THE METABOLISM OF FOLIAR-APPLIED UREA. I. RELATIVE RATES OF $C^{14}O_2$ PRODUCTION BY CERTAIN VEGETABLE PLANTS TREATED WITH LABELED UREA[1]

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Introduction

A number of horticultural crops respond favorably to foliar applications of urea. Considerable variability exists among crops, however, in the tolerances of the foliage to spray concentrations of urea as well as in the apparent rate of utilization, and in the growth and fruiting responses obtained (**2**, **3**, **4**). Plants showing the least tolerance to urea, in general, respond most favorably to physiologically tolerable amounts of it. The greater effectiveness of the nitrogen in urea when foliar-applied, as compared with the usual inorganic ionic forms, is generally believed to reside in its nonpolar organic properties. The first step in the utilization of the nitrogen in urea by the leaves of plants presumably is hydrolysis by the enzyme urease giving ammonia as one of the products.

$$(NH_2)_2C{=}O + H_2O \xrightarrow{\text{urease}} 2\,NH_3 + CO_2$$

In view of the almost immediate responses obtained with urea sprays on a number of crops, this reaction must proceed rapidly, and one of the factors limiting the reaction rate may be the urease activity in the leaves. Furthermore, rapid hydrolysis, possibly induced by high urease activity, may cause the accumulation of hydrolytic products conducive to the foliage injury easily achieved on most crops but which varies among species with regard to the highest concentration which can be applied without visual burning of the foliage.

As a measurement of the urease activity, and, similarly the rate of hydrolysis and possible utilization of urea applied to the leaves of several vegetable plants, carbon-14-labeled urea was employed, and the relative rates of the evolution of radioactive carbon dioxide were determined.

Materials and methods

Cucumber, bean, tomato, sweet corn, celery, and potato plants were grown to comparable sizes in sand in four-inch clay pots with complete

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nutrients in the greenhouse. Normal plants four to six inches in height and growing vigorously were selected for treatment.

A weighed quantity of C^{14} urea was dissolved in 0.25 to 0.5 milliliters of a 0.3% Dreft solution. The amount of solvent used was dependent upon the ability of the leaf surface to retain the solution. The urea concentrations varied from 0.1 to 0.5%, but were always held below the predetermined concentration considered toxic when applied as a spray to the leaves of a particular plant. The C^{14} urea was purchased from the Los Alamos Scientific Laboratory and had a specific activity of 1 mc. per 18 milligrams. Measured quantities of the solutions were applied immediately after preparation to a given area on the upper surface of the leaf or leaves of individual plants. An attempt was made to have the total area of application as well as the physiological age of the leaves of the various species as constant as

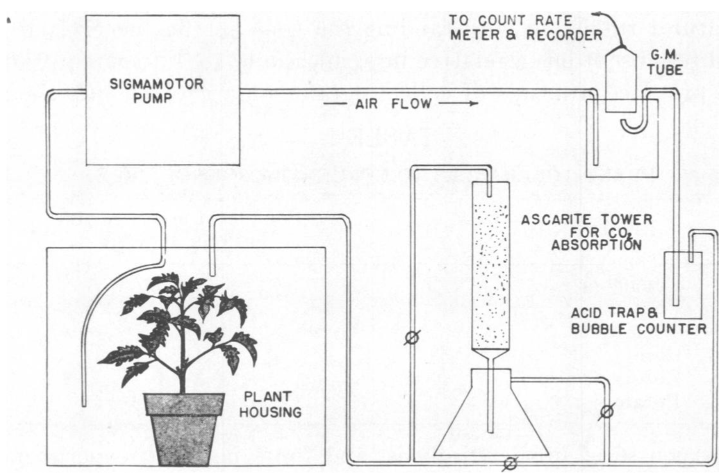

FIG. 1. Schematic drawing of closed system used to measure accumulation of $C^{14}O_2$ produced by hydrolysis of C^{14} urea.

possible. Young leaves, not fully expanded, were always selected for treatment. Before treating, all plants were held in the dark for at least one hour. Initial tests suggested that such a preconditioning greatly minimized the possible dark fixation of CO_2.

Following treatment and during the measurement of hydrolysis of urea the plants were held in the dark in order that the $C^{14}O_2$ evolved would not be re-utilized in photosynthesis. Several (never less than three) replicate runs were made on a single species to determine the pattern of hydrolysis including the approximate rate and kinetic order of the reaction.

The comparative rates of accumulation of $C^{14}O_2$ as a result of urea hydrolysis by the leaves of the various plants were measured by the apparatus shown in figure 1. A Nuclear Instrument and Chemical Corporation count rate meter (model 1615A) with an attached Esterline-Angus A. W. recorder was used in the design. For uniform voltage output for the count rate meter, a Solavolt constant voltage transformer was connected in the circuit. To provide a slow circulation of the atmosphere, which was continuously monitored in the closed system, a Sigmamotor pump controlled by a Boston Gear Works reductor (model LW13) was found satisfactory. This combination circulated the enclosed volume at a slow rate so that a representative sample was measured by the count rate meter and recorded by the attached recording instrument at all times. A typical activity curve thus obtained showing the accumulation of $C^{14}O_2$ in the system subsequent to the application of 10 μc. of C^{14} urea to the leaf of a tomato plant is illustrated in figure 2 A. A Nuclear Instrument and Chemical Corporation radiation counter with a D-34 mica window having a thickness of 1.4 mg./cm.^{2} was used. Ten microcuries of $C^{14}O_2$ urea when applied to a leaf in the typical manner gave from 200 to 500 counts per minute with this apparatus. Neoprene tubing was employed throughout the system since it was found superior to natural rubber in withstanding the constant flexing exerted by the pump and because it has a relative impermeability to diffusion of CO_2. The acid trap provided a means of collecting ammonia evolved into the system

TABLE I

PLANT TOLERANCES TO CONCENTRATIONS OF UREA.

Plant	Pounds of urea per 100 gallons of water
Cucumber	3–4
Bean	4–5
Tomato	4–6
Corn	4–6
Celery	8–14
Potato	8–14

during the course of urea hydrolysis, and following each experimental run this solution was always checked by Nessler's reagent for the presence of ammonia. At the conclusion of each experiment the entire enclosed volume was shunted through the ascarite tower for CO_2 absorption.

Urea toxicity tests were conducted under greenhouse conditions on 10 plants of each of the six species. Plants of the same age and size and which had been grown under similar conditions of temperature, sunlight, mineral nutrition, and moisture to those used in the C^{14} urea studies were selected for treatment. Concentrations of urea ranging from 2 to 20 pounds per 100 gallons of water with Dreft added as a wetting agent were applied by momentarily dipping the tops of the plants into the various solutions. Treatments were applied three times at weekly intervals. In table I the lowest concentrations causing some marginal leaf burning are listed for each crop.

Experimental results and discussion

The comparative rates of hydrolysis of C^{14} urea by the leaves of bean, corn, tomato, and cucumber plants as determined by the accumulation of

$C^{14}O_2$ in the closed system were recorded. In figure 2 A is presented a typical activity-time curve for the hydrolysis of urea applied to the leaves of a tomato plant and the portion of the curve used for determining its slope and rate constant. The slopes of similar curves are portrayed, and rate con-

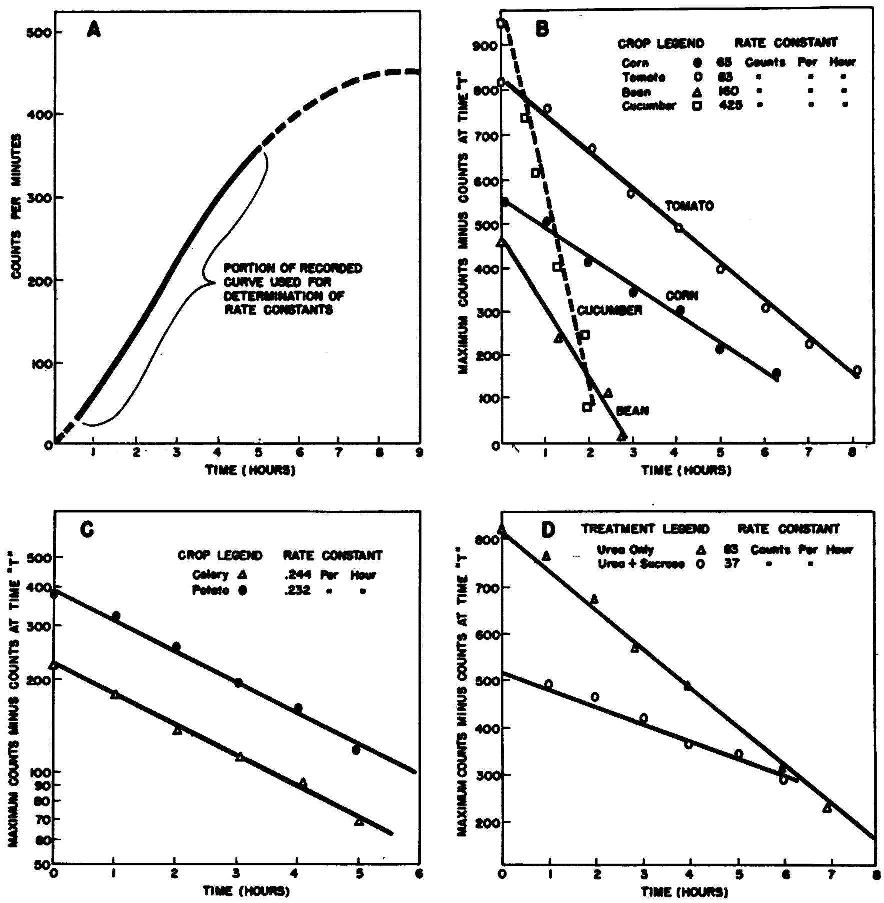

FIG. 2. The rates of hydrolysis of C^{14} urea applied to the foliage of several vegetable plants as illustrated by the initial slopes of the activity-time curves, showing the accumulation of $C^{14}O_2$ in a closed system. A. A zero order activity-time curve showing the approximate portion of the recorded curve used for determination of rate constant. B. Typical zero order rate constants and curve slopes for corn, tomato, bean, and cucumber. C. First order rate constants and slopes for celery and potato. D. Comparative rate constants and curve slopes for the tomato with urea only and with urea and sucrose.

stants are listed in figure 2 B for cucumber, bean, tomato, and corn plants. The reaction rates for these four plants were typically of zero order. The most rapid rate of hydrolysis occurred when urea was applied to the cucumber leaf, followed by the bean, tomato, and sweet corn. Within the range of concentrations used in these tests, the calculated rate constants were found to be relatively independent of the amount of urea originally applied to the leaves, and definite slopes of the curves were found to be characteristic for a given species and easily reproducible.

It is of interest to note that within two to three hours subsequent to treatment, foliar-applied urea apparently is completely hydrolyzed by cucumber leaves, and of all plants tested the leaves of the cucumber exhibit the greatest urease activity. This is of further significance since observations of tolerance to spray concentrations reveal (table I) that cucumber leaves are the most sensitive of the six plants tested; they are injured by concentrations greater than three to four pounds of urea to 100 gallons of water.

The rapid hydrolysis of urea by bean leaves (variety, Black Valentine) and their sensitivity to urea (table I) is not surprising in that urease was first crystallized from Jack bean meal (**5**). The tomato and sweet corn plants show considerably lower rate constants, but it is significant, even with these plants, that the hydrolysis of foliar-applied urea proceeds rapidly, that within a few hours following application the reaction is complete, and that tomato and sweet corn plants are relatively sensitive to low concentrations of urea.

The leaves of celery and potato were found to be much more tolerant to successive sprays of urea (table I). The hydrolyses of urea as measured by the evolution of $C^{14}O_2$ from the C^{14} urea applied to these leaves were found to have different reaction rates and gave straight lines when the initial log counts were plotted against time (fig. 2 C). The kinetics of such reactions are of first order, and the rate constants suggest a remarkable similarity in the urease activities of the foliage of celery and potato. The low rate constants for urease activity may indicate a basis for the high tolerance of the foliage of these crops to urea sprays. At low concentrations of urea (0.02%) applied to the leaves of celery and potato, the kinetics appeared to be of zero order with a rate constant of approximately 20. It is possible with these plants that the amount of the enzyme rapidly becomes a limiting factor as the reaction proceeds.

Tolerances of the leaves of plants to urea as related to the time-activity curves, which are in turn determined by the hydrolysis rate constants, are of special interest in view of the report that sucrose applied in equal molar quantities to urea protects the foliage of the tomato plant against possible injury by urea sprays (**1**). A solution containing 0.1 molar sucrose and C^{14} urea was prepared, applied in the usual manner, and the rate constant was derived from the initial slope of the time-activity curve and compared with that characteristic of the usual application of C^{14} urea on the leaves of the tomato plant. These data are presented in figure 2 D. As was characteristic of the tomato, both reaction rates were typically zero order, but urea hydrolysis was significantly reduced by the addition of sucrose in equal molar quantities. Possibly sucrose exerts its effect in preventing foliage burning of the tomato by partially inhibiting urease activity, which may in turn prevent the rapid accumulation of toxic hydrolytic products of the reaction. In practice this effect of sucrose may be advantageous in that higher concentrations of urea may be applied and urea utilization extended for a longer period of time. Similarly, the immediate and characteristic responses from nitrogen applications to leaves may not be so readily apparent with urea if sucrose is added.

Of special significance in these studies appears to be the relationship apparently established between the reaction rates characteristic of urea hydrolysis by the leaves of various plants, the growth responses of these plants to urea and the concentrations of urea which can be applied without burning injury to the foliage. The data reported would suggest that plants most easily injured by urea sprays are apparently those which have the highest urease activity in the leaves. Conduciveness toward injury is indicative of rapid hydrolysis denoting rapid utilization and distinguishes the plants most readily benefited if below-toxic concentrations are employed. With the plants studied, urea hydrolysis as it occurs in the leaves of plants subsequent to foliar applications, appears to be the equivalent of utilization. In no instance was any ammonia collected in the acid trap (fig. 1) during the course of urea hydrolysis in the atmosphere of the closed system employed in these tests.

Summary

The first step in the utilization of the nitrogen in urea applied to the leaves of plants presumably is hydrolysis by the enzyme urease giving NH_3 and CO_2. The comparative rate constants of the activity-time curves of the enzymatic hydrolysis of carbon-14-labeled urea applied to the leaves of cucumber, bean, tomato, corn, celery, and potato were determined by continuously monitoring the air and recording the accumulation of $C^{14}O_2$ in a closed system.

The initial reaction rates for cucumber, bean, tomato, and corn, the leaves of which were found relatively intolerant to urea, were typically of zero order. With celery and potato plants having considerably more tolerance to foliar-applied urea, the kinetics of the enzymatic hydrolysis of urea by the leaves were of first order. Equal molar concentrations of sucrose used in solution with urea and applied to the foliage of tomato plants significantly reduced the rate of urea hydrolysis.

It is suggested that plants most easily injured by urea sprays are apparently those which have the highest urease activity, and the initial kinetics of the reaction are of zero order. Conduciveness toward urea injury is indicative of rapid hydrolysis and may denote rapid utilization. Hydrolysis as it occurs in the leaves of plants subsequent to foliar applications of urea appears to be equivalent to utilization.

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cago, were utilized in designing the apparatus (fig. 1) for continuously monitoring the accumulation of $C^{14}O_2$ in a closed system.

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