AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date thesis is presented 10/22/62

Title The Differential Response of Ligustrum ovalifolium and Pyracantha spp. to Simazine

Abstract approved ______________
(Major professor)

The phytotoxicity of simazine, 2-chloro-4, 5-bis(ethylamino)-s-triazine, has ranged from tolerant to extreme sensitivity among species. A study to determine growth and physiological responses of selected woody ornamentals to simazine has shown marked differences to exist among species. Greenhouse experiments, using *Ligustrum ovalifolium*, *Pyracantha koidzumi* 'Government Red' and *Pyracantha crenato-serrata*, showed great variation in expression of visual injury symptoms. Treatment of these species with 3 ppm simazine in nutrient solution resulted in 95, 35 and 0% of the leaf surface showing injury symptoms, respectively. Differences in growth response were not as pronounced, but *Ligustrum* did show significantly greater reduction in plant dry weight and shoot length than *Pyracantha*. These differences in growth response were not detected between the 2 species of *Pyracantha* used. However, the 3 species showed reduced extension growth and dry weight production with each increase in simazine concentration. It was concluded that reduction in growth from herbicidal application may occur without visual symptoms, thereby resulting in potential economic loss to the grower.

Studies with C^{14}-labeled simazine indicated that uptake and translocation by the 3 species was of the same order of magnitude. The accumulation of radioactivity by the two species of *Pyracantha* was identical under the conditions of this experiment. Results with simazine sensitive *Ligustrum* indicated that its initial uptake

was slightly greater than that of the other two species, but was followed by a rapid decrease in accumulation as phytotoxicity developed. The percentages of C^{14} that were chloroform extractable from the leaves of the 3 test plants were similar after an exposure of 1 week to 1 ppm simazine in solution culture. After an exposure of 2 weeks, the extractable C^{14} in the leaves of _L. ovalifolium_, _P. koidzumi_ 'Government Red' and _P. crenato-serrata_ was 54.3, 38.9 and 21.9%, respectively. This indicated greater metabolism of the simazine by the more resistant _P. crenato-serrata_ to non-toxic degradation products. Following exhaustive extraction with chloroform, the percentages of total activity remaining in the samples were 22.6, 36.4 and 59.3 for _L. ovalifolium_, _P. koidzumi_ 'Government Red' and _P. crenato-serrata_, respectively. These levels of simazine in the leaves corresponded inversely with the total amount of leaf surface showing symptoms of chlorosis and/or necrosis and further supported the conclusion that the more tolerant species has a greater ability to metabolize simazine to non-toxic degradation products.

THE DIFFERENTIAL RESPONSE OF LIGUSTRUM OVALIFOLIUM AND PYRACANTHA SPP TO SIMAZINE

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1963

APPROVED:

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Date thesis is presented 10/22/62

Typed by Jolene Wuest

ACKNOWLEDGEMENT

Appreciation for assistance in preparation of this dissertation is extended to major professor Dr. A. N. Roberts. Special thanks also go to Dr. Garvin Grabtree for assistance with the greenhouse experiments and to Dr. Virgil Freed for assistance with the radioactive tracer experiments.

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INTRODUCTION

Weed control in ornamental nursery stock is responsible for one of the major production costs encountered by the industry. Woodford and Ivens (33, vol. 12, p. 139) estimated that in conifer transplant beds weeding accounts for 50% of the cost of producing two-year-old liners. Chappel (10, p. 105) reported the control of weeds in azalea beds to be a major production expense with estimates as high as $1000 per acre in some seasons. Permanent stunting and plant losses have resulted in transplant beds of ornamental stock as a result of weed competition during early phases of growth. Even more mature field stock has been seriously stunted when control of weeds was inadequate.

The development in recent years of new herbicides has given nurserymen a means of lowering labor costs. Herron (16, p. 7), studying the comparative costs of weeding *Taxus* plant beds found that the total cost of weed control on the sesone (sodium 2, 4-dichlorophenoxyethyl sulphate) treated bed was 33% less than on the untreated bed. However, use of herbicides on nursery crops has been limited. Slight foliage damage or growth reduction in nursery crops may result in serious economic loss. The wide variety of plant materials being grown in the nursery has also presented problems to the nurseryman who wished to use herbicides.

One of the newer herbicides showing considerable promise is simazine, 2-chloro-4, 6-bis(ethylamino)-s-trizine, which was first registered for use on ornamental nursery stock in March, 1958 (24, p. 207). Little interest in chemical weed control was shown by nurseryment until that time (15, p. 137).

The tolerance shown by certain plant species toward simazine is one of the outstanding advantages of the chemical. Corn has shown remarkable tolerance to this material, even at relatively high rates of application, while such plants as barley and oats are very sensitive. Since reports of use of simazine on woody ornamentals revealed considerable variation in plant responses, it was reasonable to assume that they possess the same differential tolerance to simazine shown by other plants.

The experiments reported here were designed to study the responses of certain woody ornamentals to simazine treatment and to determine, if possible, the nature of the tolerance shown by certain species. Greenhouse solution cultures were used to study the effects of simazine concentrations on plant growth and development of visual symptoms of injury. Further experiments using

C^{14}-labeled simazine were used to compare the rates at which simazine was absorbed, translocated and metabolized by these species as a possible explanation of their relative tolerance.

REVIEW OF LITERATURE

Properties of simazine. The triazine compounds were synthesized and tested for herbicidal properties in 1952 by the Geigy laboratories in Basel, Switzerland. Gast, Knusli and Gysin (13, vol. 15, p. 107) first reported on this work in the spring of 1955, and described the herbicidal properties of simazine. Simazine, 2-chloro-4, 6-bis(ethylamino)-s-triazine, is a crystalline solid melting at 225° C., and having a solubility of 5 ppm in water at 20-22° C. It is only slightly soluble in organic solvents: 400 ppm in methyl alcohol, 2 ppm in petroleum ether and 900 ppm in chloroform. This material is non-inflammable, non-corrosive and has a low order of toxicity to mammals. The acute oral LD_{50} was found to be in excess of 5000 milligrams per kilogram of body weight for both mice and rats (14, vol. 3, p. 329).

Plant tolerance levels and species differences. Certain plant species have shown a remarkable degree of tolerance to simazine. Davis, Funderburk and Sansing (11, vol. 7, p. 308) found corn to be very resistant to the phytotoxic effects of simazine, while cucumber was very sensitive and cotton intermediate in sensitivity.

Barley was used in studies by Moreland et al. (21, vol. 34, p. 432-435) and found to be very sensitive. Ashton (3, vol. 9, p. 614) described the use of Kanota oats for a bio-assay determination of simazine. Of the plants tested by Burnside and Behrens (7, vol. 9, p. 152), corn was found to be the least sensitive to simazine injury. The plants tested, in increasing order of sensitivity were: corn, sorghum, flax, soybean, rye, wheat and barley.

Results of screening trials, using simazine on woody ornamentals, have been reported by many investigators. Britt (6), using 58 species of ornamentals growing in containers, found simazine was toxic to a relatively large number of species tested. Reports by Ticknor et al. (32, p. 1-23) and Ries, Grigsby and Davidson (25, vol. 7, p. 417) also showed considerable differences in response to simazine by a large number of woody ornamental plants. Reports by Runge (27, vol. 111, p. 16 and 28, p. 167) listed 25 and 26 species of ornamentals, respectively, that were treated with simazine without apparent injury, while Harris (15, p. 137) reported injury to 6 species of woody ornamentals. In a review of chemical weed control in nursery plantings in Connecticut and elsewhere, Ahrens (1, p. 36-38) listed 55 species and varieties of woody ornamentals that have shown differences in tolerance to applications of 2 to 3 pounds of simazine per acre.

Considerable difference in response of Ligustrum and Pyracantha species to treatment with simazine have been reported. Britt (6) found no injury to Pyracantha graberi with rates of simazine as high as 8 pounds per acre, while Ries, Grigsby and Davidson (25, vol. 7, p. 412) reported simazine caused severe damage to Pyracantha coccinea lalandi at 4 pounds per acre. Chadwick (9, p. 50) found Pyracantha coccinea lalandi was seriously injured when planted in soil immediate following application of simazine. Foliage on the injured plants was chlorotic and many leaves developed necrosis which resulted in reduced plant growth.

Richards (24, p. 207-212) listed Ligustrum sp. among ornamental plants tolerant to simazine. Ticknor et al. (32, p. 18) reported no injury to L. ovalifolium from applications of simazine at 10 pounds per acre on a clay loam soil. Bing (4, p. 410) reported no simazine injury to L. ovalifolium at rates up to 6 pounds actual per acre. In a later report (5, p. 150) he shows L. ovalifolium liners to be injured by 8 pounds actual simazine per acre, whether used in granular or spray form. Pridham (22, p. 175) found both L. ovalifolium and Euonymus fortunei to be very sensitive to simazine. Britt (6) found that 4 pounds per acre of simazine was sufficient to produce toxicity symptoms on *L. tosanum*.

Much of the variation in plant response reported in the literature may be the result of soil differences, since many herbicides are adsorbed by soil colloids. Burnside and Behrens (7, vol. 9, p. 156) found that soils high in organic matter and/or clay content were associated with less phytotoxicity after simazine treatment than soils low in these components. Other factors, such as soil moisture, plant maturity, time of herbicidal application and soil nutritional status may also contribute to differences in plant response. Ahrens, Sweet and Harris (2, p. 199-200) used rooted cuttings of *Ligustrum ibolium* to study the effect of soil fertility on simazine injury to nursery plants. Severe injury was observed at the lowest fertility level when treated with simazine at 2 pounds per acre, while no injury was found at the highest level of fertility.

Species differences may account for some of the variation in response reported above. Britt (6) found injury on *Juniperus tamariscifolia* at one pound per acre while injury symptoms were not expressed on *J. pfitzeriana* with rates as high as 8 pounds per acre. Ries, Grigsby and Davidson (25, vol. 7, p. 409-417) list *J. chinensis pftzeriana*, *J. communis*, and *J. virginiana glauca* as resistant and *J. horizontalis plumosa* as susceptible to simazine injury. Britt (6) also found injury on *Mahonia aquifolium* at 4 pounds and no injury symptoms on *M. baelei* at 8 pounds per acre simazine. Studying weed control in lining-out stock of *Taxus capitata* and *T. media* 'Hicksi', Ries and Watson (26, p. 50) reported outstanding differences in susceptibility between the 2 species of *Taxus*.

Symptoms of injury. Davis, Funderburk and Sandsing (11, vol. 7, p. 308) reported the characteristic symptoms of simazine toxicity to be marginal necrosis of the leaves followed by eventual death of the whole plant. They found that simazine or a biologically active C^{14}-labeled degradation product accumulated in the areas where necrosis first occurred. This was the same accumulation pattern reported by Sheets (31, vol. 9, p. 6). The typical plant symptoms of simazine toxicity described by Schneider (29, p. 418) included chlorosis, which started at the leaf tip and progressed along the margins to the base of the leaf. Necrosis of tissue also occurred in the chlorotic areas. This chlorosis spread rapidly over the entire leaf surface and was followed by death, first of the leaf and eventually the entire plant. In older plants the chlorosis first appeared on the older leaves and progressed upward to the growing point.

Nature of plant tolerance. Serious attempts have been made to determine the factors responsible for the differences in plant tolerance to triazine herbicides. Three possibilities were proposed by Gysin and Knusli (14, vol. 3, p. 334-335): (1) Sensitive plants are able to pick up the chlorotriazines through their roots and so may accumulate a lethal dose in the plant, while nonsensitive plants are unable to take up the chemical through their root systems and remain unaffected. (2) A sensitive plant may be out of the sphere of influence of a herbicide because of special morphology, for example, because of its very deep root system. (3) The nonsensitive plant may possess a mechanism which detoxifies the accumulated chemical into a nonphytotoxic material, while a sensitive plant is not able to metabolize the herbicide to harmless compounds.

It has been demonstrated by various workers that tolerant, as well as sensitive plants are able to absorb and accumulate substantial amounts of simazine. Using the C^{14}-labeled herbicide, Montgomery and Freed (20, vol. 9, p. 233) demonstrated that corn, a tolerant plant, growing in soil treated with 8 pounds per acre simazine was able to accumulate up to 15.6 ppm. From work conducted by Roth and others, Gysin and Knusli (14, vol. 3, p. 336) reported that the total amounts of simazine taken up by various plants were of the same order of magnitude. Davis, Funderburk and Sansing (11, vol. 7, p. 302) found no obvious correlation between susceptibility and the amount of simazine absorbed by the plant. C^{14}-labeled simazine moved readily into the roots of the 3 species used; corn, cotton and cucumber. Radioactivity was observable in corn leaves after 8 hours and in cucumber leaves after 0.5 hour. Sheets (31, vol. 9, p. 6), using oat plants grown in nutrient solution treated with C^{14}-labeled simazine, found accumulation in the leaf tips beginning within 48 hours after exposure.

Since simazine moves slowly in soil, the deep root system of many woody ornamentals offers an excellent opportunity for selective weed control. Montgomery and Freed (19, p. 79) applied C^{14}-labeled simazine to the surface of soil columns at rates equivalent to 10 pounds per acre and followed this with an application of 12 inches of water within a 3-day period. One-inch increments of the soil columns were then examined for the presence of simazine, and although the compound had penetrated to a depth of 7 inches, the maximum concentration of the chemical was found in the surface-to-one-inch depth of soil. Sheets (30, vol. 7, p. 189) found that simazine moved downward at a greater rate and was more toxic to oats and soybeans in sandy soil than in clay loam soil. Yet many shallow rooted plants such as azaleas and rhododendrons have shown tolerance to simazine (1, p. 37-38).

Of the 3 theoretical possibilities proposed by Gysin and Knusli (14, vol. 3, p. 334-335), the apparent ability of tolerant plants to detoxify the chemical to non-phytotoxic materials appears the most reasonable. Montgomery and Freed (20, vol. 9, p. 231-237) found that appreciable amounts of C^{14}-labeled herbicide were taken up by corn from soil treated with simazine. They were able to demonstrate by means of ion exchange and paper chromotographic methods that extensive degradation of simazine was possible in corn. This was established conclusively by additional experiments, which demonstrated that the corn plant was capable of complete degradation of simazine to $C^{14}O_2$. Since the C^{14} was uniformly labeled in the triazine ring, the evoluation of $C^{14}O_2$ must have resulted from splitting the ring, indicating complete decomposition of the basic structure of simazine. Ragab and McCollum (23, vol. 9, p. 83) demonstrated that both resistant and susceptible plants decomposed simazine to a nontoxic product. They found the initial production of $C^{14}O_2$ to be considerably greater with cucumber than corn. After a high initial production of $C^{14}O_2$, cucumber showed a gradual decrease in production while that from corn gradually increased. Their findings suggested that both resistant and susceptible plants were able to metabolize simazine but that the susceptible cucumber may absorb simazine at a more rapid rate than corn, as indicated by greater radioactivity in the plants.

Gysin and Knusli (14, vol. 3, p. 337), in reviewing the work of Roth, reported that extensive degradation of simazine occurred when added to freshly pressed juice from young corn plants. After 100 hours only a small percentage of the simazine added could be isolated unchanged from the corn juice. Another experiment using freshly pressed juice from wheat plants gave a recovery of over 90 percent unchanged simazine. These findings were later substantiated by Castelfranco, Foy and Deutsch (8, vol. 9, p. 586). Davis, Funderburk and Sansing (11, vol. 7, p. 308) analyzed the leaves of selected plants to determine what fraction of the radioactivity was simazine or other chloroform soluble compounds. The average percentages found were 3, 23 and 64, respectively for corn, a resistant plant; cotton, intermediate in susceptibility; and cucumber, a highly sensitive plant.

MATERIALS AND METHODS

On the basis of preliminary experiments, the woody ornamental species _Pyracantha crenato-serrata_, _Pyracantha koidzumi_ 'Government Red' and _Ligustrum ovalifolium_ (California Privet) were selected for use in these studies. _L. ovalifolium_ gave evidence of being susceptible to simazine injury at concentrations of 1 ppm in nutrient solution. In contrast, no injury was noted on _P. crenato-serrata_ at concentrations of 4 ppm.

Cuttings of the selected species were taken in the fall and winter and rooted under intermittent mist. When well-rooted, the cuttings were placed in aerated nutrient solution until established and growing rapidly. Plants to be used in the various experiments were then selected for uniformity of size and stage of development and were grown in Hoagland's nutrient solution #2 (17, p. 31). The nutrient solutions were aerated continuously and changed at 2-week intervals during the course of each experiment. Day and night temperatures in the greenhouse were maintained at approximately 72° and 68° F., respectively. Supplemental lighting was provided from a bank of fluorescent and incandescent lamps supplying 500-600 foot candles.

Plant Growth and Injury Symptoms

A stock solution of simazine was prepared and aliquots were added to containers of nutrient solution to give desired concentrations. In each experiment the plants were exposed to simazine for a period of 2 weeks, after which the solutions were changed.

Prior to simazine treatment, the plants selected for use were weighed and the longest shoot on each measured. These same shoots were measured again at periodic intervals during the experiment and at the end. At the same time the plants were rated for visual symptoms of chlorosis and/or necrosis. The rating system, based on the percentage of total leaf surface affected, was as follows:

1 - no visible symptoms

10 - 100% leaf surface showing chlorosis and/or necrosis.

At the conclusion of each experiment, which will be referred to in further discussion as 'harvest', the plants were removed from the nutrient solution. After separation, the fresh weight of the root and shoot of each plant was determined. These plant parts were then dried in a forced-air drying cabinet at 70-75° C., until constant for dry weight.

Experiment 1. In the first experiment, selected rooted cuttings of *L. ovalifolium* and *P. koidzumi* 'Government Red' were grown in quart Mason jars, one plant per jar. The jars were painted with black and then aluminum paint to eliminate algae growth and to help reduce the temperature of the nutrient solution. The plants were inserted through holes in the jar lids and held upright with absorbent cotton. Plants in this experiment were treated with 5 levels of simazine (0, 0.5, 1, 2 and 4 ppm, each replicated six times) and arranged in randomized blocks on the greenhouse bench. The plants were harvested 49 days after treatment. Figure 1 shows the arrangement of the test plants on the greenhouse bench and a close-up view of the plants inserted in quart Mason jars. The method of aeration is also illustrated.

Several plants in this experiment were lost due to an unknown bacterial disease, so the values for these missing observations were calculated using the method given by Li (18, p. 209-212).

Experiment 2. Results obtained in experiment 1 did not agree with those obtained in preliminary experiments, thus suggesting a second experiment using the three test plants, *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata*. In this experiment, plants were treated with 4 levels of simazine (0, 1, 2

A

B

Figure 1. Plant culture: A. General view of arrangement on the greenhouse bench; B. Close up of transplanted plants in Mason jars with aerating system in operation.

and 3 ppm, each replicated 4 times) and arranged in randomized blocks. Half-gallon polyethylene freezer cartons, which had also been painted with black and aluminum paint, were used as containers for the solution cultures. In this experiment 1 plant of each of the 3 test species was grown in the same container to eliminate any possible error due to variation in solution concentration. These plants were harvested 34 days after treatment.

The average size of test plants used in experiments 1 and 2, based on fresh weight and shoot length, are presented in Table 1.

TABLE 1. Measurements taken of test plants at beginning of experiments 1 and 2.

	Average fresh weight (gm.)		Average shoot length (cm.)	
Test Plant	Exp. 1	Exp. 2	Exp. 1	Exp. 2
L. ovalifolium	4.26	6.80	20.07	25.55
P. koidzumi, 'Government Red'	2.96	7.92	11.23	30.35
P. crenato-serrata	---	3.93	---	19.44

Since it was found that the initial size (based on plant weight and extension growth) of the test plants had an influence on final size, statistical interpretation of results was based on analysis of covariance (18, p. 366-370).

Absorption, Translocation and Metabolism

The test plants used in the radioactive tracer experiment, *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata*, were selected for uniformity of size, stage of maturity and rate of growth, and were comparable to those used in previous experiments. Treatments were made by dissolving a mixture of the C^{14}-labeled and technical simazine in chloroform. Aliquots of this stock solution were added to each container to give the desired concentration and allowed to evaporate. The containers were then filled with nutrient solution and aerated for 24 hours prior to transfer of the test plants.

At harvest, the treated plant material was separated into stem, leaves and roots, with the root portion being discarded. Each sample consisted of a composite of 3 test plants. To estimate the total amount of activity present and its distribution in various parts of the test plants, 1 portion of each sample was dried and the amount of radioactivity in the dried tissue determined. The dried plant tissues were ground to a fine powder with mortar and pestle and packed in nickel plated planchets for counting. The radioactivity was determined with a thin window (1.9 mg/cm^2) gas flow G.M. tube. All counts were corrected for background and self-absorption.

It was also of interest to determine what fraction of the radioactivity found in the test plants was chloroform soluble. Previous work by Montgomery and Freed (20, vol. 9, p. 231-237) had shown that the traizine herbicides could be extracted quantitatively from plant tissue with chloroform in a Soxhlet extractor. The chloroform-soluble radioactivity represents the maximum possible amount of the parent triazine. A sample of the green macerated plant material was exhaustively extracted with chloroform. Aliquots of the chloroform extracts were plated and counted. Samples of the dried plant material that had been exhaustively extracted were ground and plated for counting. Again, counts were corrected for background and self-absorption.

Experiment 3. In this first experiment using C^{14}-labeled simazine, 2 test species were used; *L. ovalifolium*, which had shown the greatest sensitivity and *P. crenato-serrata*, which was tolerant. These plants were exposed to a concentration of 2 ppm simazine for a period of 2 weeks before they were harvested for analysis. Each sample consisted of a composite of 5 plants.

Experiment 4. A second study was conducted using the 3 test species, *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata*. Plants were exposed to a concentration of 1 ppm simazine and samples harvested after exposures of 1 and 2 weeks. Additional test plants, which had been grown in simazine treated nutrient solution for 2 weeks, were removed from solution, the roots rinsed in water and returned to fresh, untreated nutrient solution. These were harvested and analyzed 3 weeks after being exposed to the C^{14}-labeled simazine. Each sample consisted of a composite of 4 plants.

Additional plants were harvested after 1 and 2 weeks of exposure to C^{14}-labeled simazine and dried in a plant press. Autoradiographs were prepared by placing the dried plants in contact with no-screen x-ray film. This film was exposed for two months before developing.

RESULTS

Plant Growth and Injury Symptoms

Injury Rating. The plants in these experiments were periodically rated for visual symptoms of injury. In the first experiment, *L. ovalifolium* showed symptoms of chlorosis and/or necrosis at the lowest concentration (0.5 ppm simazine) 2 weeks after treatment. The percentage of leaf surface showing injury symptoms had increased on plants in all treatments when final ratings were made at harvest. The *L. ovalifolium* plants treated with the highest concentration of simazine (4 ppm) were either dead or seriously injured and showing no signs of recovery when harvested.

Visual symptoms of injury were found on plants of *P. koidzumi* 'Government Red' 2 weeks after treatment with both 2 and 4 ppm simazine, but no symptoms were evident on plants treated with either 0.5 or 1 ppm. When the plants were harvested at the end of 7 weeks, the percentage of total leaf surface showing symptoms had increased; the highest concentration (4 ppm simazine) resulting in less than 50% foliage injury and the lowest concentration (0.5 ppm) producing no signs of injury.

The amounts of foliage injury to L. ovalifolium and P. koidzumi 'Government Red' in experiment 1, 7 weeks after simazine treatment, are graphically shown in Figure 2. There was a progressive increase in the severity of injury to the two species with each increase in simazine concentration. However, considerable difference was noted in severity of injury symptoms produced between L. ovalifolium and P. koidzumi 'Government Red'.

When the plants in experiment 2 were first rated, 1 week after treatment, all L. ovalifolium plants treated with simazine showed injury symptoms. The percentage of leaf surface showing symptoms increased steadily until the experiment was terminated after 5 weeks. At the completion of the experiment, the L. ovalifolium plants growing in solutions containing 3 ppm simazine were either seriously injured or dead. Of those in solutions containing 2 ppm simazine, 1 was dead, while the remaining 3 were showing signs of recovery. Plants treated with 1 ppm were showing injury symptoms at harvest, but there were signs of recovery with the development of new unaffected leaves.

None of the P. koidzumi 'Government Red' plants showed visible signs of injury 1 week after treatment. When the solutions containing the simazine were changed at the end of 2 weeks, plants

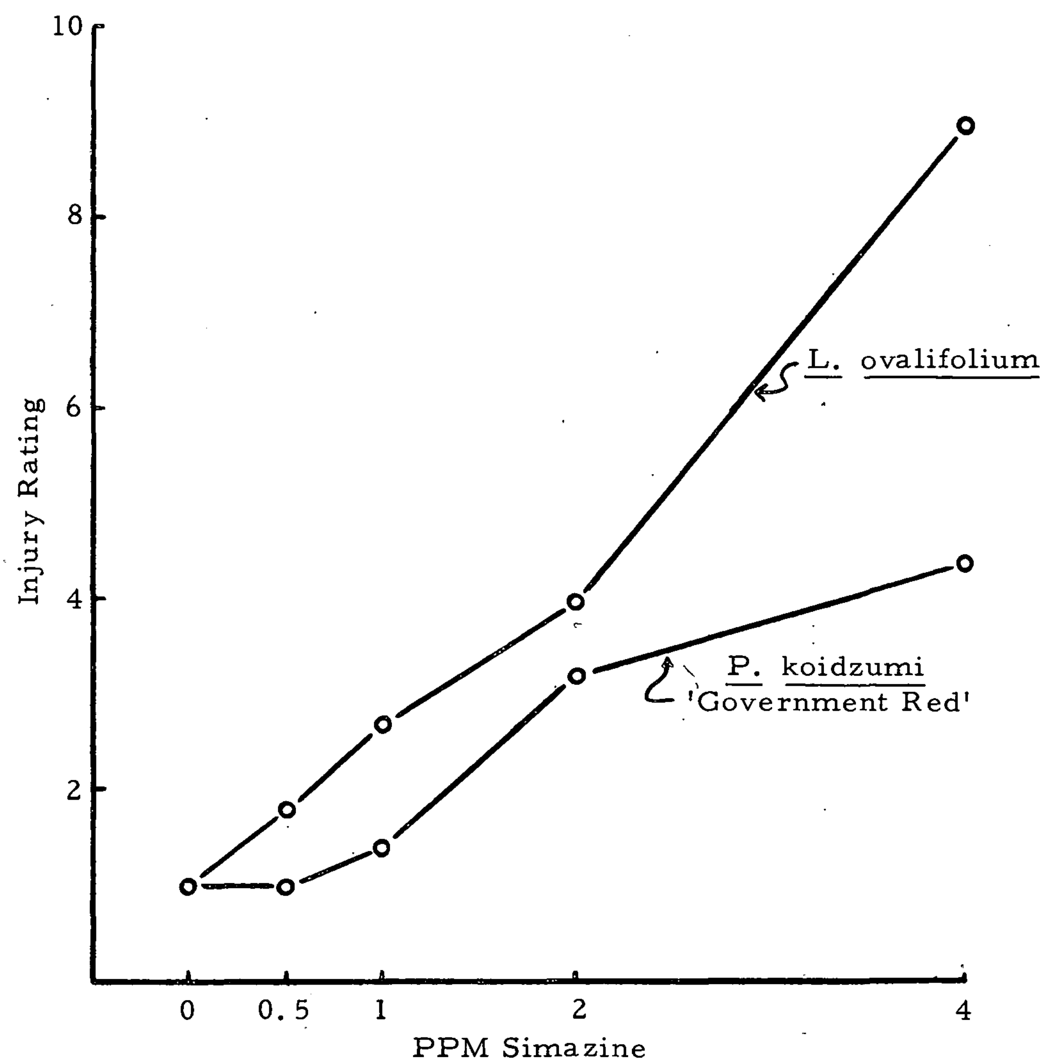

Figure 2. Experiment 1. Foliage injury symptoms on L. ovalifolium and P. koidzumi 'Government Red' 7 weeks after simazine treatment.

having received 2 and 3 ppm simazine were showing injury symptoms. Symptoms did not develop on the plants receiving 1 ppm simazine until 4 weeks after they were first exposed to the herbicide. When the experiment was terminated, the appearance of new unaffected leaves on the P. koidzumi 'Government Red' plants gave evidence of their recovery. In this experiment none of the plants of P. crenato-serrata developed visual symptoms of injury. The amounts of foilage injury to L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata 5 weeks after treatment with simazine in experiment 2 are graphically shown in Figure 3. Again, as in experiment 1, there was a progressive increase in injury symptoms on L. ovalifolium and P. koidzumi 'Government Red' foliage with each increase in simazine concentration.

Injury symptoms first appeared on plants of L. ovalifolium and P. koidzumi 'Government Red' as chlorotic or necrotic areas along the leaf margins. The second or third leaf from the stem tip, which was 1/2 to 3/4 expanded, usually developed symptoms first. In the case of the more sensitive L. ovalifolium, the higher concentrations of simazine resulted in foliage symptoms along the entire length of the stem with the older and more mature leaves

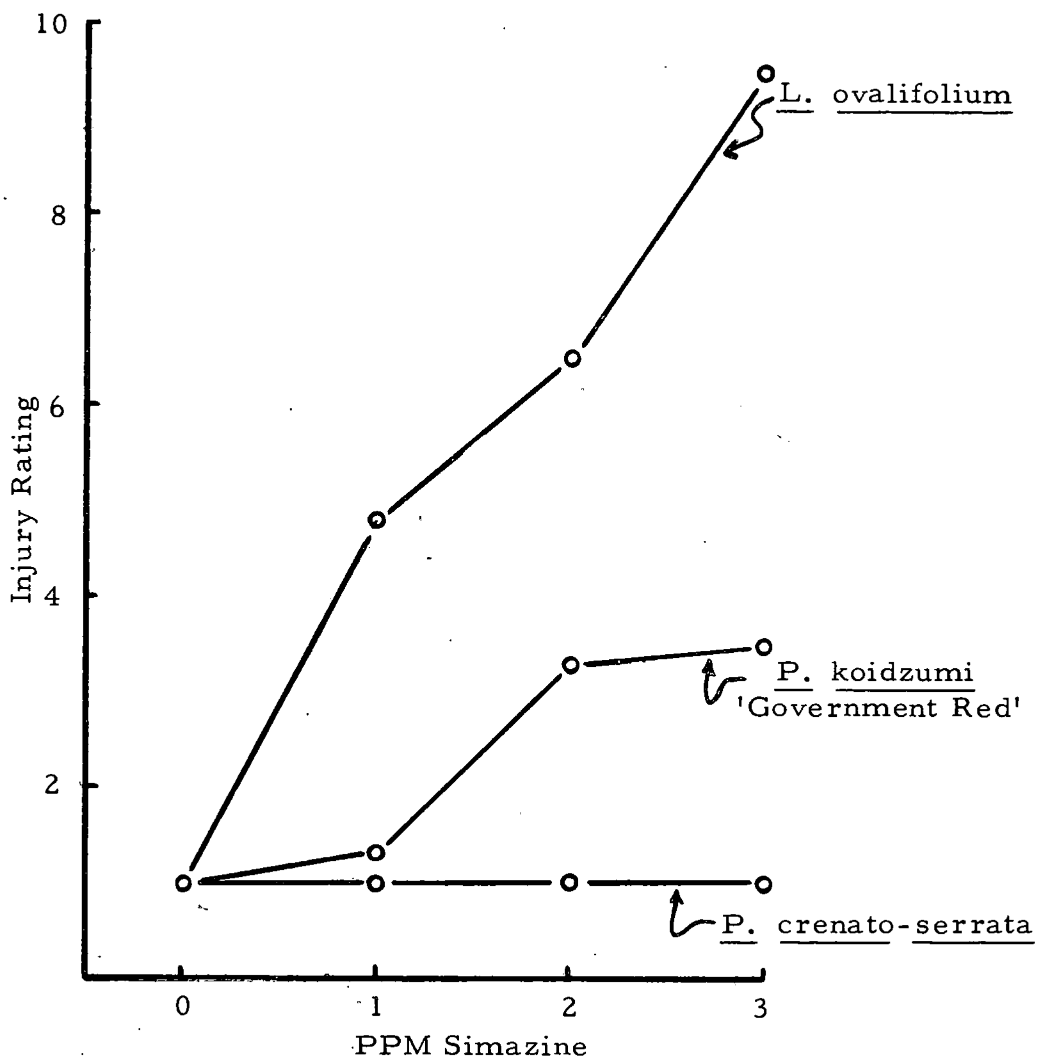

Figure 3. Experiment 2. Foliage injury symptoms on <u>L. ovalifolium</u>, <u>P. koidzumi</u> 'Government Red' and <u>P. crenato-serrata</u> 5 weeks after treatment.

affected last. This was followed by tip die-back and eventual death of the plant.

Dry Weight. Mean dry-weight values obtained for plants and plant portions grown in experiment 1 are compared in Figure 4. Dry weight of both roots and shoots of *L. ovalifolium* and *P. koidzumi* 'Government Red' were significantly reduced by simazine treatment. Simazine at 0.5 ppm significantly reduced the dry weight of *L. ovalifolium* shoots and roots. It required 0.5 ppm and 1 ppm simazine to produce a significant reduction in dry weight of *P. koidzumi* 'Government Red' shoots and roots, respectively.

Figure 5 shows the plant dry-weight values obtained in experiment 2. There was again a significant reduction in the dry weight of *L. ovalifolium* plants with simazine treatment. Comparisons of treatment means revealed that dry weight of *L. ovalifolium* was significantly reduced by all concentrations of simazine. However, in this experiment there was not a significant reduction in the dry weight of *P. koidzumi* 'Government Red' plants from simazine treatment. There was a significant reduction in the dry weight of *P. crenato-serrata* shoots from simazine treatment but not of the roots. Comparisons between treatment means revealed that the dry weight of *P. crenato-serrata* shoots treated with 2 and 3 ppm simazine were significantly less than the untreated control.

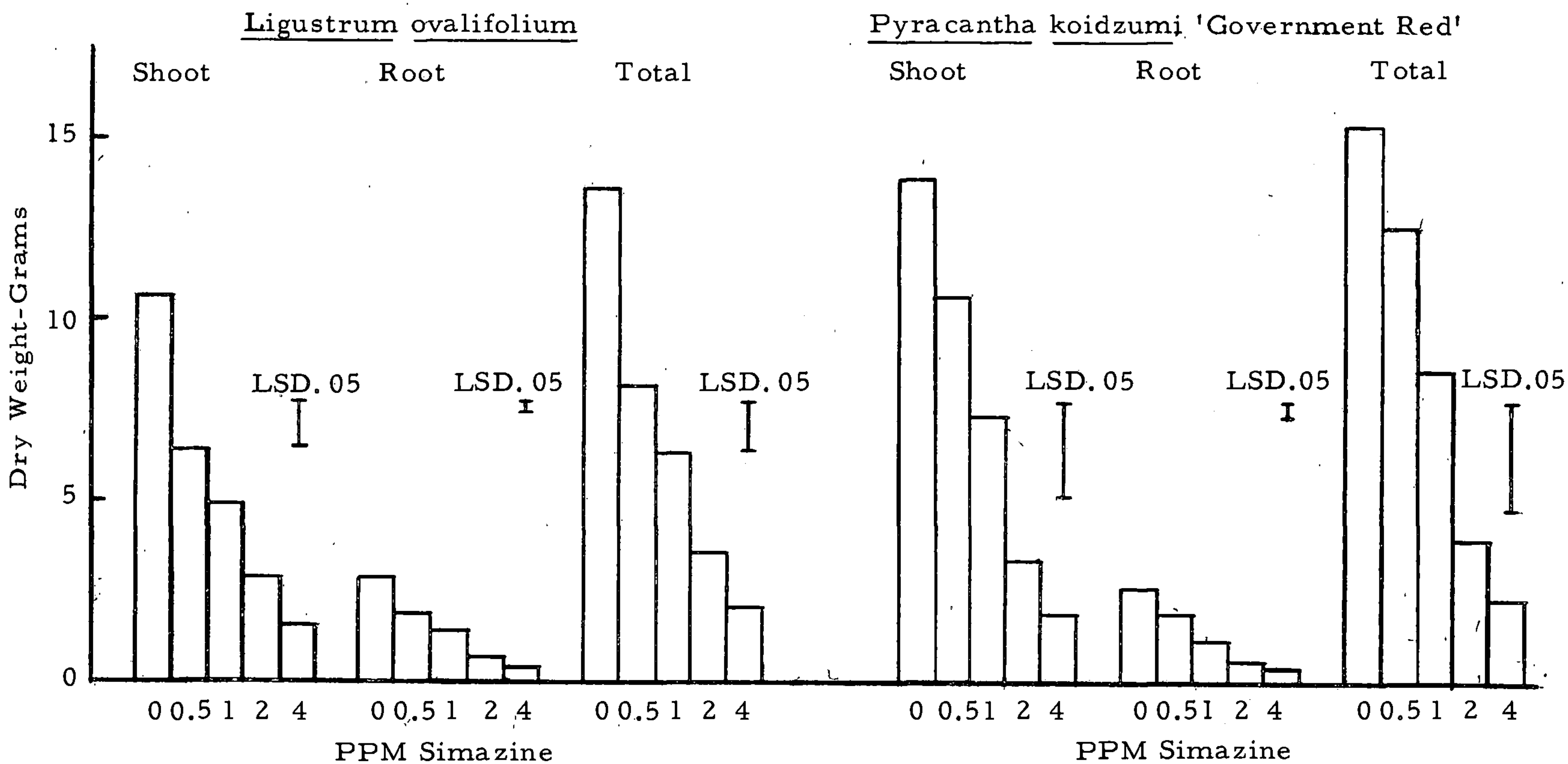

Figure 4. Experiment I. Mean dry weights for *L. ovalifolium* and *P. koidzumi* 'Government Red' plants as influenced by various levels of simazine.

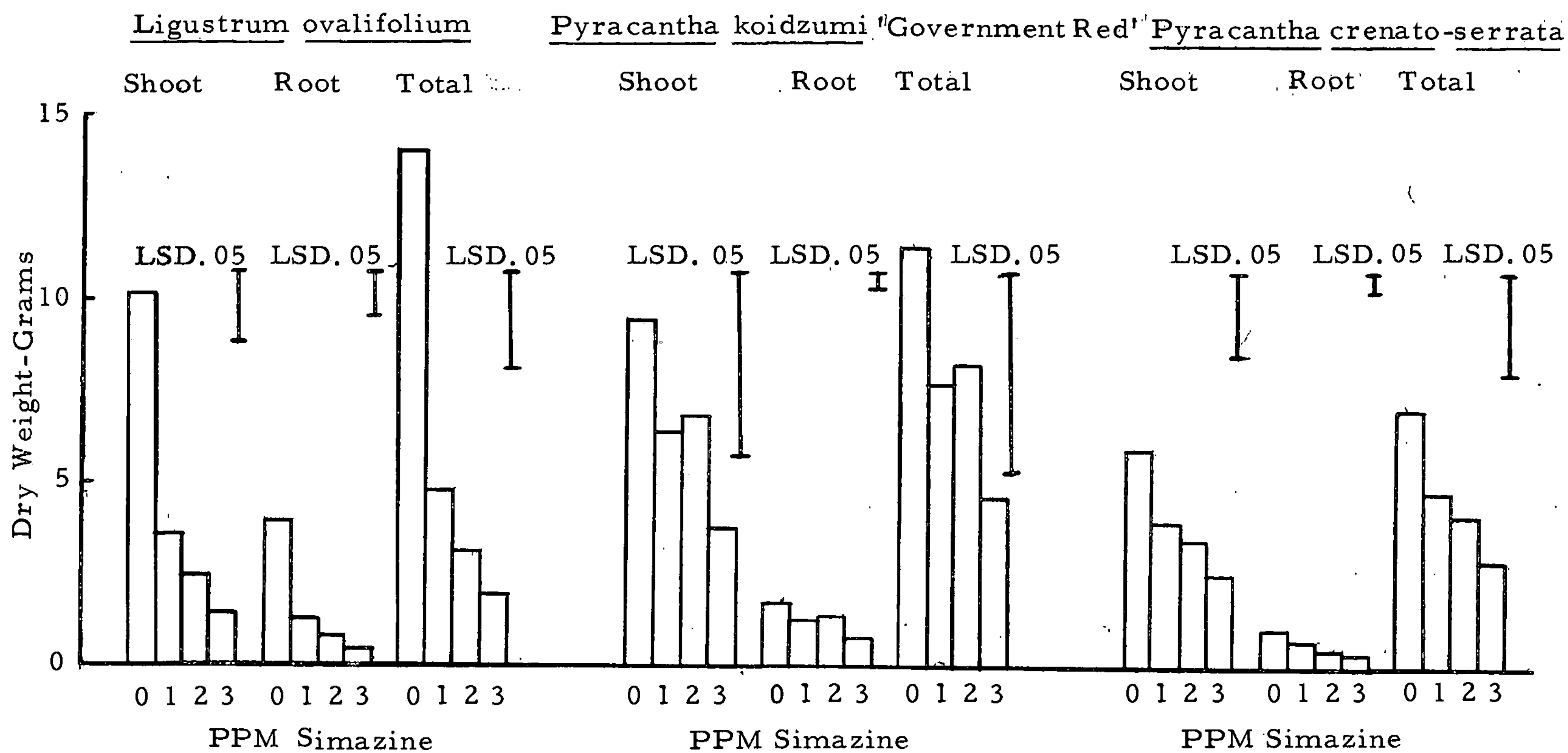

Figure 5. Experiment 2. Mean dry weights for L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata plants as influenced by various levels of simazine.

Extension Growth. In the first experiment, there was no significant reduction in shoot growth of *L. ovalifolium* and *P. koidzumi* 'Government Red' plants as a result of an exposure of 2 weeks to simazine (Table 2). Comparisons between treatment means at the end of 5 weeks showed that shoot growth of *L. ovalifolium* was significantly reduced by all levels of simazine treatment, while that of *P. koidzumi* 'Government Red' was reduced only by treatments of 2 and 4 ppm. This was still the case when the plants were harvested at the end of 7 weeks.

TABLE 2. Experiment 1. Mean shoot length for plants of *L. ovalifolium* and *P. koidzumi* 'Government Red' as influenced by various levels of simazine.

	L. ovalifolium			P. koidzumi 'Government Red'		
Simazine	2 weeks	5 weeks	7 weeks	2 weeks	5 weeks	7 weeks
ppm	cm	cm	cm	cm	cm	cm
0	25.9	40.0	52.9	17.6	38.4	50.5
0.5	24.3	27.8	37.9	18.7	40.6	54.3
1.0	24.9	27.3	31.8	19.0	32.3	42.7
2.0	23.3	23.9	26.8	15.9	23.6	34.8
4.0	25.0	22.3	23.6	16.6	21.5	28.7
LSD .05	3.5	5.6	5.9	3.7	7.4	6.8

The 3 species used in experiment 2 showed no significant reduction in shoot growth from simazine until the fourth week (Table 3). At this time, both L. ovalifolium and P. crenato-serrata showed significant growth reduction, while shoot growth of P. koidzumi 'Government Red' was not affected. Comparison of treatment means revealed that growth reduction in L. ovalifolium was significant at all levels of simazine treatment, whereas shoot growth of P. crenato-serrata was reduced only at 3 ppm concentration. The same observation was made when the plants were harvested at the end of 5 weeks.

Absorption, Translocation and Metabolism

Appreciable amounts of C^{14}-labeled simazine were taken up from nutrient solution by the 3 test species. The uptake of simazine by the 2 speices of Pyracantha was almost identical (Figure 6). At the end of 1 week, the total amount of simazine taken up by Ligustrum was slightly greater than that found in Pyracantha. Pyracantha absorbed simazine at a more or less constant rate during experiment 4, while the rate of uptake by Ligustrum was reduced by about 50% during the second week. Figure 6 shows that plants grown for an additional week in untreated nutrient solution showed no increase in radioactivity.

TABLE 3. Experiment 2. Mean shoot length for plants of *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* as influenced by various levels of simazine.

Plant	Simazine	Weeks after treatment			
		2	3	4	5
	ppm	centimeters			
L. ovalifolium	0	32.3	33.4	36.3	41.5
	1	26.3	26.4	28.0	28.4
	2	27.6	27.7	27.8	28.2
	3	23.4	23.3	23.3	23.5
	LSD.05	3.3	3.1	4.5	6.0
P. koidzumi	0	40.6	45.8	49.7	52.9
'Government Red'	1	37.3	42.1	47.5	51.8
	2	36.9	38.4	39.8	42.0
	3	37.6	38.1	38.9	41.4
	LSD.05	5.7	9.1	10.7	12.4
P. crenato-	0	29.2	36.2	42.9	47.4
serrata	1	28.3	34.0	40.5	46.8
	2	26.6	30.0	35.0	40.2
	3	24.2	26.3	27.7	32.0
	LSD.05	4.9	7.5	8.9	10.1

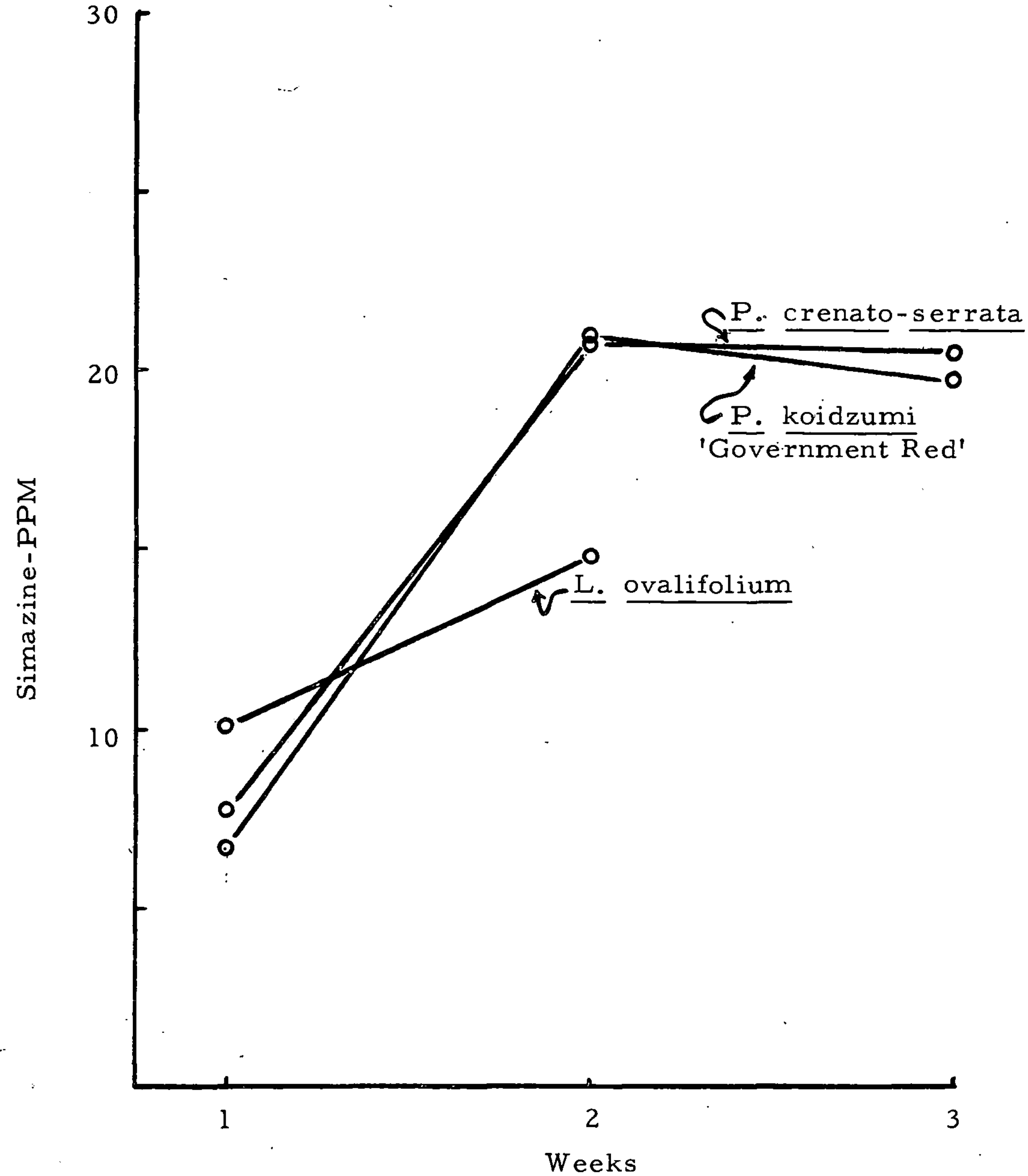

Figure 6. The uptake of C^{14}-labeled simazine by L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata treated with 1 ppm radioactive simazine.

The percentages of total activity that was extractable from the leaves of the 3 test species with chloroform at the end of 1 week of treatment were comparable (Figure 7). However, Pyracantha leaves showed a marked decrease in the percentage of activity that was chloroform extractable after 2 weeks, while the amount extractable from Ligustrum leaves remained constant. The portions of total activity that were extractable from the leaves of the 3 test species after an exposure of 2 weeks to C^{14}-labeled simazine in nutrient solution were 54, 39 and 22% for L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata, respectively. Attention should be called to the fact that the chloroform soluble radioactivity was expressed as if it were all in the form of the parent triazine. This fraction represented any unchanged triazine as well as other chloroform soluble degradation products.

That portion of the total activity present in the leaves of Pyracantha in a non-extractable form was approximately 4 times that of Ligustrum, 1 week after treatment with 1 ppm C^{14}-labeled simazine in solution culture (Figure 8). There was no difference between the 2 species of Pyracantha in this respect; the P. koidzumi 'Government Red' having 47% and the P. crenato-serrata 44% of non-extractable activity present in the leaves. However, after an exposure of 2 weeks, considerable difference was noted in the

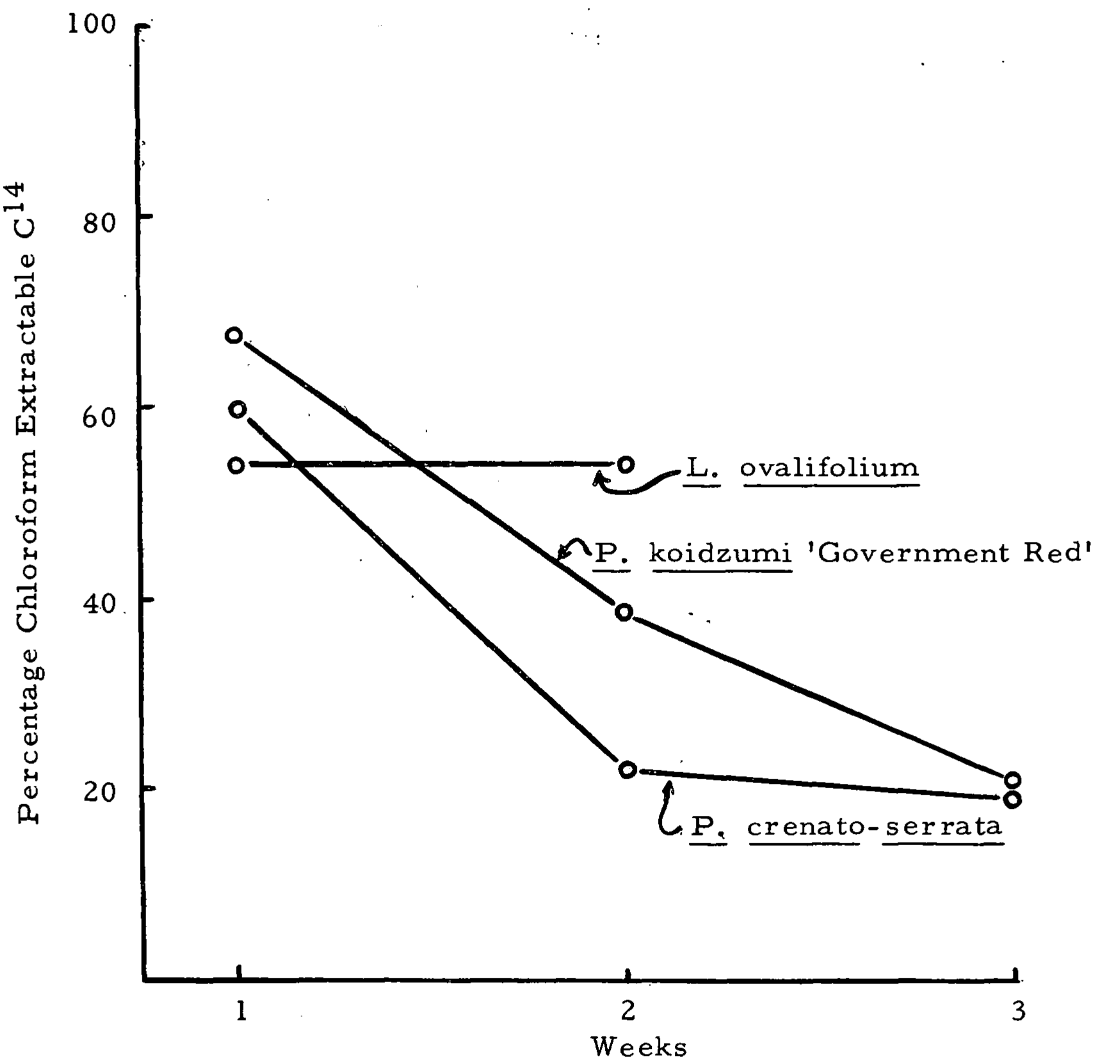

Figure 7. The percentage of chloroform extractable C^{14} in *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* foliage after treatment with 1 ppm radioactive simazine.

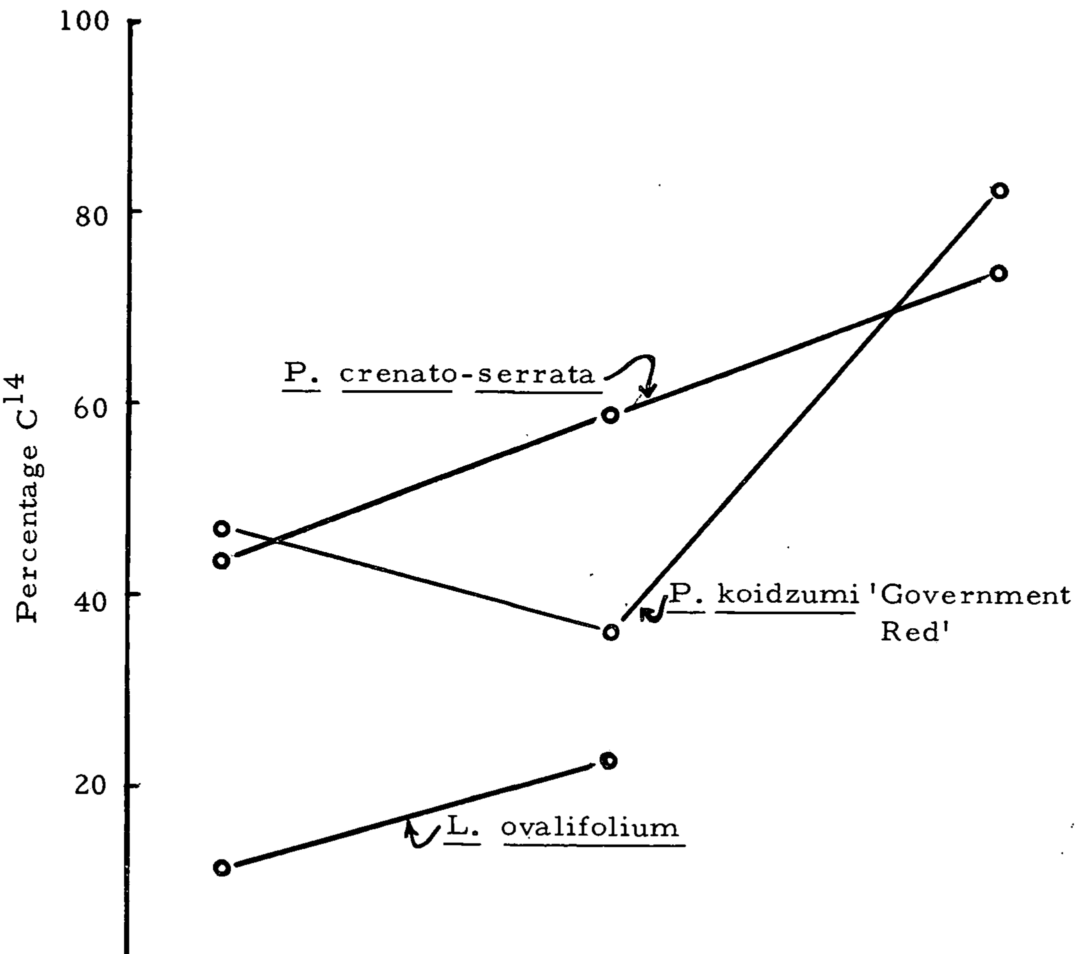

Figure 8. The percentage of C^{14} in foliage of *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* after exhaustive chloroform extraction.

amount of non-chloroform-soluble activity in the 3 species. This fraction represented 23% of the total activity found in L. ovalifolium leaves, 36% in P. koidzumi 'Government Red' and 59% in P. crenato-serrata leaves.

The autoradiographs in Figures 9 and 10 reveal that the radioactivity is distributed throughout the entire plant. They also show that accumulation of simazine by the 3 species is greatest along the leaf margins as shown by the lighter areas in the figures.

Figure 9. Autoradiograph of: A) L. ovalifolium, B) P. koidzumi 'Government Red' and C) P. crenato-serrata after an exposure of 1 week to 1 ppm C^{14}-labeled simazine.

Figure 10. Autoradiographs of: A) L. ovalifolium, B) P. koidzumi 'Government Red' and C) P. crenato-serrata after an exposure of 2 weeks to 1 ppm simazine.

DISCUSSION AND CONCLUSIONS

There were striking differences in severity of injury symptoms shown by the test plants used in these studies. L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata treated with 3 ppm simazine had 95, 35 and 0%, respectively, of their leaf surface showing injury at harvest. Characteristic toxicity symptoms observed on L. ovalifolium and P. koidzumi 'Government Red' were similar to those reported by Davis, Funderburk and Sandsing (11, vol. 7, p. 308) and Schneider (29, p. 418). These symptoms also resembled those reported by Chadwick (9, p. 50) on P. coccinea lalandi and Ahrens, Sweet and Harris (2, p. 199-200) on L. ibolium.

The autoradiographs showed that the 3 woody ornamental species, L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata, accumulated the highest concentrations of radioactive simazine or degradation products along the leaf margins. This corresponded to the areas in which visual symptoms of chlorosis and/or necrosis first appeared. Davis, Funderburk and Sansing (11, vol. 7, p. 308) also found that simazine or a biologically active C^{14}-labeled degradation product accumulated in the areas where necrosis first occurred. The autoradiographs of the 3 woody ornamentals also showed a general distribution of radioactivity throughout the entire plant. Davis, Funderburk and Sandsing (11, vol. 7, p. 303) suggested that this represented biologically inactive degradation products.

The response of L. ovalifolium to simazine in experiments 1 and 2 corresponded closely. In both experiments this species showed significant decrease in dry weight and shoot growth as a result of simazine at all concentrations used. The average reduction in dry weight of L. ovalifolium plants receiving simazine treatment was 53 and 56% for experiments 1 and 2, respectively. Similar reductions were also obtained in shoot length. The degree of foliage injury in the 2 experiments was comparable.

The response of P. koidzumi 'Government Red' to simazine was considerably different in experiments 1 and 2. In experiment 1 there was a significant reduction in plant dry weight and shoot length as a result of simazine treatment. This was not the case in experiment 2. The average reduction in dry weight of P. koidzumi 'Government Red' plants treated with simazine was 44 and 18% for experiments 1 and 2, respectively. However, a comparison of visual injury ratings for the 2 experiments did not reveal this difference. These data showed the extent of injury symptoms to be similar for the 2 experiments.

Although cuttings of the plant materials used in the 2 experiments were started at the same time, experiment 2 was conducted some weeks after the first experiment; thus the plants in experiment 2 were treated at a more advanced stage physiologically. This difference in plant fresh weight and shoot length from one experiment to the other is shown in Table 1. Since the L. ovalifolium and P. koidzumi 'Government Red' plants were at comparable stages of development in each experiment, it appeared that the P. koidzumi 'Government Red' species was less sensitive to the phytotoxic effects of simazine as the plant became more mature, while the L. ovalifolium was very sensitive at all stages of development. Of course this is hypothetical, but does suggest possibilities for further investigation.

Although P. crenato-serrata showed significant reduction in plant dry weight and shoot length as a result of simazine treatment, visual injury symptoms were not present, even at higher concentrations. This suggested that visual symptoms can be misleading in assessing herbicidal damage. In the production of nursery stock, growth reduction as well as foliage damage can often result in economic loss to the grower. However, savings in labor from use of herbicides may more than offset any losses that might result from slight growth reductions.

The data indicated that a greater reduction in dry weight of shoots than of roots occurred in the apparently more tolerant Pyracantha species. However, this difference in response was not evidenced in the apparently more sensitive L. ovalifolium plants. This observation was not too surprising, since simazine has been found by several workers to inhibit the Hill reaction, the ability of the chloroplasts to break down water by hydrogen and oxygen in the presence of light and iron. Moreland et al. (21, vol. 34, p. 435) found simazine reduced the photochemical activity (Hill reaction) of isolated barley chloroplasts by 50% at 4.6×10^{-6} M. They also found that glucose supplied to barley plants through severed leaf tips kept plants alive and growing in the presence of lethal concentrations of simazine. If the hypothesis that this chemical kills the plant by interfering with the photosynthetic mechanism is correct, it could be concluded that plant roots are less sinsitive than foliage to the phytotoxic effects of simazine. This concept is further supported by the fact that appreciable quantities of simazine can be absorbed by the root system and translocated to the aerial portions of the plant. If the root system was sensitive to simazine, uptake and translocation might be expected to be interrupted by death of the roots, resulting in only small quantities of this chemical accumulating in the plant foliage. The reduction in root dry weight appeared to be a result of lack of photosynthetic substrate rather than simazine toxicity *per se*.

After an exposure of 1 week to C^{14}-labeled simazine in nutrient culture, *L. ovalifolium* showed a greater accumulation of simazine than the 2 species of *Pyracantha*. However, after 2 weeks *Pyracantha* plants showed considerable higher accumulations of simazine than did those of *L. ovalifolium*. This suggested a greater initial phytotoxic effect of simazine on *Ligustrum* resulting in impaired absorption. Results obtained by Ragab and McCollum (23, vol. 9, p. 80) showed that the sensitive cucumber absorbed simazine at a faster rate than the more tolerant corn plant. However, in earlier work Davis, Funderburk and Sansing (11, vol. 7, p. 302) found that there was no obvious correlation between susceptibility and the amount of simazine absorbed by the plant.

After an exposure of 1 week to 1 ppm simazine in solution culture, the amounts of chloroform-extractable C^{14} found in the leaves of the 3 test species were comparable. However, after an exposure of 2 weeks, the amounts extractable from *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* leaves were 54, 39 and 22%, respectively. Since this fraction represented the maximum amount of unchanged triazine possible, greater metabolism of simazine to non-toxic degradation products by the more resistant *P. crenato-serrata* was indicated. The amount of simazine that was extractable from the 3 test species corresponded to the degree of injury observed on the respective test plants. Davis, Funderburk and Sansing (11, vol. 7, p. 308) also found that greater amounts of activity could be extracted from the sensitive cucumber plant than the tolerant corn plant.

Work reported by Freed, Montgomery and Kief (12, p. 14-15) has shown that perennial plants tolerant to the triazine compounds do not produce abundant amounts of $C^{14}O_2$ when treated with C^{14}-labeled simazine, but rather incorporate the radio carbon into plant constituents. Therefore, any radioactive simazine that remained after the plant material had been exhaustively extracted could be assumed to represent metabolism and degradation of the triazine ring and incorporation of the degradation products into plant constituents. This report by Freed was further supported when the test species treated with C^{14}-labeled simazine showed little or no change in total amount of activity present when grown for an additional week in untreated nutrient solution.

Since the percentage of the total activity remaining in the plants after exhaustive extraction (23, 36 and 59 for L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata, respectively) corresponded inversely with the percentage of leaf area showing chlorosis and/or necrosis, it is suggested that the more resistant species have a greater ability to metabolize simazine to harmless degradation products.

SUMMARY

The phytotoxicity of simazine, 2-chloro-4, 6-bis(ethylamino)-s-triazine, has ranged from mild to extreme among plant species. A study to determine growth and physiological responses of selected woody ornamentals to simazine has shown marked differences to exist. Greenhouse experiments, using *Ligustrum ovalifolium*, *Pyracantha koidzumi* 'Government Red' and *Pyracantha crenato-serrata*, showed great variation in expression of visual injury symptoms. Treatment of these species with 3 ppm simazine in nutrient solution resulted in 95, 35 and 0% of the leaf surface showing injury symptoms, respectively. Differences in growth response were not as pronounced, but *Ligustrum* did show significantly greater reduction in plant dry weight and shoot length than *Pyracantha*. These differences in growth response were not detected between the 2 species of *Pyracantha* used. However, the 3 species showed reduced extension growth and dry weight production with each increase in simazine concentration.

Studies with C^{14}-labeled simazine indicated that uptake and translocation by the 2 species of *Pyracantha* was identical under the conditions of this experiment. Results with the simazine sensitive

Ligustrum indicated that its initial uptake was slightly greater than that of the other 2 species, but was followed by a rapid decrease in accumulation as phytotoxicity developed. After an exposure of 2 weeks, the chloroform extractable C^{14} in the leaves of *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* was 54, 39 and 22%, respectively. This indicated greater metabolism of the simazine by the more resistant *P. crenato-serrata* to non-toxic degradation products. Following exhaustive extraction with chloroform, the percentages of total activity remaining in the samples were 23, 36, and 59 for *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. creanto-serrata*, respectively. These levels of simazine in the leaves corresponded inversely with the total amount of leaf surface showing symptoms of chlorosis and/or necrosis and further supported the conclusion that the more tolerant species has a greater ability to metabolize simazine to non-toxic degradation products.

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APPENDIX

Table A. Experiment 1. Numerical rating of foliage injury symptoms on *L. ovalifolium* and *P. koidzumi* 'Government Red' following simazine treatment.

Simazine ppm	*L. ovalifolium* 2 weeks	*L. ovalifolium* 7 weeks	*P. koidzumi* 'Government Red' 2 weeks	*P. koidzumi* 'Government Red' 7 weeks
0.0	1.0	1.0	1.0	1.0
0.5	1.3	1.8	1.0	1.0
1.0	1.5	2.7	1.0	1.4
2.0	3.0	4.0	1.5	3.2
4.0	3.3	9.0	2.5	4.4

Table B. Experiment 2. Numerical rating of foliage injury symptoms on *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* following simazine treatment.

Plant	Simazine ppm	Weeks after Treatment 1	2	3	4	5
L. ovalifolium	0	1.0	1.0	1.0	1.0	1.0
	1	2.3	3.8	4.5	4.5	4.8
	2	2.5	4.5	5.8	6.0	6.5
	3	2.8	5.8	7.8	9.0	9.5
P. koidzumi 'Government Red'	0	1.0	1.0	1.0	1.0	1.0
	1	1.0	1.0	1.0	1.3	1.3
	2	1.0	1.3	2.8	3.5	3.3
	3	1.0	1.8	3.3	4.0	3.5
P. crenato-serrata	0	1.0	1.0	1.0	1.0	1.0
	1	1.0	1.0	1.0	1.0	1.0
	2	1.0	1.0	1.0	1.0	1.0
	3	1.0	1.0	1.0	1.0	1.0

Table C. Experiment 1. Mean fresh weight for plants of *L. ovalifolium* and *P. koidzumi* 'Government Red' as influenced by various levels of simazine.

Simazine	*L. ovalifolium*			*P. koidzumi* 'Government Red'		
	Shoot	Root	Total	Shoot	Root	Total
ppm	grams			grams		
0.0	38.7	25.7	60.5	39.7	23.3	62.8
0.5	21.5	19.0	40.5	30.6	18.1	48.7
1.0	16.0	15.8	31.8	20.2	9.4	29.5
2.0	10.0	7.0	16.9	11.1	7.5	18.2
4.0	4.2	6.2	10.4	6.2	4.3	10.2
LSD.05	3.9	3.0	5.4	6.2	3.3	7.9

Table D. Experiment 2. Mean fresh weight for plants of *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* as influcenced by various levels of simazine.

Plant	Simazine	Shoot	Root	Total
	ppm	grams	grams	grams
L. ovalifolium	0	36.9	34.7	71.6
	1	15.2	13.6	28.8
	2	8.4	7.4	15.8
	3	4.0	5.2	9.2
	LSD.05	6.4	12.0	16.5
P. koidzumi 'Government Red'	0	30.4	14.2	44.5
	1	19.8	11.1	30.8
	2	21.4	11.8	33.2
	3	11.0	9.3	20.3
	LSD.05	16.1	6.5	22.4
P. crenato-serrata	0	18.2	8.6	26.9
	1	12.8	6.3	19.1
	2	11.2	5.4	16.6
	3	7.8	4.9	12.5
	LSD.05	6.8	3.4	10.0

Table E. Experiment 1. Mean dry weight for plants of *L. ovalifolium* and *P. koidzumi* 'Government Red' as influenced by various levels of simazine.

Simazine	*L. ovalifolium*			*P. koidzumi* 'Government Red'		
	Shoot	Root	Total	Shoot	Root	Total
ppm		grams			grams	
0.0	10.7	2.9	13.6	13.8	2.6	16.3
0.5	6.3	1.9	8.2	10.6	1.9	12.5
1.0	4.9	1.4	6.3	7.3	1.2	8.4
2.0	2.9	.7	3.6	3.3	.6	3.9
4.0	1.6	.5	2.1	1.9	.4	2.3
LSD.05	1.2	.3	1.3	2.6	.4	2.9

Table F. Experiment 2. Mean dry weight for plants of *L. ovalifolium*, *P. koidzumi* 'Government Red' and *P. crenato-serrata* as influenced by various levels of simazine.

Plant	Simazine	Shoot	Root	Total
	ppm	grams	grams	grams
L. ovalifolium	0	10.1	4.0	14.1
	1	3.6	1.3	4.9
	2	2.5	.8	3.2
	3	1.5	.5	2.0
	LSD.05	1.9	1.2	2.7
P. koidzumi 'Government Red'	0	9.5	1.8	11.4
	1	6.4	1.3	7.7
	2	6.9	1.4	8.3
	3	3.8	.9	4.7
	LSD.05	4.9	.8	5.7
P. crenato-serrata	0	6.0	1.1	7.1
	1	4.0	.8	4.8
	2	3.6	.6	4.2
	3	2.6	.5	3.1
	LSD.05	2.3	.5	2.7

Table G. Uptake of C^{14}-labeled simazine by plants of L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata plants treated with radioactive simazine.

Test plant	Portion Sampled	Weeks after treatment: Experiment 3* 2	Experiment 4** 1	2	3
		ppm	ppm	ppm	ppm
L. ovalifolium	Leaf	79.2	15.4	23.8	---
	Stem	13.6	5.0	5.7	---
	Total	46.4	10.2	14.7	---
P. koidzumi 'Government Red'	Leaf	---	6.6	30.0	29.1
	Stem	---	6.8	12.0	10.4
	Total	---	6.7	21.0	19.8
P. crenato-serrata	Leaf	78.7	9.3	28.7	29.9
	Stem	25.8	6.0	12.7	11.3
	Total	52.2	7.8	20.7	20.6

* Plants treated with 2 ppm simazine
** Plants treated with 1 ppm simazine

Table H. Percentage of chloroform extractable C^{14} in L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata plants after treatment with radioactive simazine.

Test plant	Portion Sampled	Weeks after treatment: Experiment 3* 2	Experiment 4** 1	2	3
		percent	percent	percent	percent
L. ovalifolium	Leaf	62.2	54.6	54.3	---
	Stem	75.6	---	89.7	---
	Total	64.2	---	61.8	---
P. koidzumi 'Government Red'	Leaf	---	67.5	38.9	21.0
	Stem	---	57.1	44.4	8.8
	Total	---	62.2	40.5	17.8
P. crenato-serrata	Leaf	26.2	60.1	21.9	19.5
	Stem	37.1	85.3	40.4	16.2
	Total	28.8	70.2	27.6	18.6

* Plants treated with 2 ppm simazine
** Plants treated with 1 ppm simazine

Table I. Percentage of C^{14} in plants of L. ovalifolium, P. koidzumi 'Government Red' and P. crenato-serrata after exhaustive chloroform extraction.

Test plant	Portion Sampled	Weeks after treatment			
		Experiment 3*	Experiment 4**		
		2	1	2	3
		%	%	%	%
L. ovalifolium	Leaf	29.6	11.3	22.6	---
	Stem	31.3	9.8	22.5	---
	Total	29.8	10.9	22.5	---
P. koidzumi 'Government Red'	Leaf	---	47.1	36.4	82.6
	Stem	---	35.8	47.6	75.6
	Total	---	41.4	39.6	80.7
P. crenato-serrata	Leaf	32.6	43.9	59.3	74.1
	Stem	63.9	40.9	51.7	83.8
	Total	40.6	42.7	57.0	76.7

* Plants treated with 2 ppm simazine
** Plants treated with 1 ppm simazine