MEASUREMENT OF FOLIAR ABSORPTION IN *CITRUS UNSHIU* MARC. USING ^{14}C-LABELLED UREA

Sung-Jun Song[1] and Zang-Kual U.[2]

[1]Applied Radioisotope Research Institute, Cheju National University, Cheju 690-756 REPUBLIC OF KOREA (E-mail:songjs@cheju.cheju.ac.kr)

[2]Subtropical Horticulture Research Center, Cheju National University, Cheju 690-756 REPUBLIC OF KOREA(E-mail:zkyu@cheju.cheju.ac.kr)

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ABSTRACT

Foliar application of urea for fruit trees is considered as an alternative method of soil N-fertilization causing the groundwater pollution of nitrate. As there is no suitable method to determine the absorption rate of urea by intact leaves at the practical application concentration, we have developed a technique for estimating the foliar absorption of urea using ^{14}C tracer in Citrus tree. ^{14}C-labelled urea was applied by dipping leaves in the vinyl bag containing the 1% urea solution plus 0.74MBq. The applied amount was calculated by weighing the reduction of the solution volume after treatment and the absorption rate was indirectly determined by counting the radioactivity of ^{14}C urea solution washed off from leaf surfaces. Various washing solutions(water, 0.5 mM $CaCl_2$, 0.5mM $CaCl_2$ + 1% urea, 1% urea) were compared to get no difference between them. In order to wash the leaves, mild scrubbing with soft brush in the 0.01 % commercial detergent solution was very effective, which can remove more than 98% of unabsorbed urea at one time. Urea absorption increased rapidly up to 80 % by 24 hours after application and continued to increase slowly up to 90 % until 9 days, being higher in the adaxial part than in the abaxial, and much more in expanding leaves than in the matured leaves. In conclusion, the tracer technique using ^{14}C-labelled urea can be an useful method for estimating the absorption rate of urea by intact leaves of Citrus tree at the practical application concentration.

INTRODUCTION

The heavy fertilization of N in Citrus orchard in Cheju, Korea to increase the productivity as well as to compensate low utilization efficiency(less than 20%), led to the groundwater contamination of NO_3. In general foliar spray has been used for supplementing the nutrients which were not covered by soil fertilization. On the other hand, foliar N applications have been suggested as an alternative to conventional soil fertilization to reduce NO_3 losses into the aquifers(Council for Agricultural Science and Technology, 1985). Leaf N content in citrus trees was known to increase more quickly and effectively by foliar spray of urea than by soil fertilization(Jones and Steinacker, 1953). Embleton and Jones(1974) reported that citrus production could be maintained by applying 3-6 foliar applications of urea per year, thus implying that 16%-33% of the annual requirement could be supplied with a single foliar application. Although urea absorption rate of citrus leaves(Impey and Jones, 1960) or apple leaves(Shim et. al. 1972) was estimated by dipping them in 5% urea solution and determining the urea remained in the washing by using micro-conway(Sehgal and Nayler, 1966) the concentration of urea used is suspected to be much higher than the practical concentration(0.5%-1%), and may cause leaf damages with abnormal absorption, and thus can not be applied for an

extended time. Therefore, we developed a technique for estimating the foliar absorption of urea using ^{14}C tracer in Citrus tree at the practical application concentration.

MATERIAL AND METHODS

1. Plant material

Five year-old ***Citrus unshiu*** Marc.(var. Miyagawa) trees grown in the glass house of Applied Radioisotope Research Institute at Cheju National University were used.

2. Inspection of foliar burn by urea application

0.5, 1.0, 2.0, 3.0, 5.0 and 7.0%(W/V) concentrations of urea(Hayashi Pure Chemical Industries Ltd., Japan) were applied on mature leaves. Each leaf with 5 replications was dipped in individual urea solution. After 7-day foliar burns were scored.

3. Determination of ^{14}C-labelled urea absorption

Leaf was dipped into the vinyl bag containing the 100 ml of 1% urea solution including 0.74MBq of ^{14}C-labelled urea and 20 l of Tween-20. To calculate the amount of urea applied to leaf the intial 100 ml of urea with single vinyl bag was weighed before and after the leaf was dipped. After the absorption interval the leaf was detached and washed by scrubbing with a soft brush in 100 ml of water containing 0.01 % commercial detergent (LG Chemicals. Korea) for 4 min. Five ml of washings were taken in the counting vial, and 10 ml of Aquasol-2(Packard, USA), a cocktail solution was added. Radioactivities of ^{14}C were counted with the liquid scintillation counter(Packard 2700TR, USA).

RESULTS AND DISCUSSION

1. Foliar burn by urea application

Foliar burns begun to appear at more than 1% urea concentration(table 1). More than 80% of leaves to which 3-7% urea solution were applied were damaged, showing brown spots within 2 days. Therefore, 5 % urea solution used for determining foliar absorption rate of Citrus tree(Impey and Jones, 1960) would give a phytotoxicity to leaf and it can not be thought that actual foliar urea absorption by *Citrus unshiu* Marc. is made.

Table 1. Effect of urea application on citrus leaf burn. Each leaf was dipped in the individual urea solution. The rate of leaf damage was evaluated at 5-day after application.

Urea concentration(%)	1.5	1.0	2.0	3.0	5.0	7.0
Rate of foliar burn(%)	0	0	20	80	85	90

2. Effect of washing conditions on foliar urea absorption by *Citrus unshiu* Marc.

Our primary objective is to develop a method for determination for estimating the foliar absorption of urea using ^{14}C tracer in *Citrus unshiu* Marc. at the practical application concentration. Therefore, we tried to optimize washing conditions of *Citrus unshiu* Marc. leaves.

a. Washing solutions and frequencies

Effect of washing solutions and frequencies on the washing of ^{14}C-urea remained on leaf surface was shown in table 2. For the composition of washing solution the addition of calicum maintaining the membrane stability(Marschner, 1995) was tested and non-labelled urea solution was used for replacing effectively the ^{14}C-labelled urea bound nonspecifically on the leaf surface. When water, 0.5

mM $CaCl_2$, 0.5mM $CaCl_2$ plus 1% urea and 1% urea as washing solutions were compared, there was no difference among them. Also, more than 98% of ^{14}C-urea of total washings was collected at the first washing, twice or more washing was not thought to be required.

Table 2. Effect of washing solutions and frequencies on the washing of ^{14}C-urea remained on leaf surface. Citrus leaves were detached 2-day after application and washed in the water, 0.5mM $CaCl_2$, 0.5 mM $CaCl_2$ plus 1% urea and 1% urea solution three times, respectively. The three step of washing were conducted and the relative distribution(%) of ^{14}C-urea in each washing was shown.

Washing solution	Washing frequency	Distribution of ^{14}C-urea In the washings(%)
Water	1	99.0
	2	0.80
	3	0.20
0.5 mM $CaCl_2$	1	98.3
	2	0.89
	3	0.81
0.5mM $CaCl_2$ + 1% Urea	1	98.3
	2	1.25
	3	0.45
1% Urea	1	98.5
	2	1.50
	3	0

b. Washing method

For the effective washing of ^{14}C-urea on leaf surface, shaking and scrubbing with mild brush in the washing solution were compared(table 3). Scrubbing method was more effective than shaking one irrespective of washing solutions.

Table 3. Effect of the washing methods on the washing of ^{14}C-urea remained on leaf surface. Leaves were detached 2-day after application and washed by mild-shaking in the shaker or by scrubbing with soft brush in the water or 0.5 mM $CaCl_2$ for 4 min.

Washing method	Washing solution	Relative washing efficiency
Shaking	Water	1.00
	0.5 mM $CaCl_2$	1.05
Scrubbing	Water	1.53
	0.5 mM $CaCl_2$	1.43

c. Use of detergent for washing

In general detergent solution has often been used for leaf washing. Therefore, effect of detergent addition on the washing of ^{14}C-urea remained on leaf surface was examined(table 4). Scrubbing leaves with mild brush in the solution of 0.01% detergent was more effective as compared with water.

Table 4. Effect of detergent addition on the washing of ^{14}C-urea remained on leaf surface. Leaves were detached 2-day after application and washed by scrubbing with soft brush in water and water plus 0.01% detergent for 4 min.

Detergent addition	Relative comparison of washing
Water	1.00
Water with 0.01% detergent	1.43

d. Washing time

Table 5 shows relative comparison of washing on depending on the foliar washing time. Four min or over of washing time gave almost same effect. As extended washing time may induce effux of

urea absorbed and urea absorption by leaf may be underestimated, 4 min is recommended for the washing time of ^{14}C-urea remaining on leaf surface.

Table 5. Effect of detergent addition on the washing of ^{14}C-urea remained on leaf surface. Leaves were detached 2-day after application and washed by scrubbing with soft brush in water plus 0.01% detergent for various time length.

Washing time(min)	Relative comparion of washing
2	1.00
4	1.68
6	1.40

2. Foliar urea absorption by *Citrus unshiu* Marc.

a. Urea absorptions by abaxial and adaxial part of leaf

Urea absorptions by abaxial(upper surface) and adaxial(lower surface) of citrus leaf were presented in Table 6. Absorption of urea by the adaxial of leaf in Washington navel orange was rather higher than by abaxial after 30 hrs (Impey and Jones, 1960). But absorption by the adaxial of citrus unshiu Marc. Leaf was slightly higher than by the abaxial after 24 hrs.

Table 6. Relative comparion of urea absorption between abaxial and adaxial. 1% urea solution was applied to abaxial and adaxial part of leaf by painting with small soft brush on them. Leaves were detached 2-day after application and washed by scrubbing with soft brush in water plus 0.01% detergent for 4 min.

Leaf part	Relative absorption
Abaxial	1.00
Adaxial	1.09

b. Urea absorptions depending on leaf age

Table 7 shows relative comparison of urea absorption among expanding leaf, fully expanded leaf and matured leaf. Urea absorption in expanding leaf was higher than in matured leaf. This is similar to Lea-Cox and Syversten(1995)'s finding that uptake of 15N per unit leaf area of 2 month-old leaves was 1.6 to 6-fold greater than that of older leaves.

Table 7. Relative comparison of urea absorption depending on leaf age. 1% urea solution was applied to expanding, fully expanded and matured leaves . Leaves were detached 2-day after application and washed by scrubbing with soft brush in water plus 0.01% detergent for 4 min.

Leaf age	Relative absorption
Expanding leaf	1.25
Fully expanded leaf	1.07
Matured leaf	1.00

c. Changes of foliar urea absorption of *Citrus unshiu* Marc. with time

Foliar urea absorption of *Citrus unshiu* Marc. is given in Figure 1. According to the obeservation of Impey and Johnes(1960), in Washington navel orange initial absorption of urea by leaves was so rapid that 70-80% of 5% urea applied could be absorbed within 2 hrs. Thereafter, absorption proceeded at slow but continous rate, being virtually complete within 30 hrs. In this experiment, initial absorption of *Citrus unshiu* Marc. leaves was much slower than that of Washington navel orange and 80 % of 1% urea solution applied was absorbed within the first 2 days after application. Thereafter, absorption became slower and reached more than 90% after 9-day.

It indicates that foliar urea absorption may be affected by several factors(e.g. temperature, humidity and plant species). Therefore, more studies on the

above factors to be considered are needed for estimating the amount of urea absorbed in the orchard.

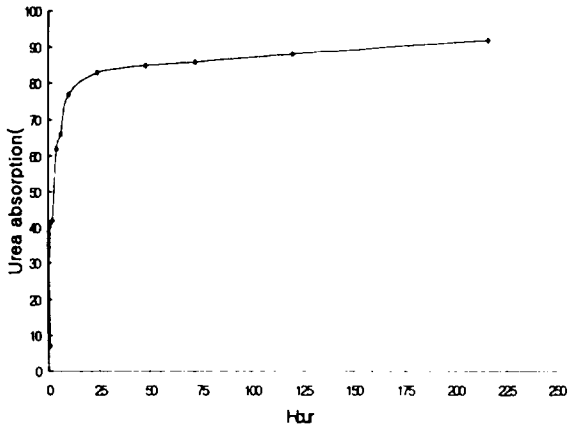

Figure 1. Foliar urea absorption(1%) of *Citrus unshiu* Marc. with time elapsed. 1% urea solution was applied to leaves, which were detached 2-day after application and washed by scrubbing with soft brush in water plus 0.01% detergent for 4 min.

CONCLUSION

A method for measuring foliar absorption of urea using ^{14}C-tracer is suggested at the practical application concentration unlike other's previous works in which 5% urea solution was applied to leaves((Impey and Jones, 1960; Shim et. al. 1972). Use of 5% urea solution to measure foliar absorption is recommendable because more than 2% urea can bring about foliar burn. However, in our experiment, factors affecting absorption rate of urea were not studied in details. Further experiment will be needed to determine foliar N absorption under the different conditions such as the temperature, humidity, N nutrient stress and leaf age.

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