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## Response of 'second-leaf' 'McIntosh' apple trees to soilincorporated simazine.

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Robinson, Donavan Errol, "Response of 'second-leaf' 'McIntosh' apple trees to soil-incorporated simazine." (1969). Masters Theses 1911 - February 2014. 3258. Retrieved from [https://scholarworks.umass.edu/theses/3258](https://scholarworks.umass.edu/theses/3258?utm_source=scholarworks.umass.edu%2Ftheses%2F3258&utm_medium=PDF&utm_campaign=PDFCoverPages) 

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## RESPONSE OP 'SECOND-LEAP\* 'McINTOSH\* APPLE TREES TO SOIL-INCORPORATED SIMAZINE

<sup>A</sup> Thesis Presented

By

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Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of MASTER OP SCIENCE

May, 1969

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### TABLE OP CONTENTS



#### INTRODUCTION

Simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) has gained widespread acceptance for the control of weeds in tree fruits and other horticultural crops (2), Rates of simazine required to control different weed species are known but the problem is essentially one of crop safety.

Numerous investigators (5,20,47,1) have determined the tolerance of fruit trees to simazine applications on the soil surface, but determinations of the approximate threshold level for simazine toxicity to tree fruit have been infrequent. Lange and Crane (28) reported that in general 0.5 ppm was near the critical level for peach, almond, apricot, cherry, pear and black walnut seedlings growing in sand culture. To the author's knowledge however, the threshold level for simazine toxicity to apple trees grown in soil has not been established. Furthermore, fruit species (47,28), fruit cultivars (14,33), and rootstocks (29), vary in their susceptibility to herbicide injury.

Considerable attention has recently been given to the effect of simazine on the nitrogen (N) metabolism of tolerant plant species. <sup>A</sup> number of workers (18,37,21) have reported increases in growth and <sup>N</sup> content of plants treated with sub-toxic concentrations of simazine. To the contrary. Lord et al. (30,31) in field studies, obtain-

ed no differences in nutrient levels or in growth of apple and peach trees that could be attributed to simazine. High leaf N, good tree vigor and simazine adsorption by organic matter were given as possible explanations for this lack of response (30).

The objectives of the present study were: (1) to determine the threshold level for simazine toxicity to 'McIntosh' apple trees on EM Vll rootstock grown in soil; (2) to compare the threshold level for simazine toxicity for 'McIntosh' apple trees on EM 1X, EM V11 and MM 106 rootstocks; and (3) to determine the effect of simazine on the growth and <sup>N</sup> level of 'McIntosh' apple trees.

#### REVIEW OP LITERATURE

Chemical nature of simazine: Simazine belongs to a group of heterocyclic compounds known collectively as the triazines. The ring structure of the triazine molecule is characteristically 6-membered, and contains <sup>3</sup> nitrogen atoms. The various members of the triazines are distinguished according to the groups attached at positions <sup>4</sup> and <sup>6</sup> of the ring structure. The simazine molecule has these positions occupied by ethylamino groups as shown below. Accordingly the organic nomenclature of simazine is 2-chloro-4,6-bis(ethylamino)-s-triazine.



Fig. 1. Structure of the simazine molecule.

Simazine and related triazines were developed in Switzerland, and the first field tests with this herbicide in the United States were made in 1956 on corn (27). Subsequent investigations have shown that simazine provides season-long control of weeds in sugar cane,

orchards, woody ornamentals, Cranberries and a number of other crops  $(27,13)$ . It is also an effective soil sterilant when used at relatively high rates (27). Mode of action: Moreland et al. (32) and Exer (17) reported that simazine inhibited the photochemical activity of isolated chloroplasts. Exer (17) further pointed out that the inhibition involved the photochemical reduction of NAD and that simazine does not influence catalase activity nor does it inhibit respiration. Gast (19) showed that in Coleus blumei simazine blocked starch production in the light and that this effect was overcome by the addition of sucrose to starch-free leaves.

Studies conducted by Singh and West (43) revealed that simazine altered chloroplast protein of oat plants and caused marked differences in the protein and amino acid incorporation ability of these chloroplasts. An alteration in total RNA content and synthesis, as measured by  $\rm p^{32}$  incorporation, was also noted by the same workers. However, this study did not evaluate the specific RNA fraction affected.

Selectivity; Studies relating to selectivity indicated that plants resistant to the s-triazines contain a mechanism capable of rapidly degrading these chemicals. Castelfranco et al. (11) noted that some plant species degrade simazine by a non-enzymatic transformation of the molecule from the chloro- to the hydroxy-derivative.

Since the hydroxy-derivative is non-phytotoxic its formation represents a detoxification mechanism. According to Roth and Knusli (40), this transformation results from the activity of a cyclic hydroxamate in the sap of the tolerant plant. The following nucleophilic attack on the triazine ring has been proposed by Castelfranco and Brown  $(10)$ .





Fig. 2. Proposed detoxification mechanism of simazine in tolerant plant species.

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The structure of the compound  $(2)$  has been worked out by Gysin and Knusli (24), and is now established as 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one.



### Pig. 3. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazine-3-one molecule

Hydroxy-simazine as well as the cyclic hydroxamate and its 2-glucoside have been extracted by Hamilton and Moreland (25) from treated corn seedlings. Cyclic hydroxamate and its 2-glucoside are capable of splitting chlorine from simazine in vitro. Castelfranco et al.(ll) found no such destruction of simazine in Avena, a susceptible species.

Some plants are also protected from simazine injury by virtue of their deep root system, since simazine usually remains near the soil surface.

Degradation: Several processes such as volatilization,

adsorption, leaching, photodecomposition, chemical reaction and absorption by microorganisms and higher plants are responsible for loss or inactivation of herbicides  $(41)$ . The majority of investigations of simazine degradation indicate that slow microbial decomposition is the principal process responsible for the inactivation of this herbicide (7,23,34).

Investigations with  $C^{14}$  labeled atrazine and simazine indicate that the triazine ring is quite resistant to microbial degradation (28,34). However, similar investigations with chain  $c^{14}$  labeled simazine have shown that certain organisms are able to metabolize the side chain of simazine (12,26).

Of the soil microflora, the soil diptheroids (Corynebacteriaceae) and the soil Pseudomonads (Pseudomonas spp.) contribute greatly to the degradation of herbicides (35). The microbial degradation of simazine appears to be related to the quantity of carbon in the soil, and may explain the rapid disappearance of simazine in light organic soils (23).

Armstrong et al. (3) have presented considerable evidence to show that chemical hydrolysis is an important mechanism of atrazine degradation in soil It is likely that simazine is also degraded in the same manner.

Persistence and Toxicity: The Processes responsible for the loss of <sup>a</sup> herbicide from soil or for its inactivation are influenced considerably by such variables as kind of herbicide, rate of application, formulation, weather, soil type, soil climate and soil microorganisms.

Simazine is one of the most persistent herbicides and generally requires <sup>3</sup> to 12 months for inactivation (42), but under some environmental conditions it has persisted in sufficient quantities to be toxic to certain plants for <sup>1</sup> year or more after application (44,15,45).

Application rate is an important factor affecting simazine presistence. The decomposition of a herbicide in soil occurs as <sup>a</sup> first order reaction (9). This means that the same percentage of the original dosage. will remain in the soil after a given length of time regardless of concentration applied. Simazine when applied at high concentrations prevents growth of all green plants and this effect may either be temporary or may last several years, depending on the rate used.

Lord et al. (30) reported that the persistence of the granular formulation of simazine was greater than that of the wettable powder formulation. The reson for this is not clear. Buchholtz (6) suggests the following explanation for the greater persistence of granular over wettable powder herbicide formulations. Each granule contains a high concentration of active material which

is released into the soil immediately surrounding the granule. This may result in greater adsorption by the soil colloids than would be the case with a more uniformly applied wettable powder formulation of simazine. A greater period of time may then be required for desorp-' tion and a longer period of residue might be expected.

Simazine is washed into the soil by rain or irrigation where it is subject to adsorption by soil colloids. Organic matter content and perhaps to some extent the amount and nature of clays present largely determine the capacity of soil to adsorb simazine. The findings regarding the relationship of clay content and herbicidal activity are not consistent, however. Upchurch and Mason (49) and Grover (22) have reported that the dosage of simazine required to produce a given plant response was essentially unrelated to clay content. To the contrary, Talbert and Pletchall (46) found no adsorption by kaolinite clay but Putnam, illite and montmorillonite clays were increasingly adsorptive in that order. More recently, Day et al. (16) found a negligible correlation between simazine phytotoxicity and soil  $pH$  and clay content. There was a marked interrelationship between organic matter, cation exchange capacity, the equilibrium concentration of simazine in the soil solution, and phytotoxicity. Simazine phytotoxicity was more closely correlated with percent organic matter than with any

other factor.

Simazine adsorption by soil colloids offers resistance against leaching and, therefore, it remains in the / upper soil profile (8,30,39) and Causes limited injury to deep-rooted plants. Furthermore, adsorption may lead to a rapid reduction in initial level of simazine residue (22,46) but may also allow a marginal amount of residue to persist for many months due to slow desorption (6). The adsorptive reaction has been found to be fully reversible by increasing temperature, water elution and elution with a number of organic solvents  $(46)$ .

Simazine toxicity is greater under conditions of high moisture content than under low moisture conditions  $(22)$ . Bailey and White  $(4)$  have advanced the hypothesis that simazine (an organic solute) and water (a highly polar solvent) compete for the adsorption sites on the colloids and on this basis the availability of simazine under varying conditions of organic matter, clay and soil moisture can be explained.

METHODS AND MATERIALS

To determine the threshold level for simazine toxicity to 'second-leaf' 'McIntosh' apple trees on EM Vll rootstock and the effect of this herbicide on tree growth and <sup>N</sup> level, fall-dug trees were purchased in February, 1968 and stored in a nursery cellar until establishment of the treatments on March 5, 1968, After adhering soil and sawdust were washed from the roots, the trees were pruned uniformly and divided into <sup>5</sup> size-groups of 14 trees each according to weight. The trees in each size group were divided into <sup>2</sup> groups of approximately equal weight. This procedure resulted in <sup>5</sup> replicates of trees and 2 groups of approximately the same weight within each replicate of trees.

The trees in one group for all replicates were planted in Hinckley gravelly loamy sand, and those of the other group were planted in Woodbridge fine, sandy loam. Simazine, 80% wettable powder had been thoroughly incorporated into these soils with a mechanical cement mixer at concentrations of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 ppm on an ovendry basis. Untreated soils served as controls. The trees were greenhouse-grown in 30 lb frozen food cans at T the University of Massachusetts, Amherst, until the com-

<sup>1</sup>'Second-leaf' is a nurseryman's term for the second growth year of the scion variety.

pletion of the study on July 13, 1968. A night temperature of 65°F was maintained during the early part of the study but during the summer months night temperatures frequently exceeded 65°F. Day temperatures varied greatly throughout the study. The photoperiod was 14 hours until early May, 1968 and thereafter the trees received natural daylength. The trees were watered when needed and a modified Hoagland's solution applied weekly. Frequent fumigation and spraying were required to control red mites and aphids.

Periodic phytotoxicity ratings were made ( $0 = no$ effect; 10 <sup>=</sup> dead plant), and at the completion of the study a sample of uninjured mid-terminal leaves was obtained from each tree except those grown in soil with  $6.4$ ppm simazine; <sup>N</sup> content was determined by the micro-Kjeldahl method. The foliage of trees growing in soil containing 6.4 ppm simazine was severely necrotic, and was not considered suitable for analysis of <sup>N</sup> content.

The trees were removed from the soil, adhering soil washed from the roots and their fresh weights and terminal growth recorded. Woodbridge soil at  $0 - 3$ " and  $9 - 12$ " depths in the 30 lb frozen food cans containing 1.6 and 3.2ppm simazine was sampled for bioassay of simazine content. Since the bioassay is accurate only within a narrow range of simazine concentrations (0 -0.3 ppm), the samples were diluted with appropriate proportions of untreated soil. Series of known concentrations were prepared by using dilutions (in talc) of 80% wettable powdersimazine. The series ranged from 0 - 1.0 ppm simazine based on oven-dry weights of Woodbridge soil. Twelve oat seeds were planted in each pot containing 500g of ovendry soil with known and unknown simazine concentrations. Hoagland nutrient solution was added weekly, and all pots were uniformly watered every other day. At the end of <sup>4</sup> weeks the oat plants were cut at soil level and their fresh weights determined. A standard curve of herbicidal toxicity was constructed by plotting the fresh weights of the oat plants as a function of known herbicide concentrations. Simazine concentrations in the Woodbridge soil was estimated by fitting the fresh weight of the oats to the standard curve. The standard and unknowns were run in triplicate, and the values for the three pots were averaged.

To determine the effect of rootstock on simazine phytotoxicity to'second-leaf' 'McIntosh' apple trees and the effect of this herbicide on the tree growth and N level, 35 trees on EM Vll, EM IX and WH 106 rootstock were pur chased in February and stored and prepared for planting using the previously described procedures. The trees on each rootstock were divided into <sup>5</sup> size groups of <sup>7</sup> trees, each according to weight, making <sup>5</sup> replicates containing <sup>7</sup> trees on each rootstock.

Oven-dried Woodbridge fine sandy loam was mixed with simazine to prepare concentrations of 0.8, 1.2, 1.6, 3.2

and 6,4 ppm of this herbicide and untreated soil served as controls. The trees were planted into 30 lb frozen food cans on April 23, 1968 and grown under field conditions until the completion of the study on August 26, 1968. The trees were watered and sprayed with insecticides as required and one watering with Hoagland's solution was administered during the first week of growth. Following previously described procedures, data for fresh tree weight, terminal growth and N levels were obtained.

The data from both the greenhouse and field studies were subjected to analysis of variance and the mean differences were compared by the Duncan Multiple Range Test.

RESULTS AND DISCUSSION Phytotoxicity symptoms: Approximately 5 weeks after planting, the greenhouse-grown 'McIntosh' apple trees on EM Vll rootstock developed distinctive symptoms of simazine phytotoxicity. The symptoms first appeared on trees growing in Hinckley soil containing 6.4 ppm simazine. <sup>A</sup> week later trees growing in Woodhridge soil containing 6.4 ppm simazine, and those in Hinckley soil with 3.2 and 6.4 ppm simazine showed simazine injury. The first indication of toxicity was slight chlorosis along the leaf margin. As the condition worsened, interveinal yellowing and the retention of green coloration by the veins became the distinguishing pattern of injury (Figure 4). Acute toxicity caused marginal and interveinal necrosis and leaf abscission (Figure 5). The phytotoxicity symptoms first appeared on the suckers at the tree base and then progressed from the lower to the upper branches of the tree, with the basal leaves being affected first.

The foliar damage was more severe on trees in Hinckley soil, and was present at a lower simazine concentration than on those in Woodbridge soil (Table 1). This difference in phytotoxicity ratings may have been due to differences in characteristics of the two soils (Table 2). Hinckley soil was considerably higher in sand than the Woodbridge soil, while the clay and organic matter contents were lower. Organic matter and clay in soils adsorb herbicides and reduce their phytotoxicity (46,49).

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Pig, 4. Early leaf symptoms of simazine phytotoxicity to xariy rear symptoms or simazine phytotoxic interveinal yellowing with veins remaining green. (Photograph by Louis J. Musante, School of Education, Univ, of Massachusetts.)



Fig. 5. Advanced leaf symptoms of simazine phytotoxicity to 'second-leaf' 'McIntosh' apple trees. Leaves showing marginal and interveinal necrosis. (Photograph by Louis J.Musante, School of Education, Univ. of Massachusetts.)



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Five trees per treatment.

 $z_{\text{Pytotoxt}}$  as rated  $0 - 10$ , where  $0 =$  no effect. Phytotoxicity was rated 0-10, where 0 = no effect.

Characteristics of Hinckley and Woodbridge soils used to determine the threshold<br>level of simazine phytotoxicity to greenhouse-grown 'second-leaf' 'McIntosh'<br>apple trees on EM VII rootstock, March 5 - July 13, 1968. Table 2.



It can also be noted from Table 1 that between May <sup>23</sup> and June <sup>26</sup> the foliar damage became less acute. Several processes such as volatilization, adsorption, leaching, photodecomposition, chemical reaction, and absorption and metabolism by microorganisms and higher plants are responsible for the degradation of herbicides (41), and because of this degradation plant recovery may occur.

Simazine remaining in the soil at the completion of the study represented only 50 to 59 $%$  of the original amount (Table 3) and may account for the partial recovery of the trees between May 23 and June 26 from simazine toxicity (Table 1). The data in Table 3 also indicate that little leaching of simazine occurred in the 30 lb frozen food cans during the course of the study. Since the simazine was thoroughly incorporated in the soil processes other than photodecomposition, volatilization and leaching were probably responsible for the degradation of simazine.

The phytotoxicity ratings for 'McIntosh' apple trees on EM Vll rootstock grown under field conditions are basically in agreement with the ratings made on the greenhouse-grown trees, except the symptoms were less severe (Table 4).

Concentration of simazine in Woodbridge soil at 2 depths in 30 lb frozen food cans<br>in which 'second-leaf' 'McIntosh' apple trees were greenhouse-grown from March 5 -<br>July 13, 1968. Table 3.



 $z_{\text{Determined}}$  by bioassay.

Simazine phytotoxicity to 'second-leaf' 'McIntosh' apple trees on EM Vii rootstock<br>in Woodbridge soil grown in 30 lb frozen food cans under field conditions,<br>April 23 - August 26, 1968. Table 4. Simazine phytotoxicity to 'second-leaf' 'McIntosh' apple trees on EM Vil rootstock in Woodbrldge soil grown in 30 lb frozen food cans under field conditions, April 23 - August 26, 1968\* Table 4.

 $11$ 



V<sub>Five</sub> trees per treatment.

<sup>z</sup> Phytotoxicity was rated  $0 - 10$ , where  $0 =$  no effect. Phytotoxicity was rated 0-10, where 0 = no effect

Influence of simazine on tree growth: The visual phytotoxicity ratings (Tables <sup>1</sup> and 4) indicated that the threshold level for simazine phytotoxicity to 'secondleaf\* 'McIntosh\* apple trees on EM Vll rootstock was between 0,8 and 1.6 ppm for the Hinckley soil, and between 1.6 and 3.2 ppm for the Woodbridge soil. To the contrary the data for fresh weight increase (Table 5) indicate that the threshold level for simazine toxicity did not differ for the two soil types, being between 0.8 and 1.6 ppm. The data for fresh weight increase for field grown 'McIntosh' trees are not in exact agreement with these findings (Table 6), possibly due to differences necessary forsignificance. Variability in time of bud break on the trees used in the study was considerable and made large growth and weight measurement differences necessary for significance among treatments. Nevertheless the data for fresh weight increase may be a more reliable laboratory measurement of herbicide injury than terminal growth and visual symptoms of injury.

Ries et al. (38) reported that sub-toxic levels of simazine increased growth of non-bearing apple and bearing peach trees. Although the data in Table <sup>6</sup> indicate that 0.8 ppm simazine increased fresh weight of the 'McIntosh' apple trees, the increase was not significant. The most striking response of the 'McIntosh' trees to 1.6, 3.2 and 6.4 ppm simazine was the restriction of root development (Pig. 3).



VFive trees per treatment.

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 $z$ Means in any column followed by unlike letters are significantly different at the  $5%$  level.



Table 6. Effect of rootstock on simazine phytotoxicity to 'second-leaf\* 'McIntosh'apple trees

Table 6.

Effect of rootstock on simazine phytotoxicity to 'second-leaf' 'McIntosh'apple trees

<sup>ZMeans in any column followed by unlike letters are significantly different at the 1% level.</sup> ^Means in any column followed by unlike letters are significantly different at the 1/^ level.



Pig. 3. Effect of simazine on root development of 'secondheaf' 'McIntosh' apple trees. Note the limited root development of the trees grown in soil containing 3.2 and 6.4 ppm simazine in comparison to the tree in untreated soil. (Photograph by-Louis J. Musante, School of Education, Univ. of Massachusetts.)

Effect of rootstock on tree response to simazine; The data in Table 6 give a slight suggestion, but not conclusive evidence, that rootstocks may vary in simazine tolerance. 'McIntosh' on EM V11 and EM 1X in soil containing 3.2 and 6.4 ppm simazine made significantly less growth than the check trees, whereas only 6.4 ppm simazine adversely affected the fresh weight of 'McIntosh' on MM 106 rootstock. Scion-rootstock-herbicide interrelationships have been given scant attention by other researchers. Working with reciprocal grafts of peach and apricot budded seedlings. Tweedy and Ries (47) demonstrated that rootstock did not alter tolerance to simazine and prometryne. Apricot scions were more susceptible than, peach to both herbicides regardless of the rootstock on which they were grafted. They concluded that tolerance is a result of physiological resistance occurring in the scion. On the other hand, Lange and Elmore (29), working with almonds, revealed that both scion and rootstock influenced tree susceptibility to herbicides. They found that shallow rooting 'Mariana 2624' was more sensitive to simazine and isocil than the deeper rooting 'Lovell' rootstock. In the present study, depth of rooting was not a factor affecting rootstock influence on 'McIntosh' susceptibility to simazine toxicity.

Influence of simazine on leaf N: Generally, the growth responses to herbicides have been attributed to the elimi-

nation of weed competition rather than a direct effect of the herbicide treatments. In 1962, however, Ries et al. (38) reported that sub-toxic concentrations of simazine increased leaf N and growth of non-bearing apple and bearing peach trees and leaf N in bearing apple trees. It was suggested that this herbicide, influenced the <sup>N</sup> metabolism of these trees. In later work, Tweedy and Ries (4'7) showed that these responses to simazine occur in plants grown with nitrate, but not in plants grown with ammonium nitrogen, and are greatest when nitrate and temperatures are at sub-optimal levels. To the contrary, Vorob'ev et al. (50) working with corn, and Lord et al. (30,31) in studies with mature apple and peach trees ob tained no response in N content of leaves to sub-toxic simazine applications. The latter author suggested that high leaf N, good tree vigor and simazine adsorption by clay and organic soil fractions as possible explanations for this lack of response.

In the present study a significant N response to simazine was obtained with both the greenhouse and fieldgrown 'McIntosh\* trees (Tables <sup>7</sup> and 8). V/hether or not differences in growing conditions can explain the difference in N response between the greenhouse and field-grown trees is not known, since data for this variable were beyond the scope of the experiment.

It is evident from this study and that of Ries et al.

(38) that simazine applications will increase <sup>N</sup> level in fruit trees. In the field studies by Lord et al. (30, 31), simazine may not have been available for sufficient uptake of this herbicide by the tree roots to affect N metabolism.

With the greenhouse-grown 'McIntosh' trees the N response was present only in those in Hinckley soil (Table 7), and only at 3.2 ppm simazine, which also caused foliar damage (Table 1). With the field grown trees on Woodbridge soil, the N effect of simazine was more striking (Table 8) and was evident at sub-toxic simazine concentrations- (Table 4).

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Table 7. The effect of soil type on N response of "second-leaf' greenhouse-grown, 'McIntosh' apple trees on EM Vll rootstock to simazine. Eppre trees on Em wir rectstoon to Simmary in 30 lb frozen food cans.



 $\mathbf{y}_{\text{Five trees per treatment.}}$ 

 $z$ Means in any column followed by unlike letters are significantly different at the  $1\%$  level.

**Contract Contract** 

Table 8. The effect of simazine on N response of 'second-leaf\* 'McIntosh\* apple trees on <sup>3</sup> clonal rootstocks under field conditions. Trees grown from April 23 to August 26, 1968, in 30 lb frozen food cans.



V<sub>Five</sub> trees per treatment.

 $z$ Means in any column followed by unlike letter are significantly different at the  $1\%$  level.

SUMMARY

'Second-leaf\* 'McIntosh\* apple trees were grown under greenhouse and field conditions in <sup>30</sup> lb frozen food cans containing soil-incorporated simazine at concentrations of  $0.2$  -  $6.4$  ppm. Data for terminal growth, fresh weight increase and visual symptoms of phytotoxicity indicated that fresh weight increase was a more precise method for the detection of simazine injury than the other 2 measurements. The threshold level for simazine phytotoxicity to the trees were between 0.8 and 1.6 ppm for the <sup>2</sup> soils used in the study. Root development was drastically reduced at the higher simazine concentrations. There was a suggestion that trees on MM.105 rootstock were more tolerant to simazine than those on EM V11 and EM 1X rootstocks. A significant leaf nitrogen response to simazine was obtained but with the greenhouse-grown trees this response was found only on the lighter of the <sup>2</sup> soils and at toxic simazine levels.

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#### **ACKNOWLEDGEMENTS**

The author wishes to express his genuine gratefulness to Dr. William J. Lord for his excellent advice, help and personal encouragement given throughout the preparation of this thesis; to Drs. John H. Baker and Jonas Vengris for their helpful suggestions and criticisms; and to Messers Anthony Rossi and Leslie Cromack for their general assistance and work on the analysis of leaf nitrogen.

The author is also indebted to the many other people in the Department of Plant and Soil Sciences who contributed towards this study through their advice and guidance.

Donavan E. Robinson

# RESPONSE OF 'SECOND-LEAF' 'MCINTOSH' APPLE TREES TO SOIL-INCORPORATED SIMAZINE

A Thesis

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May 1969