
Aug. 15	2.56	1.70	2.03	1.96	1.03
	2.60	1.66	2.00	1.97	1.10
	2.52	1.74	1.82	2.07	1.08
Ave.	2.56	1.70	1.95	2.00	1.07
Sep. 15	2.81	2.00	2.10	2.08	1.11
	2.86	1.94	2.03	2.10	1.17
	2.76	2.06	2.02	2.18	1.11
Ave.	2.81	2.00	2.05	2.12	1.13
Oct. 15	2.82	1.93	2.12	2.30	1.07
	2.88	1.92	2.00	2.32	1.15
	2.79	2.00	2.09	2.40	1.08
Ave.	2.83	1.95	2.07	2.34	1.10

Table 12a. Chlorophyll Content of 4-year-old Sour Orange Seedlings for Studying the Effects of Wetting Agent-Sucrose Combinations in Urea Sprays on the Absorption of Nitrogen by the Leaves
 ——Original Data. (Mg./100 cm²)

A. 15 lbs. Urea per 100 gallons

Sampling Date	+ W. Agent - Sucrose	- W. Agent + Sucrose	+ W. Agent + Sucrose	- W. Agent - Sucrose
Mar. 15, 1952.	0.77	0.88	0.88	0.78
	0.82	0.82	0.84	0.75
	0.81	0.82	0.83	0.75
Ave.	0.80	0.84	0.85	0.76
Apr. 15	0.91	0.90	0.94	0.92
	0.94	0.88	0.90	0.89
	0.91	0.86	0.86	0.89
Ave.	0.92	0.88	0.90	0.90
May 15	0.95	0.91	0.88	1.00
	1.02	0.87	0.87	0.98
	1.00	0.86	0.83	0.96
Ave.	0.99	0.88	0.86	0.98
June 15	1.36	1.06	1.00	1.27
	1.40	0.99	0.98	1.22
	1.35	0.95	0.96	1.23
Ave.	1.37	1.00	0.98	1.24
July 15	1.94	1.77	1.56	1.52
	2.00	1.66	1.50	1.50
	2.00	1.70	1.50	1.45
Ave.	1.98	1.71	1.52	1.49
Aug. 15	1.94	1.74	1.80	1.90
	2.01	1.68	1.78	1.80
	1.99	1.68	1.70	1.76
Ave.	1.98	1.70	1.76	1.82
Sept 15	2.20	1.66	1.86	2.00
	2.28	1.66	1.80	1.94
	2.24	1.60	1.74	1.94
Ave.	2.24	1.64	1.80	1.96
Oct. 15	2.17	1.78	1.87	2.04
	2.20	1.70	1.78	1.99
	2.17	1.68	1.75	1.94
Ave.	2.18	1.72	1.80	1.99

Table 12a. Chlorophyll Content of 4-year-old Sour Orange Seedlings for Studying the Effects of Wetting Agent-Sucrose Combinations in Urea Sprays on the Absorption of Nitrogen by the Leaves
 —Original Data. (Mg./100 cm²) — Continued.

B. 10 lbs. Urea per 100 gallons

Sampling Date	+ W. Agent - Sucrose	- W. Agent + Sucrose	+ W. Agent + Sucrose	- W. Agent - Sucrose	Checks
Mar. 15	0.77 0.80 0.80	0.84 0.81 0.81	0.88 0.83 0.81	0.80 0.80 0.77	0.79 0.80 0.84
Ave.	0.79	0.82	0.84	0.79	0.81
Apr. 15	0.95 1.00 0.99	0.88 0.85 0.85	0.93 0.90 0.87	0.95 0.91 0.90	0.81 0.81 0.84
Ave.	0.98	0.86	0.90	0.92	0.82
May 15	1.26 1.30 1.28	0.92 0.89 0.86	0.90 0.90 0.87	1.15 1.09 1.06	0.77 0.80 0.80
Ave.	1.28	0.89	0.89	1.10	0.79
June 15	1.53 1.59 1.56	1.07 0.97 0.96	1.04 1.01 1.01	1.26 1.23 1.20	0.81 0.82 0.83
Ave.	1.56	1.00	1.02	1.23	0.82
July 15	2.06 2.16 2.11	1.52 1.50 1.42	1.65 1.60 1.55	1.80 1.66 1.64	0.76 0.76 0.79
Aug. 15	2.18 2.30 2.21	1.68 1.58 1.57	1.90 1.86 1.70	2.00 1.90 1.89	0.71 0.74 0.74
Ave.	2.23	1.61	1.82	1.93	0.73
Sept 15	2.20 2.26 2.20	1.80 1.72 1.70	1.90 1.88 1.80	2.07 1.97 1.96	0.72 0.80 0.82
Ave.	2.22	1.74	1.86	2.00	0.78
Oct. 15	2.18 2.28 2.26	1.78 1.70 1.68	1.90 1.89 1.76	2.00 1.97 1.97	0.77 0.79 0.78
Ave.	2.24	1.72	1.85	1.98	0.78

Table 13a. Analysis of Variation: Effect of Wetting Agent-Sucrose Addition to Urea Solution on Absorption of Nitrogen by Sour Orange Leaves from Foliar Sprays, as Indicated by the Increase in Trunk Circumference of Young Seedlings.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Main Plot				
Concentration	1	0.01	0.01	0.02
Replication	2	0.02	0.01	0.02
Main plot error	2	1.04	0.52	
Subplots				
Treatment	3	2.03	0.68	22.7 ^{**}
T x C	3	0.78	0.26	8.7 ^{**}
Subplot error	12	0.35	0.03	

Least Difference Required for Significance Between Treatment Means:
 1% = 0.31 cm.
 5% = 0.22 cm.

Table 15a. Analysis of Variation: Effects of pH of Urea Spray Solution on Nitrogen Absorption by Duncan Grapefruit Leaves.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	23	1.4582		
Treatment	7	0.7839	0.1120	2.66 ^{**}
Error	16	0.6743	0.0421	

Least Difference Required for Significance Between Treatment Means:
 1% = 0.50 mg./10 cm²
 5% = 0.36 mg./10 cm²

Table 16a. Analysis of Variation: Effect of Soil and Foliar Applications of Nitrogen on the Nitrogen and Chlorophyll Contents of the Leaves and the Dry Weight of the Trees of Sour Orange Seedlings.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	DFN Value
<u>A. Nitrogen</u>				
Total	7	3.0313		
Treatment	3	2.8703	0.9568	23.7**
Error	4	0.1610	0.0403	
<u>B. Chlorophyll</u>				
Total	7	1.4944		
Treatment	3	1.4761	0.4920	106.9**
Error	4	0.0183	0.0046	
<u>C. Dry Weight</u>				
Total	7	337665.0		
Treatment	3	334812.5	111604.2	156.5**
Error	4	2852.5	713.1	

L.S.D. for Nitrogen:

1% = 0.92 mg./10 cm²

5% = 0.56 mg./10 cm²

L.S.D. for Chlorophyll:

1% = 0.32 mg./100 cm²

5% = 0.19 mg./100 cm²

L.S.D. for Dry Weight:

1% = 115.1 grams

5% = 69.4 grams

Table 17a. Analysis of Variation: Effect of Soil and Foliar Applications of Urea on Nitrogen Content of Ruby Grapefruit Leaves.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	62	22.137		
Treatment	2	12.125	6.061	3031**
Sampling Date	6	4.983	0.831	416**
T x D	12	4.944	0.412	206**
Error	42	0.085	0.002	

L.S.D. for Treatment Means:

1% = 0.029 mg./10 cm²

5% = 0.014 mg./10 cm²

L.S.D. for Sampling Date:

1% = 0.056 mg./10 cm²

5% = 0.042 mg./10 cm²

Table 19a. Analysis of Variation: Nitrogen Content of Leaves of Valencia Orange Trees at Various Intervals after the Application of Urea Spray at a Concentration of 10 lbs. per 100 Gallons of Water.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	23	0.0050		
Treatment	7	0.0016	0.00023	1.09
Error	16	0.0034	0.00021	

No significant difference between treatment means.

Table 20a. Analysis of Variation: Percent of Applied Nitrogen Absorbed by Valencia Orange Leaves at Various Intervals under Greenhouse Conditions.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	"F" Value
Total	23	10520.74		
Treatment	7	10508.55	1501.22	1975.3**
Error	16	12.19	0.76	

Least Difference Required for Significance Between Treatment Means:
 1% = 1.26 percent
 5% = 0.91 percent

BIOGRAPHICAL ITEMS

Wilfred Ching-ching Chen was born on July 3, 1924, in Peiping, China. After he had finished his high school education in Toy-Shan Middle School, he enrolled in the Nantung Agricultural College at Shanghai in September, 1940, where he spent one and a half years with his major subject as Agronomy. In the spring of 1942, he transferred to the National Sun Yat-Sen University at Canton, China, where he received his B.S. degree in Horticulture in June, 1944.

Following his graduation from college, he was called into service and was sent to India by the Chinese Army. On returning from India in October, 1945, he was appointed as Assistant in Horticulture by the National Sun Yat-Sen Memorial Park at Nanking, China. He was employed by this institution as Assistant Horticulturist from October, 1946, until June, 1949.

He came to the U.S. in September, 1949, for further studies in his field of horticulture. He received his Master of Agriculture degree from the University of Florida in July, 1950. Since that time he has pursued his studies in the University of Florida leading to a degree of Doctor of Philosophy.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Date _____

C. V. Noble
Dean

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