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THE ABSORPTION, TRANSLOCATION AND METABOLISM OF C^{14}-LABELED SIMAZINE (2-chloro-4,6-bis-(ethylamino)-s-triazine) IN ORCHIDS

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF THE
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By
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BIOGRAPHICAL SKETCH

Sheila W. Chao was born in China on April 3, 1940. She attended elementary school in Chengtu, China. Her family moved to Taiwan in 1949 because of the communist occupation of mainland China. In Taiwan she attended the Taipei Provincial First Girls Middle High School and graduated in 1957. She obtained a Bachelor of Science degree from the National Taiwan University in June 1961. While an undergraduate at National Taiwan University she conducted research on the physiological changes of banana fruits during shipment. After graduation she was employed as a teaching assistant for one year in the Department of Botany of the university.

Sheila was awarded an East-West Center scholarship to pursue graduate studies leading to a Master's Degree in Horticulture at the University of Hawaii. She obtained the degree in June 1965.

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INTRODUCTION

Simazine, 2-chloro-4-bis-(ethylamino)-s-triazine, is synthesized by reacting cyanuric chloride with two equivalents of ethylamine in the presence of an acid acceptor. Simazine is recommended for use as a selective herbicide for weed control in a long list of major crops by the Geigy Chemical Company. Based on preliminary observations by Gilmy (1963), the Geigy Company disclosed the possibility of using simazine for weed control in orchids.

From tests at the Hawaii Agricultural Experiment Station, Murashige, Sheehan and Kawamoto (1963) established that simazine could be used as a herbicide in vanda, cattleya and dendrobium orchids. The effective killing of algae, mosses and a variety of broadleaved weed species was observed within a month after treatment. Simazine controlled weeds for one year in the investigations. Gilmy, as reported by Murashige (1963), observed a delay in flowering of the orchids as a result of the influence of simazine.

Simazine has been extensively recommended and used for weed control in corn, Zea mays L. The most widely accepted theory of explaining the resistance of corn to simazine is that corn has a mechanism which detoxifies or degrades the simazine to hydroxysimazine, a non-toxic form.

The main purpose of this study was to determine if the selectivity in orchids was due either to a limited absorption of simazine or to the metabolism of the herbicide to a non-toxic compound such as hydroxysimazine. Since orchids have waxy leaf cuticles and some

have terrete leaves and aerial roots, it was postulated that the selectivity may be a result of little or no uptake of simazine.

REVIEW OF LITERATURE

Chemical and Physical Properties

Simazine, 2-chloro-4,6-bis-(ethylamino)-s-triazine, has the following chemical structure:

The technical material is a white, crystalline substance which is non-combustible and non-corrosive. At 25° C. its solubility in water is 5 ppm, methanol 400 ppm, and in chloroform 900 ppm.

Simazine sublimes at high temperatures in basic media. When heated in acidic or basic media it hydrolyzes to the herbicidally inactive 2-hydroxy-4,6-bis-(ethylamino)-s-triazine. The solubility of hydroxysimazine at 25° C. is 4 ppm in water, 11 ppm in ethanol and 0.3 ppm in chloroform.

Absorption and Translocation

Simazine is most effective when applied before weeds emerge. Although simazine does not prevent the germination of weed seeds, it destroys weed seedlings after gaining entrance through the root system.

Absorption of a lethal concentration of simazine results in chlorosis and eventual death of the seedling weeds shortly after emergence. Simazine has little if any foliage contact action.

Crafts (1959) found that labeled simazine penetrated the leaf cuticle quite readily and moved within the leaf only in an acropetal direction. The distribution of simazine in leaves suggested that simazine, upon entering the mesophyll, diffused along the cell walls and did not enter the living symplast.

Davis, et al. (1959) studied the absorption and translocation of C^{14}-labeled simazine by corn, cotton and cucumber plants. They concluded that the toxicity symptoms expressed as marginal leaf necrosis on cucumber and cotton plants were due to heavy accumulation. The only toxic effect on corn was a stunting of the plant after a prolonged period in a nearly saturated solution of simazine. The leaves of the three crops were far more susceptible than the roots. They also showed that simazine entered the stele roots and was translocated in the xylem.

Sheets (1961) studied the uptake and distribution of simazine-C^{14} by oat and cotton seedlings and found that it was absorbed by the roots and distributed throughout oat seedlings within three hours. The concentration of C^{14} in the oat leaves, a susceptible species, was approximately three times greater than in cotton leaves, a semitolerant species. Absorption and translocation of simazine-C^{14} from solution were greater at 37° C. than at 26° C. At 37° C., absorption and translocation were greater in plants which were grown in a 33 percent

relative humidity environment than at 66 percent relative humidity. However, Clor, Crafts and Yamaguchi (1962) found with cotton seedlings that the translocation of labeled 2,4-D and urea applied to the foliage was greatly increased under high humidity conditions.

Smith and Buchholtz (1962) studied the transpiration rate of atrazine in plants. They concluded that atrazine acts as an inhibitor of photosynthesis and showed that it reduced the transpiration rate both in tolerant and susceptible plants. The reduction in transpiration occurred within one to three hours after application and the transpiration rate was reduced by 50 percent after six hours.

Ville and Davis (1962) studied the influence of atrazine on the water uptake of corn, cotton and soybean plants. They found that the water loss from atrazine treated plants was reduced in light but not in the dark. Microscopic examination revealed that atrazine caused closure of the stomata. It was also shown that the decrease in transpiration corresponded in time to the effect of atrazine on photosynthesis.

According to Roth (1958), simazine inhibited photosynthesis in Elodea and had a stimulating effect on respiration. Esser (1959) reported that simazine inhibited the photochemical activity of isolated chloroplasts. He also presented evidence that the inhibited reaction involved photochemical reduction of DPN, diphosphopyridine-nucleotide. Moreland, et al. (1959) reduced the photochemical activity of isolated barley chloroplasts by 50 percent in a 4.6×10^{-6} M solution of simazine. Glucose supplied to the barley plants through severed leaf

tips kept the plants alive and growing in the presence of lethal concentrations of simazine. They postulated that the blocking of photosynthesis was a main weed killing power of simazine. However, Crafts (1961) stated that the true mode of action of triazine herbicides involved more than an inhibition of photosynthesis in that, some weeds are killed before they appear above ground. Apparently the blocking of photosynthesis is simply a secondary response as regards the herbicidal activity of triazines.

Gast (1959) reported that simazine blocked starch production in the light in Coleus blumei Benth. This effect was overcome by the addition of sucrose to starch-free leaves in the dark, thereby indicating that simazine inhibited sugar formation. These results also indicated interference of photosynthetic CO_2 fixation by simazine.

Ashton, et al. (1960) studied the effect of certain triazines on $C^{14}O_2$ fixation in red kidney beans. They concluded that simazine and related triazines drastically inhibited CO_2 fixation in the light. This is undoubtedly an important factor in the phytotoxic properties of these compounds.

Allen and Palmer (1963) studied the mode of action of simazine in barley. They confirmed that light is essential for the lethal action of simazine. The inhibitory effects of simazine in barley could be prevented either by excluding light or by supplying glucose.

In short, simazine inhibits the Hill reaction which is an important reaction associated with photosynthesis.

Metabolism

As reported by Crafts (1961), Roth (1958) added simazine to freshly pressed corn juice and after 100 hours only a limited amount of the original simazine was recovered. Over 90 percent was recovered from fresh wheat juice tests. From these results Gysin and Knusli concluded that the selectivity between species treated with triazine herbicides was principally the result of differential breakdown.

Roth (1959) found that simazine sensitive plants exhibit a higher catalase activity than resistant plants. Since catalase formation is dependent upon light, a change in the catalase activity of a plant may be a result of the inhibition of photosynthesis by simazine. He extracted a polyphenol fraction from corn which was able to break simazine down in vitro and this polyphenol was identified as a definite spot on a chromatograph. Plants having varying degrees of tolerance showed a significant content of polyphenols accompanied by peroxidase activity. Roth proposed that triazine herbicides interfere with the redox-potential level required for normal chlorophyll function.

According to Roth and Knusli (1961), Virtanen and his co-workers (1955-1960) found that 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one, isolated from corn sap, detoxifies the herbicide simazine in vitro. 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one and its corresponding natural precursors and glucosides behave in the same manner. It is likely that these glucosides and aglucones play a role in the resistance phenomenon of some plant species against simazine and related phytotoxic compounds.

Khudyan, Kearney and Sheets (1961) studied simazine degradation by soil microorganisms. They found that Aspergillus fumigatus Fres. is effective in the degradation of simazine. It was postulated that this fungus may possess a degradation mechanism unlike that which occurs in corn plants. Couch, et al. (1963) studied the metabolism of atrazine and simazine by soil fungi. They found that four kinds of soil fungi degraded the side chains of simazine, but ring cleavage did not occur to any appreciable extent.

Hagen and McCallum (1961) studied C^{14}-labeled simazine degradation in sweet corn, cucumber plants, and soil samples. They found more activity in plant roots than in tops when treatment was via the roots, and cucumbers showed more activity than corn. By paper chromatography it was shown that extracted plant parts had simazine in leaves and stems along with undetermined labeled components. Work with sterile and non-sterile soil proved that microorganisms play an important role in simazine breakdown in soil.

Sheets (1961) studied solvent extracts of simazine treated plant tissues. He showed that measurable amounts of the absorbed simazine were metabolized during a 96 hour exposure period. The simazine molecule was apparently changed more rapidly in roots than in leaves.

Montgomery and Freed (1961) studied the absorption, translocation and metabolism of C^{14}-labeled simazine and atrazine by corn plants. They found that the concentration of total C^{14} in the plants decreased after 30 days and that the chloroform-extractable fraction decreased after the first time of harvest. It was shown by ion exchange and paper chromatography that only trace amounts of these compounds remained

unchanged in the plants. They concluded that simazine and atrazine are metabolized in corn plants and eventually degraded to CO_2. This probably explains the great tolerance of corn to these compounds.

Castelfranco, et al. (1961) studied the non-enzymatic detoxification of simazine by Zea mays extracts. A system was found which was able to convert simazine to the corresponding 2-hydroxy analogue in the extracts. The active constituent was dialyzable, soluble in 90 percent acetone, extractable with ether and ethyl acetate and totally destroyed by ashing. In the crude extract the active constituent was destroyed by boiling. No loss of activity was found after the preparation was cleaned up by acetone precipitation and boiled for five minutes. These properties indicated that the active constituent was not a protein. Extract of Avena, a simazine susceptible species, contain none of the simazine destroying activity which is present in the extracts of Zea. The significance of these findings with respect to the toxicity of simazine may play a major role in determining the resistance or susceptibility of a particular species.

MATERIAL AND METHODS

The ensuing studies were designed to attempt to determine the cause of orchid plant tolerance to simazine.

A preliminary phytotoxicity test was conducted to become familiar with the use of simazine for weed control in orchid culture. Young Dendrobium plants were selected as the test plant to repeat and further study the previously reported leaf burn and a delay in time of flowering caused by the application of simazine to young orchid plants. (Muramoto, et al. 1963).

Absorption, translocation and metabolism of simazine were of interest in an attempt to explain the tolerance of orchids to simazine. Since it was postulated that the tolerance might be partially due to little or no uptake of the herbicide by orchid plants, absorption and translocation studies were designed to determine if simazine was absorbed by orchid leaves and roots. Following the absorption studies, metabolism studies were initiated with the hypothesis that if simazine is absorbed by orchid plants it might subsequently be degraded to compounds with non-phytotoxic properties.

PHYTOTOXICITY

Because previous work by Muramoto, et al. (1963) showed that young Dendrobium and Vanda plants exhibited leaf burn and a delay in time of flowering when treated with a 10,000 ppm simazine suspension, the following experiment was designed to determine if a lower concentration of simazine could be used on the young orchid plants, and if repeated applications at monthly intervals would cause any observable

toxic effects. Dendrobium (Valley King Anthos x Phal. Lycn's strain) plants, grown in pots with shredded hapu (tree fern stem) were immersed for one minute in a simazine suspension at monthly intervals. A completely random experimental design was used in which the treatments were repeated four times. The orchids were dipped in a 5,000 ppm suspension of simazine 80 W for one minute after 0, 1 and 3 months. The simazine suspension was stirred before immersing the plants to the surface of pots. Special precautions were taken to never wet the orchid leaves.

The height of plants, number of flowers and length of flower stalks were recorded at various time intervals after treatment.

ABSORPTION AND TRANSLOCATION

It was postulated that very little simazine would be absorbed and translocated by the waxy-terrete leaves of Vanda orchids and similarly by the thickly cutinized leaves of Dendrobium orchids. If this hypothesis were proven, there would be no need to use carefully directed time consuming spray applications or pot dips. A series of experiments were designed to study the absorption and translocation of simazine labeled with C^{14} at the 2, 4 and 6 position in the triazine ring. Root uptake was also studied with the aid of water culture solutions. Three time series experiments were conducted to study absorption and translocation:

Time Series Experiment No. 1

In the laboratory under controlled temperature and light conditions, a 12 hour day was provided with a light intensity of

approximately 450 foot candles, a temperature range of 75° to 80° F. and a relative humidity from 50 to 70 percent.

Seven Vanda cuttings (ca. 20 cm long) and seven Dendrobium plants were divided into two groups -- one received leaf applications of simazine-C^{14} and the other a root application. The base of the freshly cut Vanda plants were placed in half strength Hoagland's solution for one day before treatment. A single plant served as a control.

Using masking tape, each plant was attached to the inside of a 2 x 4 inch bottle wrapped with aluminum foil to exclude the light. Fifty ml of half strength Hoagland's solution were used as a medium, non-aerated.

The specific activity of the simazine-C^{14} supplied by the Geigy Agricultural Chemical Co. was 7.85 uc/mg. A stock solution was prepared by dissolving 12.7 mg of the simazine in 2 ml of DMF (Dimethyl formamide) and heating slightly. The treatment solution was prepared by adding 0.8 ml of methanol to 0.2 ml of the stock solution. This resulted in a specific activity of 0.1 uc for each 10 ul of treatment solution. The leaf application was applied at the base of the 2nd leaf of each plant with 10 ul of treatment solution in a 1 cm diameter lanolin ring. The root application was made by adding 10 ul of treatment solution to the growing medium containing 50 ml of half strength Hoagland's solution.

After absorption periods of 1, 4 and 16 days, the lanolin ring was removed with facial tissue and the plants were removed from the culture medium. Immediately after removal the plants were rinsed with tap

water six times and then wrapped in paper towels to prevent radioactive contamination. The plants were then killed in a freeze-drier.

Exploratory trials revealed that, the heavily cutinized epidermis prevented the orchid plants from drying; therefore, just before the plants were taken to the freeze-drier, tiny holes were made over the leaf and stem surfaces with a sewing needle (ca. 1 hole/0.5 cm^2). A Sublimator Automatic Freeze-drier with condensing coils in the vacuum drum to contain the orchid plants was used to quick-kill the plants at temperatures of 0 to 5° F. The drying procedure was as follows:

1. Small holes were made on the leaf and stem surfaces before drying.
2. The condensor temperature was set at - 30° F. before the vacuum pump was turned on.
3. The vacuum drum gauge was allowed to drop below 150 microns before applying a shelf heat of 100° F.

After drying, the plants were autoradiographed following the procedures outlined by Crafts and Yamaguchi (1958). In general, the procedure included: the plants dampened in a moisture chamber, mounted on paper, pressed, redried before autoradiographing. The pressed mount was exposed to KODAK NO-SCREEN X-ray film for two weeks before developing with a standard procedure.

The resulting autoradiograms were the main source of information for interpreting the translocation of simazine in orchids.

Time Series Experiment No. 2

In the laboratory, under controlled temperature and light conditions, a 12 hour day was provided with a light intensity of

approximately 600 foot candles, a temperature range of 65° to 70° F. and relative humidities from 50 to 70 percent.

Seven Dendrobium plants (Valley King Anthos x Phal. Lyon's strain) were prepared as in the previous experiment. Among the seven plants, three received leaf applications and three received root applications with one remaining plant used as a control. The same absorption times were used as in Experiment 1. Autoradiographic procedures differed from those used in Experiment 1 only in the way of mounting. The stem was cut into cross sections 0.1 cm in thickness, mounted and oriented on paper as in the original plant.

Time Series Experiment No. 3

This study was carried out in a screen field house which had 60 percent transparency. The mean temperatures varied from 72° to 83° F. and the mean relative humidities ranged from 50 to 75 percent. No aeration equipment was used in this experiment.

The preparation following treatment of the five Dendrobium orchids was similar to those in Experiment 1. One plant served as a control and four plants received root applications of simazine-C^{14}. The absorption periods for the treated plants were 6 hours, 1, 4 and 16 days respectively with the control plant harvested at 4 days. The plants were mounted longitudinally rather than in cross section.

METABOLISM

Hamilton and Moreland (1962) isolated hydroxysimazine as the major degradation product in short-term experiments with intact corn seedlings. Freed, et al. (1961) demonstrated that hydroxysimazine

formed as the first metabolite in experiments conducted with corn. The following experiments were designed to determine if orchid plants would metabolize simazine to hydroxysimazine. Dendrobium plants were treated with labeled simazine and the metabolic products studied with strip chromatograms.

Metabolism Experiment No. 1

In the laboratory, under controlled temperature and light conditions, a 12 hour day was used with a light intensity of approximately 450 foot candles. The temperature range varied from 75° to 80° F. and the relative humidities from 50 to 70 percent.

Two Dendrobium plants were removed from their original medium and attached individually to a 4 x 10 in. glass plate which was covered with two sheets of filter paper. The roots were spread out over the upper part of the filter paper and secured by three rubber bands per plant. The glass plate containing the Dendrobium plant was placed at an angle in a 5 x 10 x 14 in. coverless glass chamber. Two hundreds ml of half-strength Hoagland's nutrient solution were added to the chamber. The nutrient solution provided the necessary nutrients to the plant and kept the filter paper moistened at all times.

The treatment solution consisted of .8 ml of stock solution diluted to 8 ml with methanol. The external surface of the plant was painted in its entirety with a brush. One ml of treatment solution was used per plant per day. The solution was applied for four days and the plants harvested after allowing one additional day for absorption. The filter paper was detached from the plant before rinsing with tap water and crushing in an electric blender.

The Dendrobium macerated tissue was transferred to 250 ml of absolute methanol with constant stirring. After waiting for a period of two hours, 200 mls of the filtrate were obtained through filtration. The filtrate was evaporated to dryness by using a stream of dry air. Subsequently, the dried extract was redissolved in 2 mls of methanol. A volume of 100 ul of this extract was spotted on a paper strip (Whatman No. 1, 17 inches long and 1.5 inches wide) and the descending paper chromatogram was developed for 20 hours with isoamyl alcohol saturated with 3 N HCl.

A 3C-55 AR continuous Chromatographic Scanner was used to detect the radioactivity which was graphed by an automatic recorder.

Metabolism Experiment No. 2

This experiment was carried out in the screen field house used for the Time Series Experiment No. 3.

One healthy Dendrobium plant was removed from the original shredded bagus medium and the root system was wrapped with a fitted plastic bag. 0.2 ml of simazine-C^{14} stock solution diluted to 10 mls with half strength Hoagland's solution was added to the bag. The mouth of the bag was secured tight for a period of four days. A second 10 mls of half strength Hoagland's solution without simazine-C^{14} was added to the bag for another four day period to insure that all of the original treatment solution was absorbed. The plant was harvested at the end of 8 days, rinsed with tap water and wrapped in paper towels. The plant was taken to the laboratory and chromatogramed as in the previous experiment. The only exception was that 20 ul of extract was spotted on the paper strip instead of 100 ul.

RESULTS

PHYTOTOXICITY STUDIES

Based on visual observations of the foliage, no symptoms of orchid injury were found within seven months after the simazine was applied. Excellent weed control was observed at two to three weeks after treatment application. The weed-killing power lasted in the medium over the experimental period. The treatment effects are shown in Figure 1.

Figure 1. Dendrobium plants showed no delay in time of flowering as a result of the simazine treatment. (Left to right: 2 dips, 1 dip and water control.)

The heights of the Dendrobium plants were recorded at time of treatment (September 1), and one at four months after the third dip. The number of flowers was recorded during the blooming period in November and the length of flower stalks was recorded at the end of blooming period on November 24. The data were analyzed statistically and are presented in Tables 1 and 2.

Table 1 and 1a show that there were no significant differences between the heights of the Dendrobium plants when treated with simazine. However, the total number of flowers and the total length of the flower stalks were reduced in all of the simazine treatments when compared to the water control (Tables 2 to 2c). The number of flowers were reduced by approximately one-half on the treated plants. Treatment No. 1, water control, had an average of 7.3 flowers per stalk as compared to 4.3, 3.0 and 3.3 for the 1, 2 and 3 simazine dips, respectively. Similarly, the flower stalk lengths were reduced when simazine was applied. The 0, 1, 2 and 3 dip treatments resulted in flower stalk lengths of 17.0, 14.6, 13.1 and 12.5 cm, respectively. The repeated dip treatments were not significantly different than the single dip when comparing the number of flowers produced and final length of flower stalks.

ABSORPTION AND TRANSLOCATION STUDIES

A visual inspection of the original autoradiograms from the three time series experiments showed that C^{14}-simazine penetrated the cuticlized orchid leaves and was translocated acropetally to a limited extent (Figures 2, 3 and 4). No movement occurred in a basipetal direction in any of the leaf treatments. When applied to the roots, it

Table 1. GROWTH OF DENDROBIUM PLANTS DIPPED IN 5,000 PPM SIMAZINE (80 W) SUSPENSION. (VALUES ARE PRESENTED FOR THE NET INCREASE IN GROWTH)

Treatment	Treatment mean of plant growth (cm)[1] 9/1/1964 - 12/31/1964	9/1/1964 - 3/31/1965
1. water control	3.9	6.7
2. 1 dip	3.2	4.6
3. 2 dips	7.0	9.3
4. 3 dips	4.6	6.2

[1] Each treatment mean is an average of 4 values.

Table 1a. ANALYSIS OF VARIANCE (MEAN SQUARE) FOR THE GROWTH MEASUREMENTS CONTAINED IN TABLE 1

Source of variation	d.f.	Data 9/1 - 12/31	9/1 - 3/31
Treatments	3	1.23	0.95
Error	12	1.38	2.06

$F_{.05} = 3.49$, $F_{.01} = 5.95$

Table 2. NUMBER OF FLOWERS AND LENGTH OF FLOWER SPIKES OF DENDROBIUM PLANTS DIPPED IN 5,000 PPM SIMAZINE (80 W) SUSPENSION

Treatment	Treatments[1] No. of flowers	Length of flower spikes
1. water control	7.3	17.0 cm
2. 1 dip	4.3	14.6
3. 2 dips	3.0	13.1
4. 3 dips	3.3	12.5

[1] Each treatment mean is an average of 4 values.

Table 2a. ANALYSIS OF VARIANCE (MEAN SQUARES) FOR THE FLOWERING DATA CONSIDERED IN TABLE 2

Source of variation	d.f.	No. of flowers	Length of flower stalks
Treatment	3	15.23**	15.84**
Error	12	1.35	1.86

$F_{.05} = 3.49$, $F_{.01} = 5.95$

** Significant F-value at the 1 % level.

Table 2b. TUKEY'S TEST FOR THE COMPARISON OF TREATMENT MEANS FOR NUMBER OF FLOWERS

Treatment	$\bar{x}$ 1/	$\bar{x}$ - 3.00	$\bar{x}$ - 3.25	$\bar{x}$ - 4.25
Water control	7.25	4.25* (2.44)	4.00* (2.19)	3.00* (1.79)
1 dip	4.25	1.25 (2.19)	1.00 (1.79)	
3 dips	3.25	0.25 (1.79)		
2 dips	3.00			

* Significant differences at the 5 % level.

Table 2c. TUKEY'S TEST FOR THE COMPARISON OF TREATMENT MEANS FOR THE LENGTH OF FLOWER STALKS

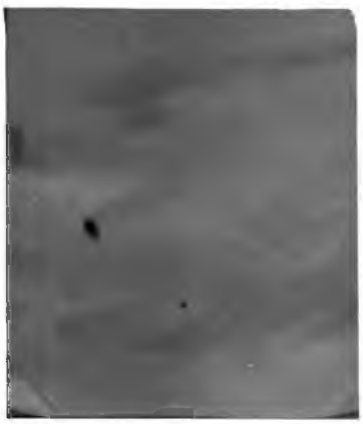
Treatment	$\bar{x}$	$\bar{x}$ - 12.50	$\bar{x}$ - 13.13	$\bar{x}$ - 14.63
Water control	16.97	4.47* (2.77)	3.84* (2.49)	2.34* (2.03)
1 dip	14.63	2.13 (2.49)	1.50 (2.03)	
3 dips	13.13	0.63 (2.03)		
2 dips	12.50			

Figure 2. Results of C^{14}-glycine applications to the roots and leaves of Dendrobium and Vanda plants. Autoradiograms of 16-day harvested plants. (0.1 uc/10 ul, 75°- 80°F., 450 fc.) Time Series Experiment No. 1. (Aa, Ab, Vanda plants; Ba, Bb, Dendrobium plants)

Aa

Ba

Ab



Bb

Figure 3. Results of C^{14}-simazine applications to the roots (Aa, Ba, Ca) and leaves (Ab, Bb, Cb) of Dendrobium plants. Autoradiograms of 1, (A)4, (B) and 16-(C) day harvested plants. (0.1 uc/10 ul, 65°- 70° F., 600 fc.) Time Series Experiment No. 2.


Aa


Ba


Ca

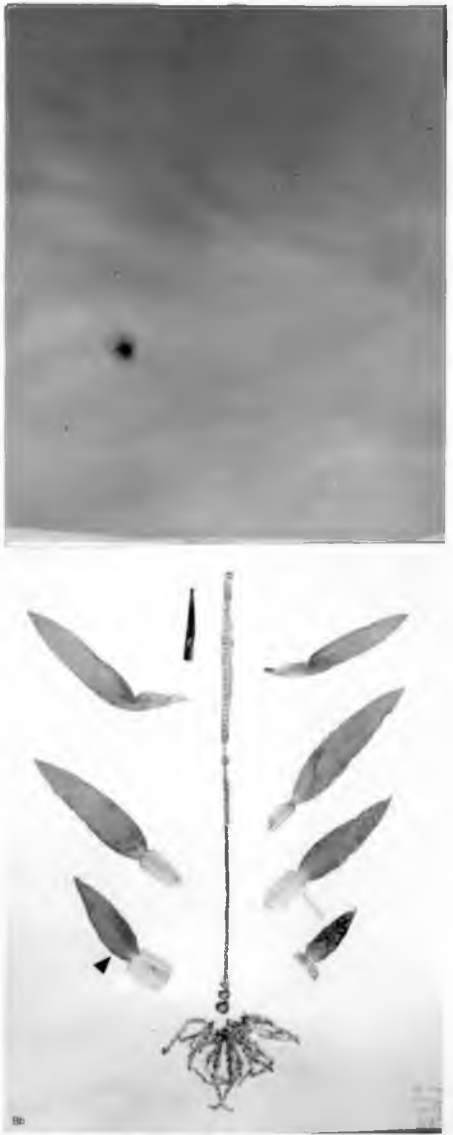
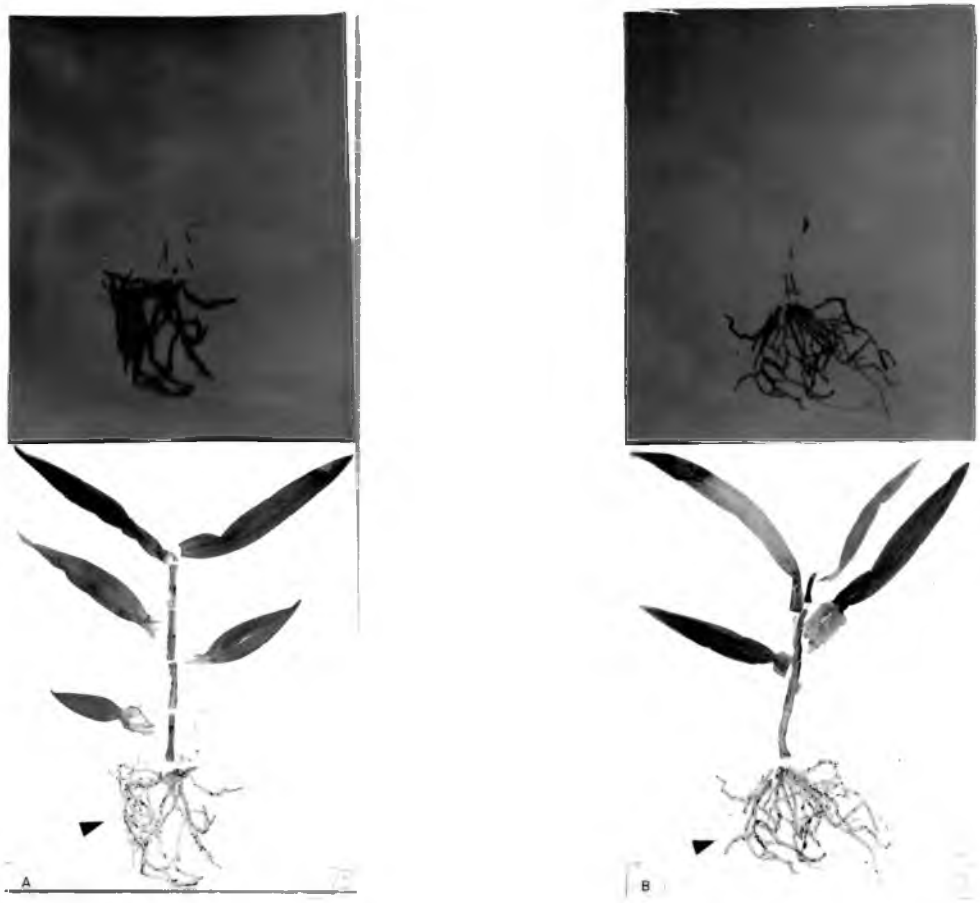


Figure 4. Results of C^{14}-cinnamic applications to the roots of Dendrobium plants. Autoradiograms of 1/4 (A), 1 (B), 4 (C) and 16-(D) day harvested plants. (0.1 uc/10 ml, 72°- 83° F. natural light) Times Series Experiment No. 3.


A
B
C
D

moved throughout the stem and leaves and there was no evidence of basipetal translocation after the simazine-C^{14} accumulated in the leaves and stems of the plant. Table 3 gives the absorption and translocation patterns for the three time series experiments.

METABOLISM STUDIES

The radioactivity chromatogram curve of Metabolism Experiment No. 1 showed that there was one major peak at Rf 0.74. The experiment failed to show any additional peaks or metabolites (Figure 5A).

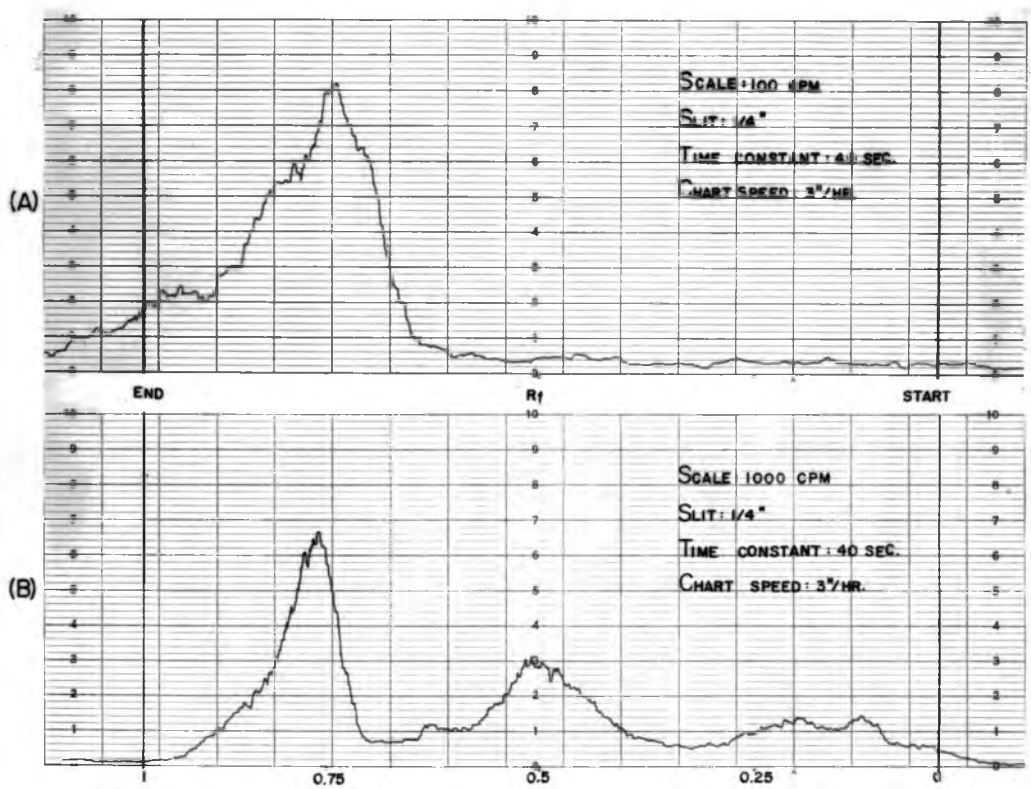


FIG: 5 CHART SHOWING RADIOACTIVITY IN THE CHROMATOGRAM. METHANOL SOLUBLE METABOLITES FROM DENDROBIUM PLANTS TREATED WITH C^{14}-RING LABELED 2-CHLORO-4,6-BIS(ETHYLAMINO)-s-TRIAZINE

However, in Metabolism Experiment No. 2, the radioactivity chromatogram curve showed two major peaks; one at Rf 0.76 and the other at Rf 0.50. Some minor peaks were also noted at Rf 0.15 - 0.20 (Figure 5B). Freed (1961) showed that the peak for C^{14}-simazine is at approximately Rf 0.80 and for C^{14}-hydroxysimazine approximately Rf 0.50. The two minor peaks were similar to that reported by Freed (1961), but as yet they have not been identified.

DISCUSSION

Controlling weeds in commercial production of orchids is a serious problem. Since hand removal of the weeds is not only time-consuming, but also often injures orchid roots, an effective herbicide for orchids is most desirable.

Murashige, Shoshan and Nakamoto (1963) established that simazine may be used as an herbicide for Vanda, Cattleya and Dendrobium orchid culture. Complete eradication of weeds was reported, but a slight delay in flowering of Vanda and Dendrobium orchids was observed. No delay and other abnormalities in flowering were observed with Cattleyas.

Based on the phytotoxicity observations on the Dendrobium studies reported herein, the number of flowers and the length of flower stalks were significantly decreased as a result of the use of simazine as an herbicide.

Certainly, the results from this single experiment are not wholly conclusive and cannot eliminate the possibility of the practice of using simazine in Dendrobium cultivation, because there are several criteria which have to be considered. First of all, the size of sampling was small. To establish the reality of this experiment, a larger sample size would be desired, and a repeat of one or more similar experiments are needed.

It was reported by Murashige, et al. (1963) that mature orchid plants were more tolerant than younger seedlings. Although the plants used in this test were two years old, they still may not have been

sufficiently mature to withstand the one minute dip in a 5,000 ppm suspension of simazine 80 W.

The concentration of the treatment solution merits consideration. The principle of deciding the concentration of the treatment solution should be determined by the minimal concentration needed for optimal weed control. Before practical recommendations might be made, further trials should be established to determine the amount of simazine needed for plants of different ages.

There is often a tendency of recovery from the toxicity caused by a herbicide. The number of flowers might increase gradually in a population treated with simazine. Since the weed killing power of simazine possesses a long-lasting characteristic, it is quite possible that the eradication of weeds might be retained for more than one blossom season. Therefore, weighing the value of benefit and loss, it may still be worthwhile to use simazine in two year old cymbid Dendrobium plants. To prove this point, a long-term experiment should be conducted to give an estimate of the economical value.

From the experimental results it was shown that the cutinized epidermis of orchids did not prevent the absorption of simazine; therefore, the primary doubt that the selectivity might be a result of little or no uptake of simazine by the leaves is subject to question. The absorbed simazine may have stayed either in the cuticular layer or in the cell wall, hence it would not influence the Hill reaction directly and cause eventual death of the plants as is common with many plants which are susceptible to simazine. A historadiographic study is desired.

When a lethal concentration of simazine is accumulated through the root, it results in chlorosis and eventual death of the plant. The amount of simazine in a plant is the result of a compensation of the amount of absorption, translocation and degradation. The speed of absorption and translocation is profoundly affected by light intensity, temperature and relative humidity. In the root application studies which were conducted under varying temperatures and light intensities, simazine entered the stele of the roots and was distributed throughout the entire plant. In Time Series Experiment No. 1 which was conducted under low light intensity (430 fc.) at a high temperature, only a trace of simazine was found in the 16-day Dendrobium plant treated with a root application. No image was obtained with Vanda plants. The possibility exists that the absorption and translocation systems of the Vandas were disturbed violently when the initial cuttings were made.

Experiment No. 2 was conducted under moderate light intensity (600 fc.) with a low temperature. The rate of translocation was much greater than in the previous experiment which had low intensity and a higher temperature, hence, it may be postulated that the light intensity had a greater influence on translocation than temperature.

One autoradiogram of a root application of a 16-day plant showed an unexpected result when compared to an autoradiogram from a 4-day plant, the intensity of blackness disappeared from the 16-day growing tip and leaves, and a black image was shown at the stem part some distance away from the stem apex (Figure 3 Ca). This phenomenon may be an individual characteristic, or the result of degradation.

In Experiment No. 3, the plants were grown in a saran house which transmitted 60 percent of natural light. The temperature was from 72° to 83° F. The autoradiogram of the 1/4-day plant showed that the absorption and translocation already took place six hours after treatment application. The amount of simazine translocation increased with time as evidenced by the autoradiograms from the 1-, 4- and 16-day plants. The distribution patterns with the exception of the 1-day plants were different from the results of Experiment No. 2. The intensity of blackness in the roots was dark and became lighter toward the upper part of plant. Since the plants grew well under these conditions, the high light intensity may have enhanced the absorption, translocation and finally the degradation of simazine.

Once simazine enters a plant, the degree of injury obtained depends on the ability of the plant to degrade it. One of the major metabolic products of simazine metabolism is hydroxysimazine. Although there is disagreement on what type of system is responsible for breaking down simazine to hydroxysimazine, the experimental findings by Roth (1958) suggested that the selectivity between species treated with simazine was principally the result of differential breakdown. The speed of degradation and the percentage of recovery were two important factors.

In Metabolism Experiment No. 1, orchid plants treated for four days with labeled simazine showed no degradation. Hydroxysimazine was found in Metabolism Experiment No. 2 when orchid plants were treated with labeled simazine for four days and left in Hoagland's solution for an additional four days with no simazine. The additional four days in

Experiment No. 2, method of simazine application or the increased light intensities may have contributed to the differential response. Nevertheless, hydroxysimazine was found to be an intermediate product in simazine metabolism in orchids.

CONCLUSION AND SUMMARY

A phytotoxicity study to determine the effect of a low concentration of simazine on young Dendrobium orchid plants, showed that the total number of flowers and the length of the flower stalks were reduced in all of the simazine treatments. Although the results from this single experiment show that careful and judicious use of simazine is required when treating young orchids, additional experimentation is suggested to verify the finding.

Three time series experiments to determine the absorption and translocation patterns of simazine in orchid plants, demonstrated that the cutinized leaf surfaces did not prevent the absorption of simazine. In root applications it was apparent that simazine entered the stele of the roots and migrated throughout the plants. No basipetal translocation of simazine was observed in orchid plants up to the termination of the experiments. Therefore, it is possible to postulate that the selectivity of orchid plants to simazine is due to factors other than the lack of absorption.

A chromatographic study was conducted to determine whether orchid plants would metabolize simazine to hydroxysimazine. A metabolite was found at Rf 0.5. Freed (1961) showed that hydroxysimazine had an Rf value of 0.5 when a similar chemical technique was used in corn studies. Minor peaks were observed at 0.15 - 0.20, but not identified. Although some further experimentation is needed to verify these results, it is believed that the orchid plant tolerance to simazine is the result of their ability to metabolize simazine to hydroxysimazine, similar to corn.

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