

FACTORS AFFECTING THE DIFFERENTIAL TOLERANCE
OF TREE SPECIES TO HERBICIDES, PRIMARILY SIMAZINE

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Fred Wesley Freeman
1963

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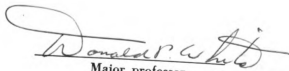
FACTORS AFFECTING THE DIFFERENTIAL TOLERANCE
OF TREE SPECIES TO HERBICIDES, PRIMARILY SIMAZINE

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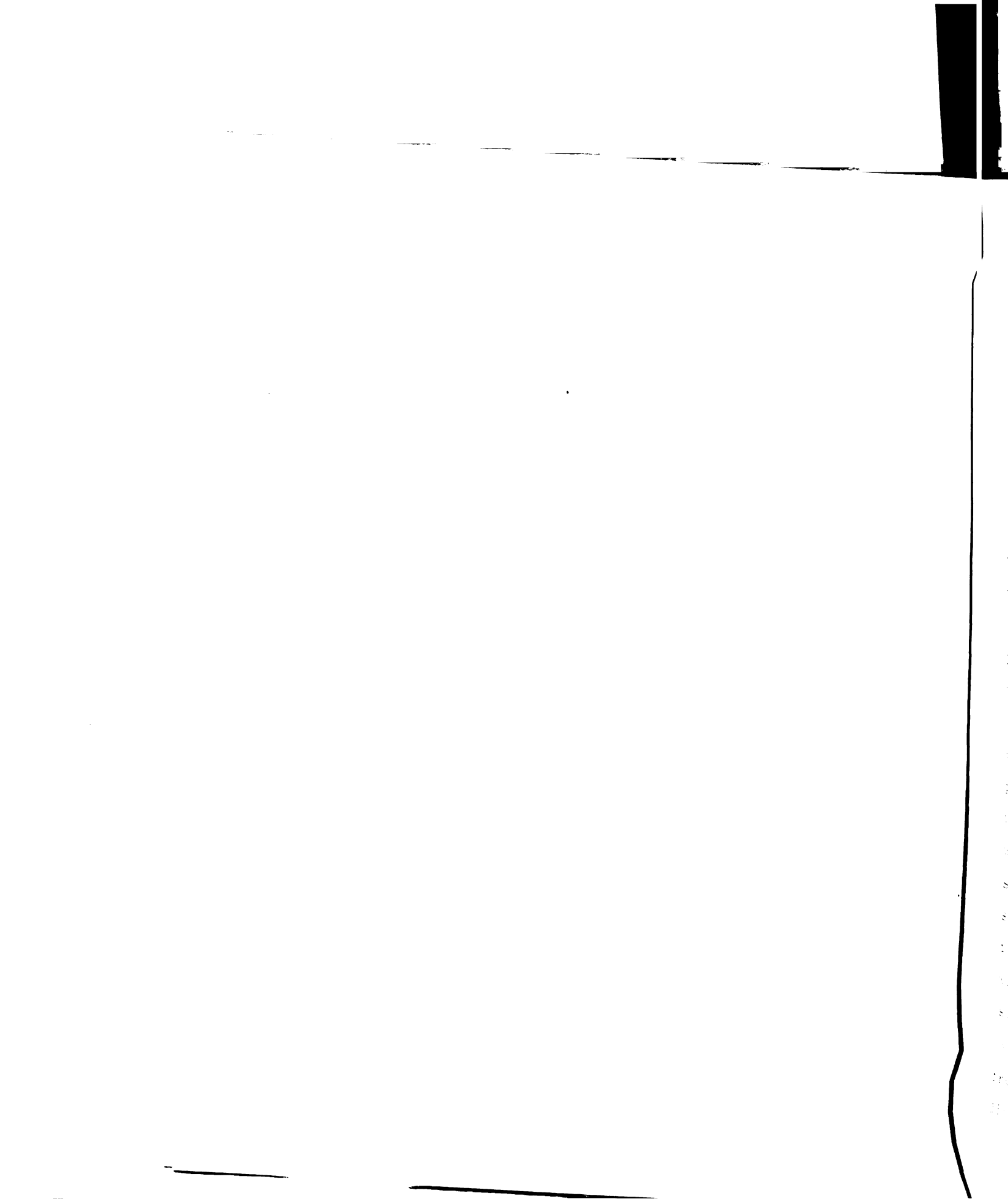
Ph.D. degree in Forestry


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Date February 19, 1963









ABSTRACT

FACTORS AFFECTING THE DIFFERENTIAL TOLERANCE OF TREE SPECIES TO HERBICIDES, PRIMARILY SIMAZINE

by Fred Wesley Freeman

Weed control in nursery management is the most laborious and expensive part of the total operation. Although chemical weed control has been practiced to some extent in the past, field evaluation and basic research are not keeping pace with the large number of new weed control agents being placed on the market. The rapidly developing chemical weed control era in agricultural crops can also be adapted, with proper research and field appraisal, to controlling weeds in woody nursery stock.

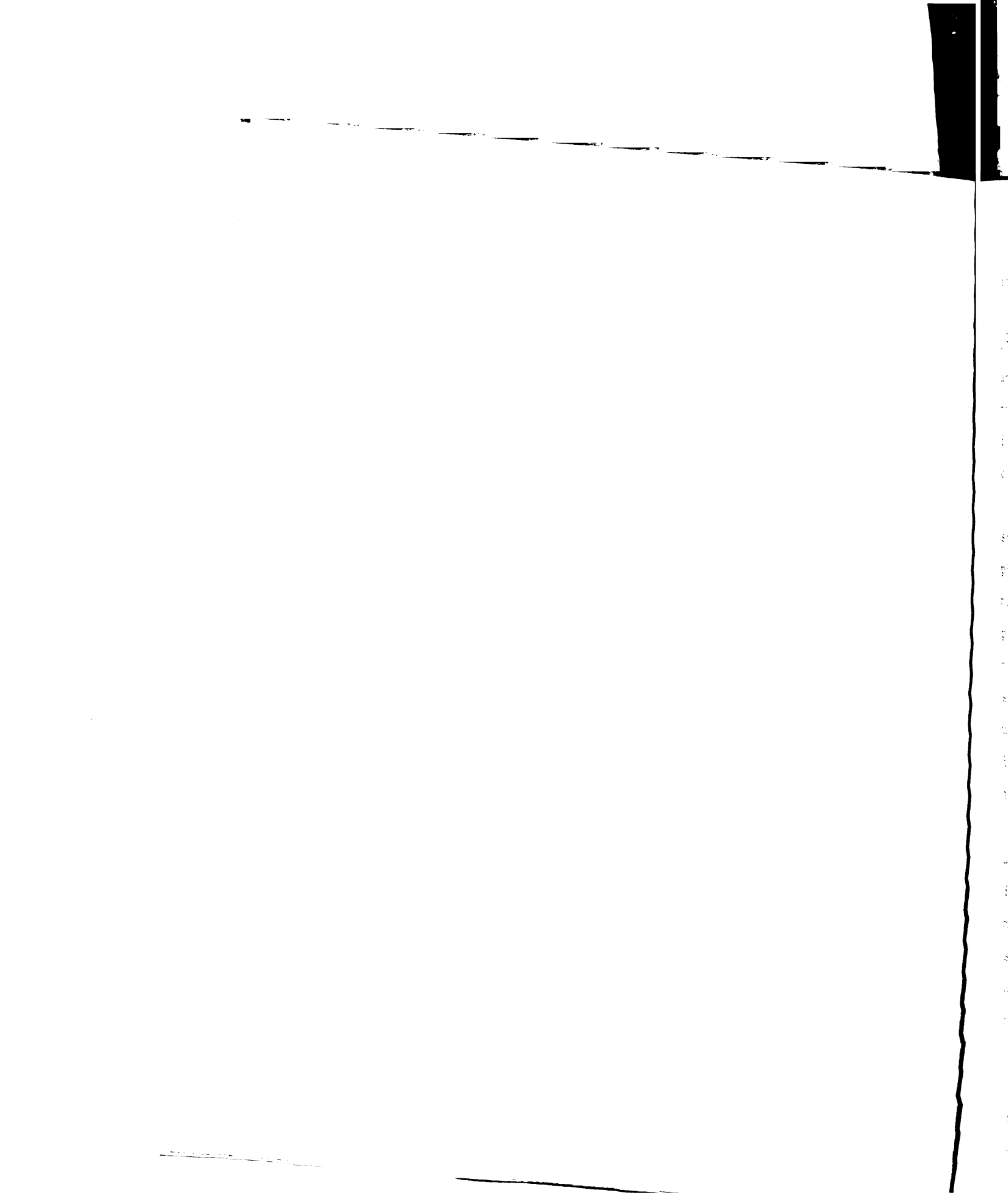
The first phase of this nursery weed research, carried on in the Bogue Forest Nursery at East Lansing, Michigan, compared some of the newer soil fumigants and organic herbicides with those used in the past. Duration and thoroughness of weed control and extent of injury to germinating coniferous seedlings were recorded and analyzed during the growing season. Of the eleven different herbicides and soil fumigants tested, alone and in combination, simazine gave the best weed control over the longest period of time. Injury to the seven species of conifers used as test plants was severe with 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine) treatments. White pine showed the greatest tolerance. 4,6-dinitro-o-sec-butylphenol, amine salt (DNBP) gave good weed control during the early growing season and caused a minimum

Fred Wesley Freeman

amount of damage to young seedlings. The results obtained with DNBP indicate that additional testing of this herbicide is warranted.

Further investigation of simazine rates and placement was made in greenhouse experiments with both conifers and hardwoods. White pine again proved to be more resistant to simazine than all other conifers tested. While all conifers except white pine were killed within five weeks after germination by both the 4 and 8 pound treatments, red, white, and bur oak were not damaged by any of the treatments. Surface applications were far less damaging to honeylocust and white pine than treatments in which simazine was mixed with the upper inch of soil.

Theorizing that the species most resistant to simazine, such as white pine and the oaks, extended their roots below the zone of high herbicide concentration more rapidly than the easily killed species, root elongation studies were made to check this point. It was found that the roots of white pine did not elongate any faster than those of simazine-susceptible Scotch and red pine. However, the roots of bur oak moved 9 inches deep in only 15 days. With the oaks, the maximum absorbing area of the root apparently moves quickly below the zone of high simazine concentration. Additional studies compared simazine toxicity symptoms of oak seedlings whose root systems were confined to the upper 3 inches of soil with those of normal growing plants. Results substantiate the belief that a good part of the tolerance in oaks is due to the root growth habit of the plant rather than



its inherent physiological makeup.

A third study was set up to investigate, by use of C^{14} -labeled simazine, the wide difference in tolerance of red and white pine to simazine. In addition, the effect of the mycorrhizal relationship associated with these two pines was investigated. Two radioassay procedures, autoradiography and counting of plant parts, were used.

Results of this study showed the total uptake of simazine to be approximately equal for both red and white pine. The needles of red pine, however, contained approximately three times the amount of C^{14} as the needles of white pine. In white pine the simazine is concentrated more heavily in the roots of the seedlings, while distribution of simazine or its degradation products in red pine is fairly uniform throughout the plant. Since simazine kills by blocking the Hill reaction during photosynthesis, the reason for the greater tolerance exhibited by white pine seems evident.

At the time of radioactive treatment, mycorrhizae had advanced only into the initial stage of development on the inoculated seedlings. Even at this early stage of development, however, the inoculated white pine seedlings contained significantly less radioactive material than the noninoculated white pines. In fact, counts of noninoculated plants were more than double those of inoculated plants.

No apparent differences were noted in simazine uptake between inoculated and noninoculated red pine. The mycorrhizal relationship either had no effect on simazine uptake with this species or perhaps develops slower on red pine and therefore had not reached a stage where it could influence uptake.

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By

Fred Wesley Freeman

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Forestry

1963



ACKNOWLEDGMENTS

The author expresses his deep appreciation to the many persons responsible for the successful completion of this study.

The Graduate Committee have given generously of their time and special skills, enabling the writer to combine all phases of the research into this final form. My sincere thanks to: Dr. Donald P. Watson, Professor of Horticulture, who encouraged me to begin work on this degree, arranged for the necessary leaves of absence and insisted on early completion of language requirements, a stumbling block for so many graduate students. Dr. Stanley K. Ries, Associate Professor of Horticulture, who first aroused my interest in chemical weed control and gave much thought, time, and equipment toward completion of the early stages of investigation. Dr. Jonathan W. Wright, Associate Professor of Forestry, whose assistance with design of experiments, program planning, and techniques of field scoring have been most helpful and time saving. Dr. Martin J. Bukovac, Associate Professor of Horticulture, who gave generously of his time in guiding me through the radioassay procedures performed at Hidden Lake Gardens and on campus.

Special thanks are due Dr. Donald P. White, Associate Professor of Forestry and chairman of the Graduate Committee, who closely supervised the development, organization, and



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writing of the complete study. At Dr. White's suggestion and with his assistance, the phase of the study dealing with the mycorrhizal relationship was developed.

The writer is grateful to the Geigy Chemical Corporation of Ardsley, New York, for their contribution of C¹⁴-labeled simazine.

An expression of gratitude is also due my wife, Jeanne, and the children, whose patience, understanding, sacrifice, and encouragement contributed much to the completion of this work.

To these and to all others who have contributed to this work but have not been mentioned, I extend my thanks.

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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	4
Comparison of s-triazines	6
Use of Simazine and Atrazine on Woody Plants	8
Chemical and Physical Properties of the s-triazines	10
Factors Affecting Herbicidal Action of Simazine	11
Deactivation of Simazine by Soil Microorganisms	14
Physiology of the s-triazines in the Plant	17
III. METHODS AND PROCEDURE	20
Comparison of Several Soil Fumigants and Herbicides in Nursery Seedbeds	20
Weed Control Ratings	24
Tree Seedling Injury Ratings	25
Statistical Analysis	25
Results and Discussion	25
Weed Control	25
Seedling Injury	33
IV. TOLERANCE OF SEVERAL HARDWOOD AND CONIFEROUS SPECIES TO SIMAZINE TREATMENTS	38
Seedling Survival and Analysis	41
Root Elongation	41
Results and Discussion	42
Seedling Survival	42
Root Elongation	46
V. THE UPTAKE AND DISTRIBUTION OF C ¹⁴ -LABELED SIMAZINE OR ITS DEGRADATION PRODUCTS IN MYCORRHIZAL AND NON-MYCORRHIZAL RED AND WHITE PINE SEEDLINGS	53



chapter	Page
Description of Growing Chamber and Growth Medium	53
Treatment with Radioactive Simazine . .	50
Autoradiograms and Counting	58
Statistical Analysis	58
Results and Discussion	50
Autoradiograms	50
Counts of Radioactive Plant Parts . . .	01
VI. SUMMARY AND CONCLUSIONS	07
LITERATURE CITED	72
APPENDIX	79

LIST OF TABLES

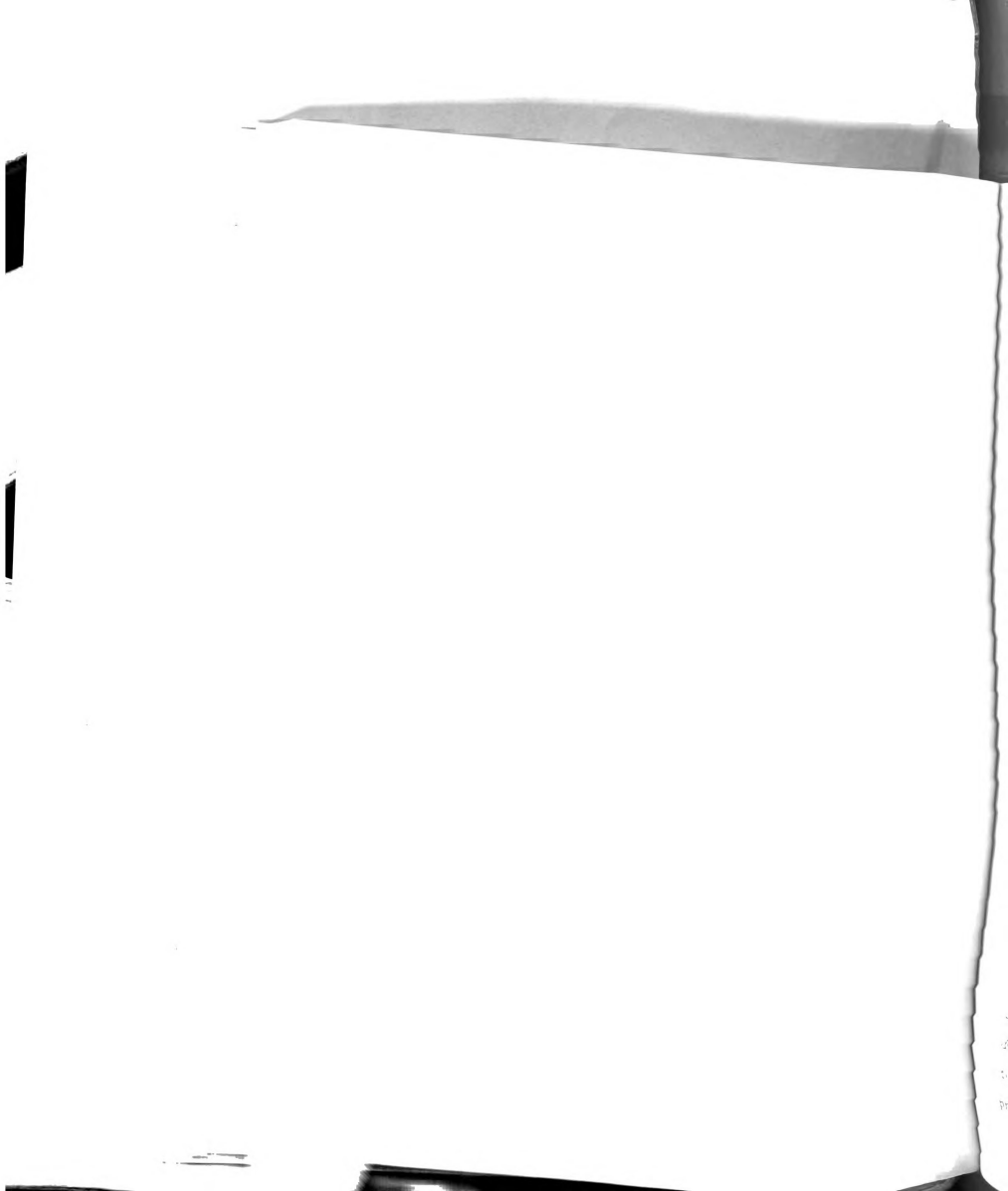
Table	Page
1 Weed control treatments used in nursery seedbeds	22
2 Tree species used in nursery weed control experiment	23
3 Mid-June weed control	28
4 Mid-July weed control	29
5 Mid-August weed control	30
6 Survival index of tree seedlings in the nursery weed control experiment	34
7 Percentage of seedlings remaining alive under DNBP and s-triazine treatments in mid-July	36
8 Tree species used in greenhouse simazine experiment	40
9 Duration (weeks) of seedling survival under various simazine treatments	44
10 Mean counts of C^{14} in red and white pine	61
11 Counts of C^{14} in roots, stems, and leaves of red and white pine seedlings	63
12 Counts of C^{14} in inoculated and noninoculated white pine seedlings	64
13 Distribution of C^{14} in red and white pine (percentage basis)	64
14 Percentage of C^{14} in leaves and roots of red and white pine	65

LIST OF FIGURES

Figure		Page
1	General view of Bogue Forest Nursery showing location of study on soil fumigants and herbicides in nursery seedbeds	21
2	Weed population in nursery seedbeds	27
3	Extremes in weed control at six weeks after treatment	31
4	Layout of greenhouse experiment testing tolerance of several hardwood and coniferous species to simazine treatments	39
5	Tolerance of honeylocust and bur oak to various simazine treatments at 3 months after application	45
6	Root elongation of Scotch pine at 3 weeks after germination	47
7	Root elongation of red pine at 3 weeks after germination	48
8	Root elongation of white pine at 3 weeks after germination	49
9	Root elongation of bur oak at 15 days	50
10	Bur oak seedlings 10 weeks after treatment with 4 pounds per acre simazine. Autoradiogram of bur oak 5 weeks after treatment with 3 pounds per acre radioactive simazine	51
11	Polyethylene greenhouse used for growing inoculated and noninoculated red and white pine seedlings	54
12	Application of radioactive simazine treatments	57
13	Autoradiograms and seedling photographs of red pine	60
14	Autoradiograms and seedling photographs of white pine	62

LIST OF APPENDICES

Appendix	Page
1 Self-absorption curve	79
2 Listing of common and chemical names of herbicides and soil fumigants mentioned in the text	80
3 Total weight, total counts per minute, background counts per minute, corrected counts per minute and adjusted counts per minute for red and white pine seedlings . . .	81





CHAPTER I

INTRODUCTION

Chemical weed control is a relatively new field in agriculture which has received much stimulus since the end of the second World War. The majority of the work done in this field has been in connection with agricultural food crops. Chemical control of weeds in woody plant nurseries has developed slowly because of complications arising from the wide variety of plants grown on a small acreage, differences in the age of plants and by the fact that these plants are perennials which may remain in the same location for several years. Because of varied growth habits and inherent differences in their physiological makeup, plants often react quite differently to the same herbicide, thus precluding one treatment over a very sizable area. In addition, the perennial nature of the plants necessitates application of herbicides on the same acreage in successive years. Repeated applications may not allow time for complete breakdown of the herbicides, resulting in build up of chemicals in the soil to levels toxic to nursery stock.

As with many other products, the demand for nursery stock has increased more rapidly than the techniques of production. Aroused public interest in conservation has tended to increase the use of forest species by state, federal, and private landowners in reclaiming submarginal agricultural



land. In addition, the trend toward suburban living has enlarged the demand for both forest and ornamental transplants. Thus number and size of nurseries has grown steadily over the years to meet the demand of the public. Since chemical weed control research in nursery management has been very limited, manual methods of weed control have continued as an expensive and laborious operation. Another facet of the problem which often goes unnoticed is the production and use of coniferous stock for forest plantations, when in some instances hardwoods would be more desirable. This has been brought on by the necessity for almost complete hand weeding of hardwood seedbeds, while some of the weed population in conifers can be taken care of with petroleum and mineral spirits. Poor survival of hardwoods in forest plantations, due largely to weed competition, has also restricted their use.

Holm (1958) sums up our stage of advancement in chemical weed control practices with the statement, "The great potential of modern methods of chemical weed control in nurseries awaits both intensive basic research and continued field testing."

The initial phase of work in this investigation was designed to compare the weed control capabilities of several soil fumigants and herbicides and the effect of these weed control agents on germinating coniferous seedlings. The most promising chemical, simazine, was then investigated in more detail. Simazine was first screened against a wide variety of tree seedlings at different rates and depths of placement.



Root elongation studies of several trees were also made to determine whether rate of root elongation had any effect on the differential tolerance of tree species to this chemical.

The final phase investigated, by use of C^{14} tracer techniques, the uptake and distribution of simazine in white pine which is relatively resistant to simazine and red pine which is easily killed by this chemical. Red and white pine seedlings, both with and without mycorrhizal inoculation, were used.



CHAPTER II

REVIEW OF LITERATURE

It has been estimated that losses to agricultural lands from weeds alone amount to almost four million dollars annually (Klingman, 1961). If losses to utility companies, highways, public recreation areas, and industrial sites were included, the figures would become even greater. The relative newness of chemical weed control is attested to by the fact that in 1955 there were only 125 federal, state, and industrial researchers and 8 state extension workers engaged in weed control work, whereas, in 1960 the Weed Society of America had over 500 active members and regional weed control conferences were meeting annually in four different sections of the United States. While some work was done in the early 1900's, the really big advance came with the advent of 2,4-D in 1941.

Early weed control practices in forest nurseries were largely confined to hand weeding, mulching, or mechanical cultivation where space was adequate. Poisonous gases and steam sterilization, where feasible, were also commonly used before 1950 for fumigation of seedbeds. Chemical control in coniferous seedbeds during this era, and to a great extent now, was accomplished with repeated applications of petroleum spirits and mineral spirits. Holm (1958) reports that organic chemicals were first tested for nursery weed control

in the Lake States Region in 1950. For example, trichloroacetic acid (TCA) was used as a pre-planting soil treatment for control of perennial grasses and subsequently 2,2-dichloropropionic acid (dalapon) and maleic hydrazide also proved effective for grass control. Sodium 2,4-dichlorophenoxyethyl sulfate (sesone) was probably the most commonly used herbicide until 1953 at which time the urea herbicides came into usage, both alone and in combination with sesone and dalapon. Recently, many of these different organic chemicals have been tested in new forest plantations with promising results (White, 1962).

Kozlowski (1960) reports that hand weeding costs in forest nurseries are as high as \$1,380.10 per acre while Taylorson and Holm (1958) cite a saving of \$450 per acre over hand weeding of coniferous transplants by use of 3-(p-chlorophenyl)-1, 1-dimethylurea (Monuron) and 2,4-dichlorophenoxyacetic acid (2,4-D). In work carried on by Havis (1961), 2-chloro-4, 6-bis(ethylamino)-s-triazine (simazine) applied during spring at 2 pounds per acre to lining-out beds with a repeat application in midsummer, required only one hand weeding during the season compared to six hand weedings for the check.

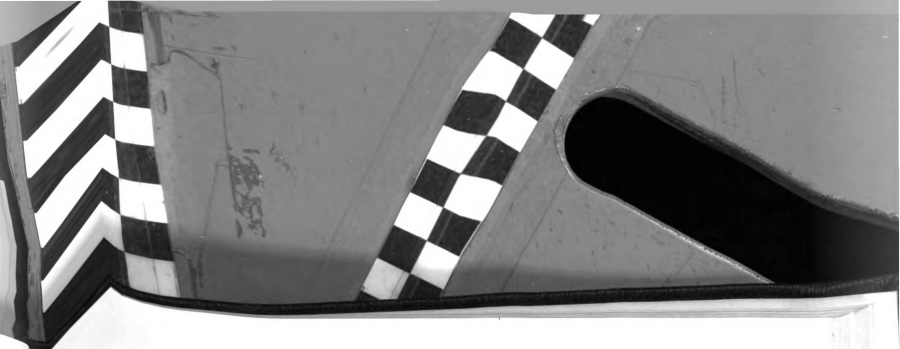
One of the newer groups of herbicides is the s-triazines. Considerable screening and some basic research has been done with simazine and atrazine, two of the more commonly used chemicals in this group. Their selectivity and long residual action are desirable traits which lend themselves

Well to the fields of nursery management and plantation establishment. Since much of the work to date has been done in fields other than forestry, it will be necessary to transgress into other agricultural fields in order to present a clear picture of all factors pertinent to their behavior.

Comparison of s-Triazines

Many investigators (Friesen, 1958), (Peters, 1957), (Taylorson and Holm, 1958), (Trevett and Burnham, 1957), (Vengris, 1957) have proclaimed the excellent weed control obtained with simazine when compared with other herbicides. This exceptional weed control is certainly due in part to the long residual action of this chemical (Switzer, 1958). Ries and Watson (1957) and Taylorson and Holm (1958) found that when simazine was applied at the rate of 4 pounds per acre to liming-out stock, weeds were satisfactorily controlled throughout the entire growing season. Noll (1960) and Talbert and Fletcher (1959), like most investigators, rate 2-chloro-4-ethylamino-o-isopropylamino-s-triazine (atrazine) equal to simazine for weed control. Schneider (1959) reports that deep rooted weeds, such as velvetleaf (Abutilon Theophrasti Medic.) are more sensitive to atrazine than simazine, probably because of the higher solubility of the former and for this same reason atrazine acts a little faster than simazine. On the other hand, simazine gives longer control of barnyard grass (Echinochloa crusgalli L.), several species of Panicum and crabgrass (Digitaria sanguinalis L.)





because of its lower solubility. Since atrazine does have value as a contact herbicide, it can be used on young weeds while simazine must be used as a preemergence treatment.

Lovely (1958) found granular formulations of simazine as effective as spray formulations. Fletchall and Schweiss (1959), however, harvested 350 pounds of weeds per acre from a granular application, compared to 50 pounds per acre from a liquid application. Unpublished work by the writer also shows superior weed control from a wettable formulation.

Probably due to limited testing and different weed populations, there is much disagreement among researchers as to the herbicidal activity of many of the other triazine derivatives. It is generally agreed that 2-chloro-4-diethylamino-6-ethylamino-s-triazine (trietazine), 2-chloro-4-diethylamino-6-isopropylamino-s-triazine (ipazine) and 2-chloro-4,6-bis (diethylamino)-s-triazine (chlorazine) are among the least active of the more common derivatives, yet Taibert and Fletchall (1959) achieved 91 percent weed control with ipazine and trietazine compared to 95 percent control with the same rate of simazine. Havis (1961) rates 2-chloro-4,6-bis (isopropylamino)-s-triazine (propazine) equal to simazine for weed control. 2-methoxy-4,6-bis(ethylamino)-s-triazine (simetone) is rated slightly less effective by Jansen et al. (1957), while 2-methoxy-4,6-bis(isopropylamino)-s-triazine (prometone) and 2-methoxy-4-ethylamino-6-isopropylamino-s-triazine (atratone) are rated by Noll (1960) as having an activity intermediate between simazine and chlorazine.

Just as the derivatives show a difference in their phytotoxic effect on weeds, they also show a difference in selectivity on crop plants. Many small fruits, tree fruits, ornamentals, and forest trees have shown a tolerance to simazine and atrazine depending on dosage used, time of application and other factors (Anonymous, 1960), (Anonymous, 1961). Larsen and Ries (1960) used simazine on young fruit trees at rates as high as 8 pounds per acre with no resultant injury. Grigsby (1958) has found that simazine is also a good algacide. At 5 p.p.m. simazine was lethal to mixtures of filamentous and unicellular green algae and this toxicity persisted for periods up to six months, whereas copper sulfate and sodium arsenite produced good initial kills but regrowth was found three to four weeks after treatment.

Use of Simazine and Atrazine on Woody Plants

A number of investigators have screened various woody species, both ornamental and forest, against simazine and atrazine when used alone and in combination with other chemicals. Ries et al. (1958) found simazine to be the best herbicide of the nine tested against four species of ornamental lining-out stock. A repeat application at 4 pounds per acre during the second growing season still caused no injury. Runge (1960) reported no injury when simazine and atrazine were applied to numerous species of established nursery stock. Taylorson and Holm (1958) report no injury to 2-year-old Douglas-fir (Pseudotsuga menziesii Mirb., Franco.). Scotch

Pine (Pinus sylvestris L.). Austrian pine (Pinus nigra Arnold). white spruce (Picea glauca Moench., Voss.) and blue spruce (Picea pungens Engelm.) when treated with simazine at 4 pounds per acre. In windbreak plantings with ten common coniferous species, Bagley and Myoshi (1959) found no apparent chemical injury from 4 pounds per acre simazine treatments with the exception of a sandy loam soil on which the survival of eastern redcedar (Juniperus virginiana L.) and red pine (Pinus resinosa Ait.) was lower than the controls. They indicate that high rainfall and shallow rooted planting stock may have been the reason for injury with the red pine. Trees in control plots were soon overtopped by weeds, vigor and growth were impaired and survival was generally lower. Holm et al. (1957) report slight injury to seedling transplants of Scotch pine with a treatment of 2 pounds per acre of simazine. In this case the spring weather was very wet. In work carried on by Ahrens (1961), the tolerance of hemlock transplants to simazine was increased greatly by delaying treatment for a longer period after transplanting. It was also noted that tolerance to this herbicide also increased with the age of the transplants. Winget et al. (1960) arrived at the same conclusion in connection with red pine.

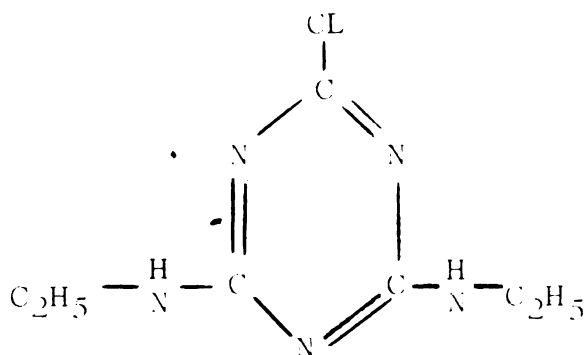
Moving from the nursery to the planting site, the competition is intensified between the transplants and the weeds for water and nutritive elements. Johnson (1960) states that as we continue to utilize the richer sites, weed problems seriously limit the early growth of tree seedlings. He cites as an example, the serious grass problems



which exist on the spruce planting sites in the Lake States. White (1960) has demonstrated on these spruce sites significantly higher survival with chemical weed control as compared with no weed control. According to White, "The effectiveness of the simazine weed control in increasing survival, growth and foliage color of spruce on the test sites commends the adoption of this technique into regular silvicultural practice." Kuntz et al. (1960) have had similar experiences of increased survival with outplantings of pines and Norway spruce (Picea abies [L.] Karst.). Hovind (1950) has shown survival percentages in 2-year-old red pine to be 50 percent with 1-1/2 pounds of simazine plus 7-1/2 pounds of dalapon per acre, 42 percent with 3 pounds of simazine per acre, as compared to 17 percent survival in the furrow with no chemical treatment and a complete loss in those plantings where no furrow or chemical was used. Jokela et al. (1961) have demonstrated increased growth of hardwood transplants by using simazine immediately after outplanting.

Chemical and Physical Properties of the s-Triazines

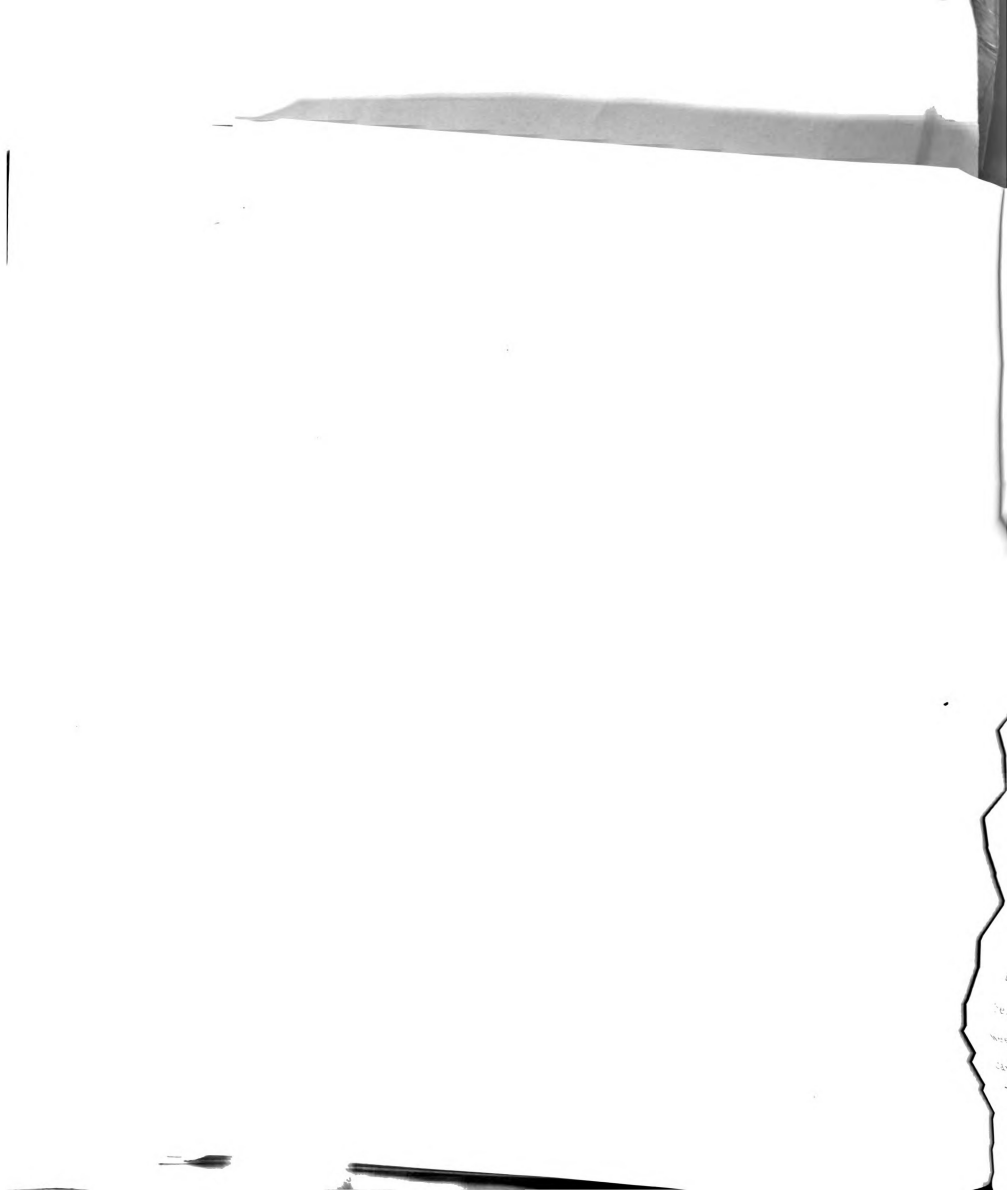
The triazines are heterocyclic nitrogen derivatives, meaning they have a ring structure composed of atoms of different kinds. If this ring is o-membered with two or more nitrogen atoms in the ring, it is known as an azine. The structural formula for simazine is:



Structurally, atrazine differs from simazine in that an NH C_3H_7 group is attached to the fourth carbon in the ring in place of the NH C_2H_5 in simazine. The technical material in both simazine and atrazine is a white crystalline substance with low solubility in water. Simazine at 5 p.p.m. has the lowest solubility of the better known s-triazines while simetone at 3200 p.p.m. has the highest. Solubilities of some of the other triazine compounds are propazine at 8.0 p.p.m., chlorazine - 10 p.p.m., trietazine - 20 p.p.m., iapzine - 40 p.p.m., atrazine - 70 p.p.m., 2-chloro-4-methylamino-o-isopropylamino-s-triazine (G-30020) - 200 p.p.m., prometone - 750 p.p.m., and atratone at 1800 p.p.m. (Richards, 1960). The decomposition of simazine occurs as a first order reaction, meaning that under comparable conditions the same percentage of the original rate will be found in the soil at a given time, regardless of whether the rate was a high or low one (Burschel, 1961).

Factors Affecting Herbicidal Action of Simazine

Some of the factors affecting herbicidal action of simazine are the amount of precipitation, fixation by clay



and organic matter, depth of placement, time of application, soil temperature, and to some extent the pH of the soil.

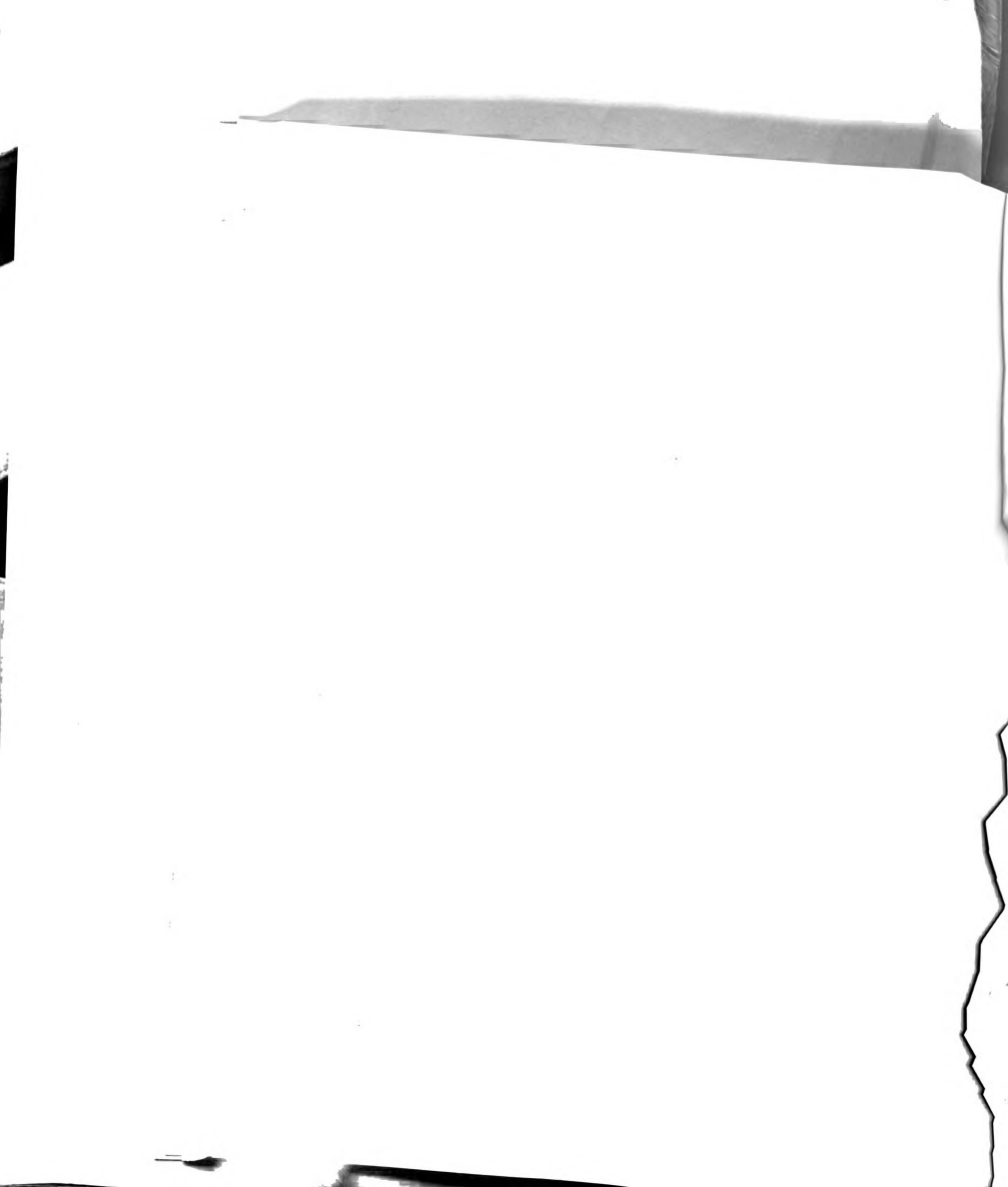
Burnside and Behrens (1961b) reported that increasing soil temperature, within limits, caused increased simazine toxicity to corn. In another paper, Burnside et al. (1961a) reported that high temperature and to a lesser extent low pH (pH 4) caused a significant deactivation of simazine in water suspensions. However, the very high temperature necessary, indicates this would not be a significant factor in the field.

Maximum results with simazine are usually obtained when this herbicide is applied during early spring before active growth begins. However, Chadwick (1958) obtained excellent weed control even after eight months from the time of application in November. Havis (1961) also secured excellent control of quackgrass (Agropyron repens L.) in established nursery stock from fall applications of simazine and atrazine at a rate of 5 pounds per acre. Lovely (1958) found that working granular simazine into the soil does not improve its effectiveness. Results of work carried on by Freeman¹ agree with this finding. When simazine in both granular and wettable form was worked into the soil and applied as a surface treatment, the surface treatment in wettable form gave the best weed control. Additional unpublished work regarding weed control as affected by depth of simazine placement, was carried on by the writer in 1958. Granular simazine was

¹Freeman, F. W., 1960. Unpublished data. Michigan State University.

placed at the rate of 4 pounds per acre at different depths in the soil mixture of one gallon tin cans. Four months after treatment, almost complete eradication of weeds was obtained from the surface application. The herbicide was of some value when placed at a depth of 1 inch, but when placed at a depth of 3 inches and below, no weed control was evident. This does not agree with the findings of Peddema (1958), who killed test weeds when simazine was placed from 4-12 inches below the soil surface.

Simazine leaches from the soil surface very slowly. Burschel (1961) found that even after application of 4 inches of water, 85-91 percent of the herbicide still remained in the upper inch of soil. Working with C¹⁴-labeled simazine and atrazine, Montgomery and Freed (1959) found maximum penetration of simazine at 7 inches and atrazine at 12 inches, after 10 pound per acre treatments of these herbicides were leached with 12 inches of water. However, maximum concentration of simazine was at the 0-1 inch level while maximum concentration of atrazine was at the 7-8 inch level. In unpublished work carried on by the writer, monthly bio-assay tests were made to determine the rate of leaching and residual action of simazine under field conditions. Soil samples were taken at monthly intervals from an outdoor nursery experiment in which simazine had been applied at 1, 2, and 4 pounds per acre. These samples were taken at the soil surface, at a depth of 1 inch and at a 2-inch depth. Cucumber (Cucumis sativus L.) seedlings were then germinated



in these soils and number of dead seedlings was recorded. All tests were replicated four times. Results showed that the 1 and 2 pound applications never moved below the 1 inch level and that at the end of four months all simazine had been broken down. In addition, the 4 pound treatment never eliminated all of the cucumber seedlings at the 2 inch depth during the four months that the bio-assays were being run. Findings of Burnside et al. (1961a) and Derscheid (1958) also attest to the slow movement of simazine through the soil profile.

Simazine at 5 and 10 pound per acre rates has remained in some soils for two growing seasons at levels toxic to germinating seedlings (Sweet et al. (1958). Low solubility and consequent slow leaching certainly contribute to the long residual action of this chemical. On the other hand, simazine and atrazine have been shown to lose their toxicity at rates of 2 pounds per acre within eight weeks, under conditions of high moisture and summer temperature (Switzer and Rauser, 1960). Stroube and Bondarenko (1960) found through oat bio-assays that twelve months after application of 4 pounds of simazine per acre, the simazine equivalent in the 0-3 inch level was 1/3 pound, 1/8 pound in the 3-6 inch level, and none in the 6-9 inch layer.

Deactivation of Simazine by Soil Microorganisms

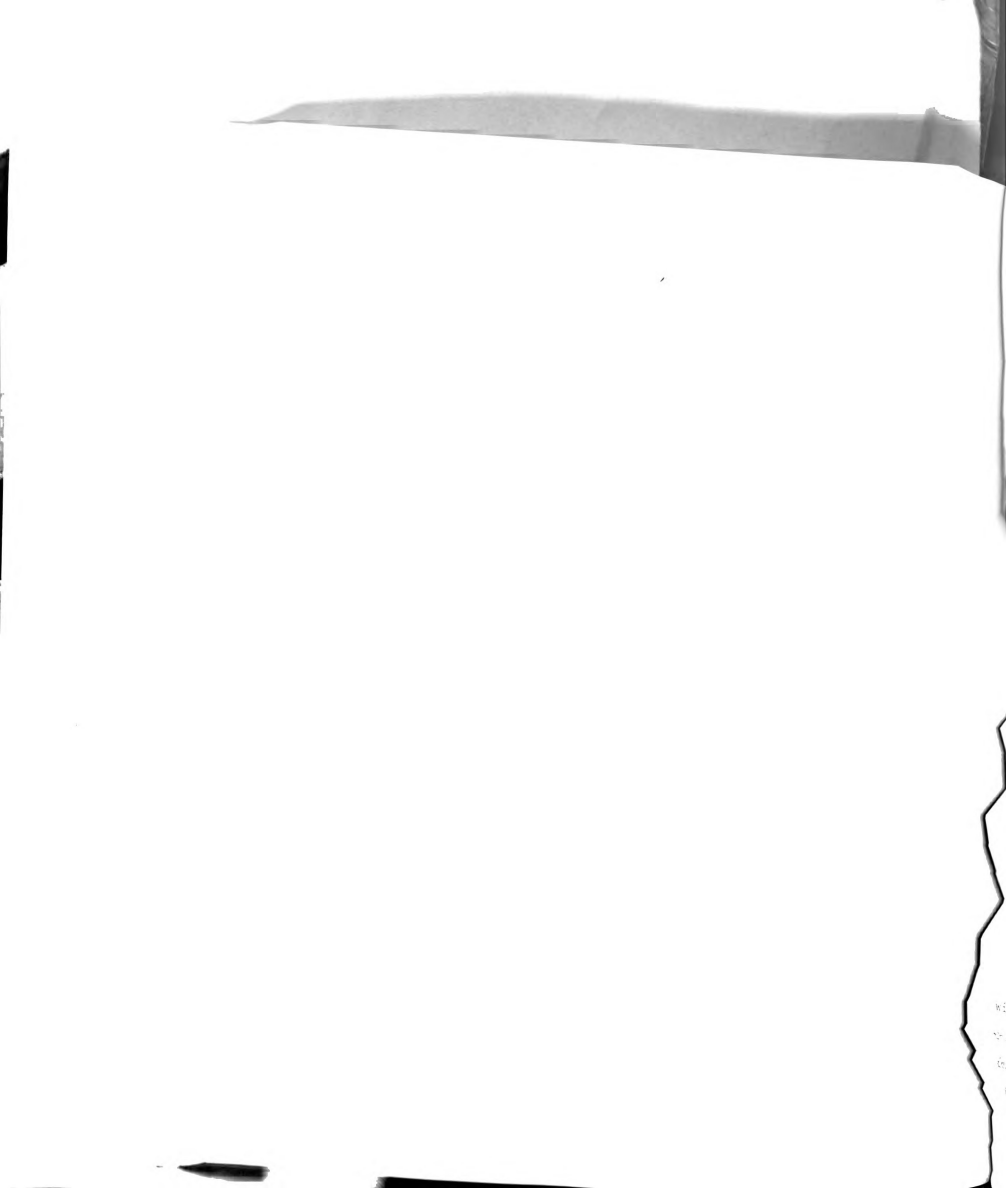
As mentioned earlier, soils high in organic matter and/or clay content caused reduced simazine phytotoxicity as



compared to soils low in these components (Burnside and Behrens, 1961b). Sheets and Danielson (1960) investigated in detail the effect of soil organic matter, clay content, cation-exchange capacity and pH on the phytotoxicity of simazine. Soil organic matter appeared to alter the initial toxicity of simazine most. They also found that in contrast to 2,4-D, repeat applications of the s-triazines are inactivated in the soil at about the same rate. Apparently the soil microorganisms capable of inactivating 2,4-D increase with repeated applications of this herbicide while those microorganisms which inactivate the s-triazines are passive in action, i.e., organisms utilize them but not selectively or preferentially. Burschel (1961) states that the decomposition of simazine in the soil is closely related to the amount of humus present. In fact, without humus, no decomposition will occur. Pure loess soil which contained no humus was analyzed for simazine 3-1/2 months after being treated with 2 and 4 p.p.m. and found to contain all of the original treatment. He found that the greater the amount of humus, and thus microorganisms, the more rapidly simazine is deactivated in the soil. Further study showed that lowering the temperature from 25°C. to 8.5°C. caused a sevenfold decrease in the rate of decomposition. Burschel attributes this decrease to the fact that microorganisms are also affected by lowering of temperature. This would indicate that fall applications of this herbicide will react much differently from those made in spring. Burnside et al. (1961a) found

that soil microorganisms deactivated simazine, but very slowly. Most of the deactivation occurred after the fourth month. Five microorganisms that were able to subsist for three months in media containing simazine as the sole source of nitrogen and nearly sole source of carbon were isolated and identified as Penicillium pupurogenum, Aspergillus ustus, and three Streptomyces species (Actinomycetes). However, these microorganisms did not deactivate simazine in solution cultures during a 30 day incubation period. According to Reid (1960), a group of soil bacteria, the soil diptheroids (Corynebacteriaceae) have been found to remove the s-triazines from the soil. Observations by Guillemat *et al.* (1960) emphasize the existence of fungi capable of metabolizing simazine and utilizing the nitrogen of this herbicide. It appears, in addition, that this degradation is tied to the abundance of carbon in the medium. The fungi listed as responsible for this degradation were Fusarium oxysporum, Fusarium avenaceum, Penicillium cyclopucin, Penicillium lanoseo-coeruleum, Cylindrocarbon radicolica, and Stachybotrys species.

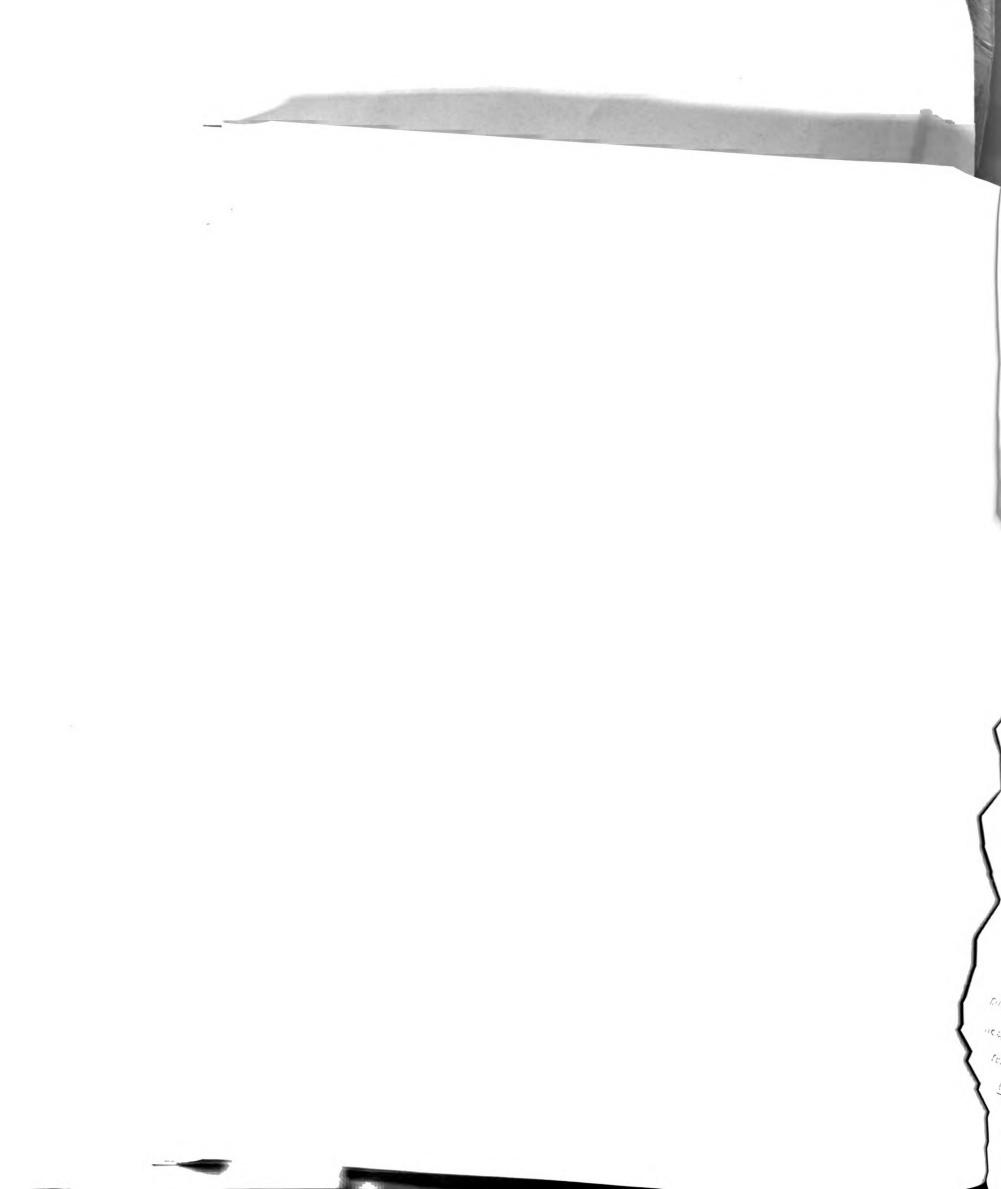
In contrast to deactivation of simazine by telluric microorganisms, Castelfranco and co-workers (1961a) have found that calcium polysulfide, a pesticide and soil corrective, also has the property for decomposing simazine. Because of its safety and relatively low cost, they feel that it could be used to hasten the breakdown of s-triazines in soil.



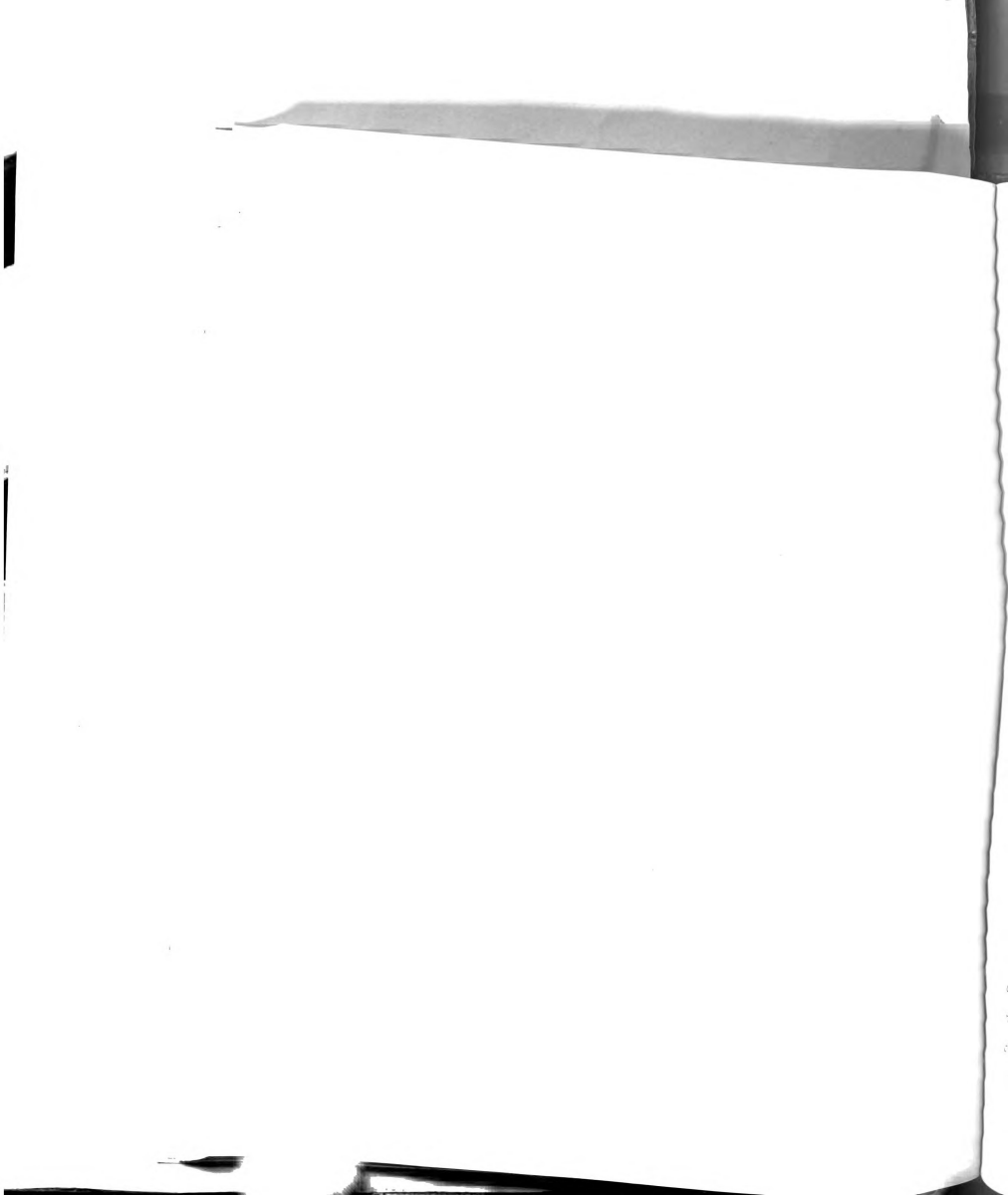
It has been generally established that simazine has no deleterious effect on the soil microflora, even when applied at extremely heavy dosages. Burnside et al. (1961a) measured carbon dioxide evolution and found it unchanged 30 days after simazine was applied at rates up to 4000 p.p.m. Nitrate formation was not impaired even by larger applications of this herbicide. After applying 300 kilograms of simazine per hectare, Guillemat et al. (1960) observed only insignificant changes to the microorganisms. On the other hand, Chandra et al. (1960) observed depressed carbon dioxide evolution in simazine-treated soils, the percent decrease generally being greatest at 28 days and decreasing thereafter.

Physiology of the s-Triazines in the Plant

Typical symptom of simazine toxicity is the chlorosis which starts at the leaf tip and progresses along the margins to the base of the leaf. Necrosis occurs in the chlorotic area and chlorosis spreads to the entire leaf. These symptoms in the plant are a result of blockage of the Hill reaction, or the ability of chloroplasts to break down water into hydrogen and oxygen in the presence of light and iron (Schneider, 1958). Ashton et al. (1960) have demonstrated with excised leaves of kidney bean (Phaseolus vulgaris L.) that the degree of inhibition of carbon dioxide fixation increases with higher concentrations of the herbicide and longer exposure time. Roth (1958) has also shown with



Elo leaves that assimilation in simazine solutions is inhibited. Work by Moreland et al. (1950) showed that glucose supplied to barley plants through severed leaf tips kept plants alive and growing in the presence of lethal concentrations of simazine for more than two months. Experiments by Gast (1958) confirm these findings. The chloroplasts of starch free Coleus Blue leaves kept on a solution containing sugar and simazine were able to form starch again. Moreland found that simazine appeared to have a similar effect on the activity of both barley and corn chloroplasts even though barley is quite susceptible to this herbicide while corn is very tolerant. Therefore, he concluded that the mechanisms which control selectivity must act before the herbicide reaches the chloroplasts. Montgomery and Freed (1961) and many other investigators have shown that the corn plant is able to degrade the s-triazines and readily metabolize these compounds. Since heating of corn sap destroys its ability to decompose simazine, Roth (1957) suggests that the resistance of corn to simazine is due to a thermolabile system, perhaps fermentative, which can transform this substance into compounds devoid of biologic action. Castelfranco and co-workers (1961b) state that the action of simazine toward different species of grasses suggests that non-enzymic detoxification reactions catalyzed by small organic molecules may play a major role in determining the resistance or susceptibility of a particular species. Davis et al. (1950) have demonstrated through the radioactivity present in leaf samples that simazine is absorbed readily



by the roots of both corn (Zea Mays L.) and cucumber (Cucumis sativus L.). However, radioactivity was observed much sooner in cucumber than in corn and simazine or the C^{14} -labeled degradation products moved more readily in cucumber than in corn. In addition, Ragab and McCollum (1961) have proved that both resistant and susceptible plants decompose simazine. The fact that cucumber metabolized simazine at a more rapid rate than corn dispels the suggestion that toxicity is associated with inability to metabolize the herbicide. They also found that cucumber plants growing in nutrient moistened glass produced adventitious roots on the stems, indicating that the herbicide is toxic to the roots. Foy (1961) has found that some of the triazine compounds accumulate in the lysigenous glands of cotton (Gossypium). Since substances deposited in these glands are apparently removed from circulation at least temporarily, he postulated that such accumulation may constitute a protective mechanism against these herbicides in normal glanded cotton. Studies with genetically glandless varieties have thus far supported this hypothesis. Another important factor which has been discovered by Rotn (1961) is that in certain cases corn absorbs relatively less simazine than other plants.

From these investigations, it becomes evident that different resistant plants have different mechanisms for neutralizing or minimizing the toxic effects of the triazine compounds.

CHAPTER III

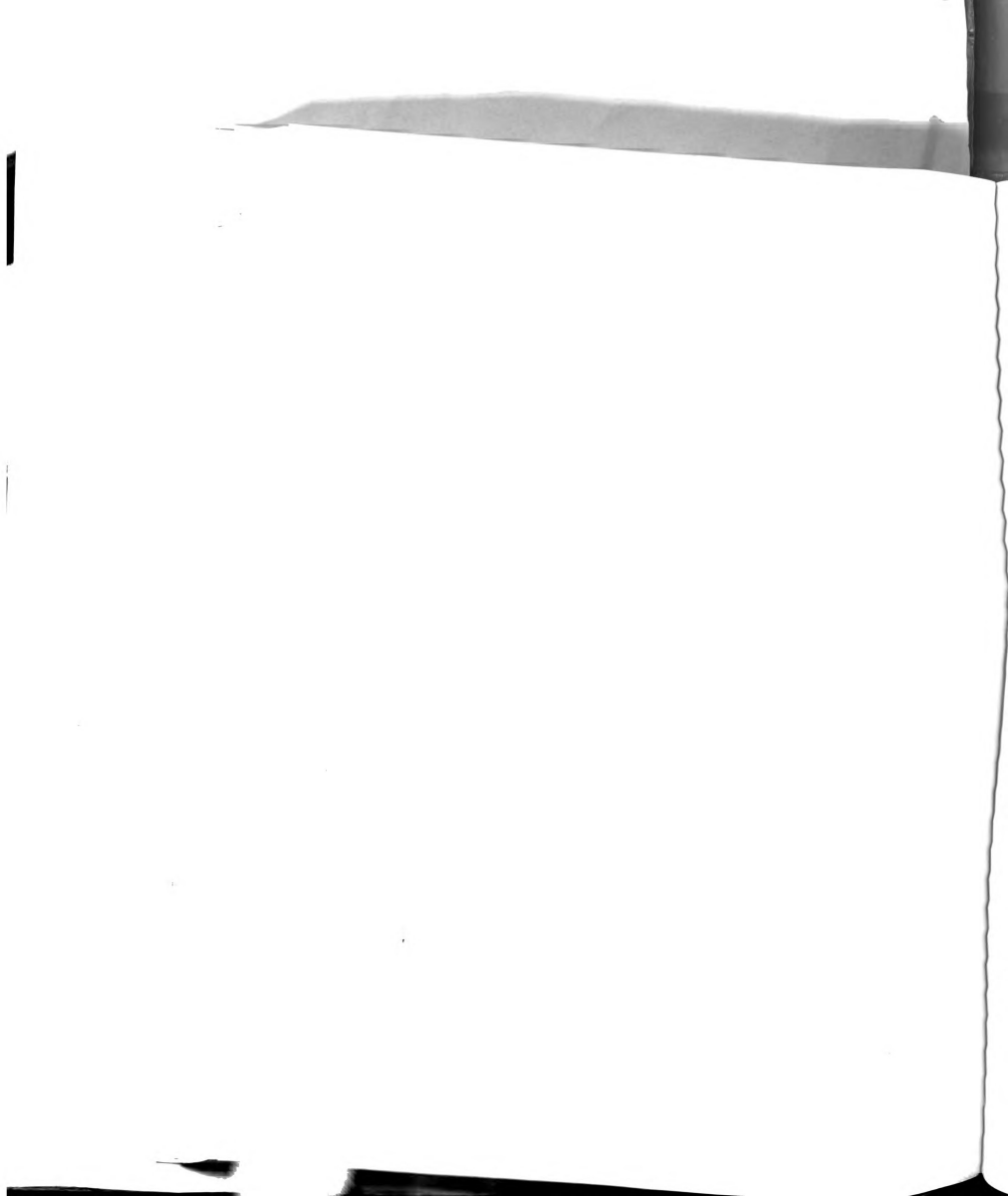
METHODS AND PROCEDURE

The excellent, long-lasting weed control obtained with simazine and atrazine in preliminary investigations, prompted a more detailed investigation of these herbicides for use in nursery seedbeds. It was decided that the logical starting point would be a comparison of these s-triazines under field conditions with other recently developed herbicides and soil fumigants in addition to those in common usage. As an adjunct to the field work, a greenhouse experiment was set up to determine the reaction and tolerance of a wide range of germinating tree species to different rates of simazine applied both as a surface treatment and intermixed with the upper layer of soil.

The differential tolerance exhibited by the tree species in the above mentioned experiments, in turn led to the third phase of work. This study investigated by use of C^{14} -labeled simazine, the uptake of C^{14} by red and white pine seedlings, and, in addition, the effect of inoculation with mycorrhizal fungi on this uptake.

Comparison of Several Soil Fumigants and Herbicides in Nursery Seedbeds

This portion of the study was set up in the Bogue Forest Nursery (Figure 1) located on the campus of Michigan State



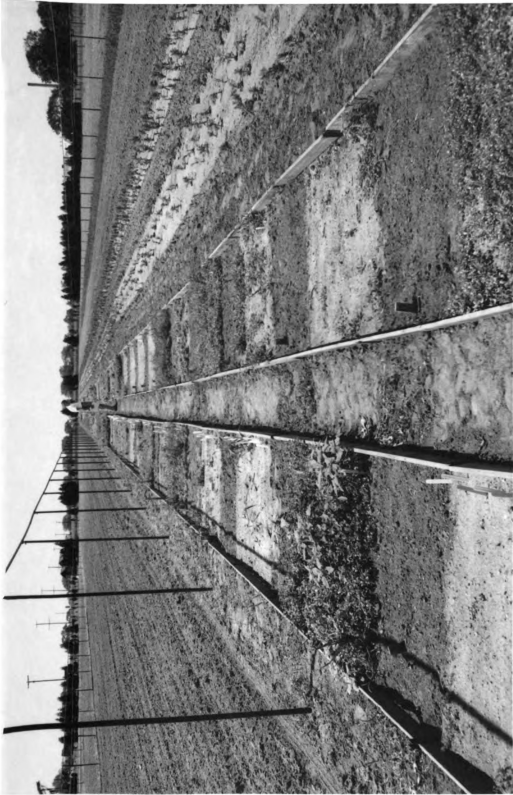


Figure 1. General view of Bogue Forest Nursery showing location of study on soil fumigants and herbicides in nursery seedbeds. Note the weed free condition of the two simazine treated spruce transplant beds which lay parallel to the seedbeds.

Table 1. Weed control treatments used in nursery seedbeds.

Treat. No.	Treatment	Rate/acre (active)	Days applied before or after seeding
1	Control	--	--
2	Handweed	--	As needed
3	Stoddard solvent	25 gal.	6 times during season
4	Methyl bromide	870 lbs.	14 days before
5	EPTC	4 lb.	At seeding
6	DMTT	280 lb.	25 days before
7	SMDC	100 gal.	25 days before
8	Chloropicrin	35 gal.	14 days before
9	Ureaformaldehyde 85	127 gal.	20 days before
10	Ureaformaldehyde 85 plus allyl alcohol plus ethylene dibromide	72 gal. 50 gal. 8.4 gal.	20 days before
11	Ureaformaldehyde 85 plus simazine	127 gal. 4 lb. W	20 days before
12	DNBP	7 lb.	At seeding
13	Simazine	2 lb. W	At seeding
14	Simazine	4 lb. W	At seeding
15	Simazine	8 lb. W	At seeding
16	Simazine	2 lb. G	At seeding
17	Simazine	4 lb. G	At seeding
18	Simazine	8 lb. G	At seeding
19	Atrazine	4 lb. G	At seeding
20	Atrazine	8 lb. G	At seeding

University. The soil type is Hillsdale sandy loam.

Twenty treatments were used, consisting of soil fumigants and herbicides tested both alone and in combination (Table 1). Each treatment was sown to 10 coniferous species. Treatment plots measured 4 feet by 6 feet and each plot was separated by a 2-foot isolation strip. The tree seed was sown in rows 4 feet long and 7 inches apart. All treatments were randomized within the block and replicated six times.

The seedbeds were prepared quite early during the spring in order to allow a safe waiting period between application of soil fumigants and sowing of the seeds. Table 2 lists the 10 coniferous species sown to each treatment and the approximate number of seeds sown in each row. The tree seeds were sown on May 17 and seedbeds were covered with slat shading during germination and early growth of the seedlings. Water was applied as needed during the growing season by an overhead sprinkling system.

Table 2. Tree species used in nursery weed control experiment.

Common Name	Scientific Name	Approx. No. of seeds sown per row
Balsam fir	<u>Abies balsamea</u> (L.) Mill.	370
White fir	<u>Abies concolor</u> (Gord.) Engelm.	137
European larch	<u>Larix decidua</u> Mill.	600
Norway spruce	<u>Picea abies</u> (L.) Karst.	420
White spruce	<u>Picea glauca</u> (Moench.) Voss.	600
Jack pine	<u>Pinus banksiana</u> Lamb.	600
Red pine	<u>Pinus resinosa</u> Ait.	302
White pine	<u>Pinus strobus</u> L.	405
Scotch pine	<u>Pinus sylvestris</u> L.	340
Douglas-fir	<u>Pseudotsuga menziesii</u> (Mirb.) Franco.	330

Weed Control Ratings

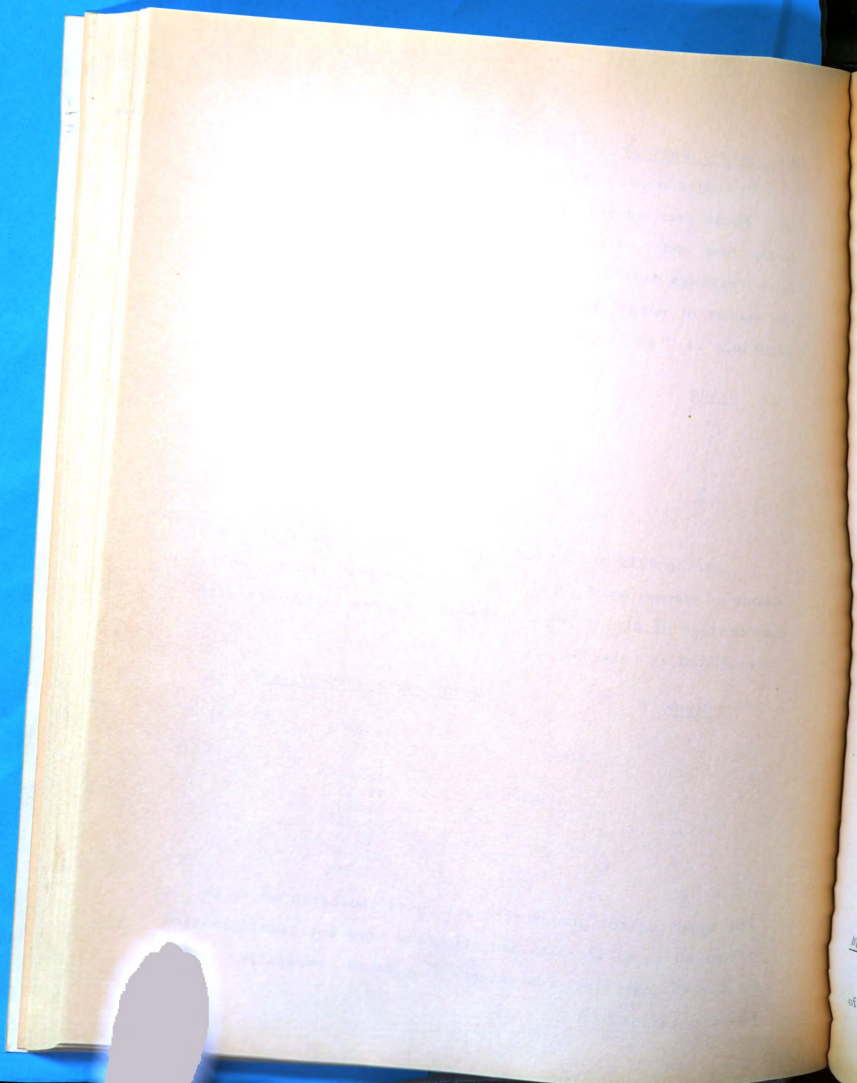
Periodic weed counts were made during the growing season. These weed control ratings were made at weekly intervals during June and semi-monthly during July and August. The weekly ratings were divided into seven categories based on the number of weeds present in the area between two rows of seedlings (1.0 sq. ft.). The seven categories are as follows:

<u>Grade</u>	<u>Weeds per 1.0 square feet</u>
0	0
1	1-3
2	4-8
3	9-15
4	16-50
5	51-100
6	100 plus

During July and August it became more convenient, because of denser weed populations, to rate weed control on the Percentage of plot covered by weeds. Eleven categories were designated as follows:

<u>Grade</u>	<u>Weed coverage in percent</u>
0	0
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

The weed control grades were set up as indicated above in order to normalize data and eliminate need for transformation prior to analysis. Convenience in ease of computation was also a factor.



Tree Seedling Injury Ratings

The coniferous seedlings were checked periodically to determine the effect of the soil fumigants and herbicides on their germination and survival. Injury ratings were determined by recording the number of live seedlings per species. The ratings were divided into six categories as follows:

<u>Grade</u>	<u>No. live seedlings per row</u>
0	0
1	1-10
2	11-30
3	31-100
4	101-200
5	200 plus

As in the weed control ratings, grades were defined for convenience of computation and to normalize data and thus eliminate need for transformation prior to analysis.

Statistical Analysis

Each set of weed coverage or injury data was subjected to analysis of variance, using plot means as items. For each analysis the degrees of freedom were as shown below:

<u>Source of Variation</u>	<u>Degrees of freedom</u>
Treatment	19
Block	5
Error	95
Total	119

Results and Discussion

Weed Control

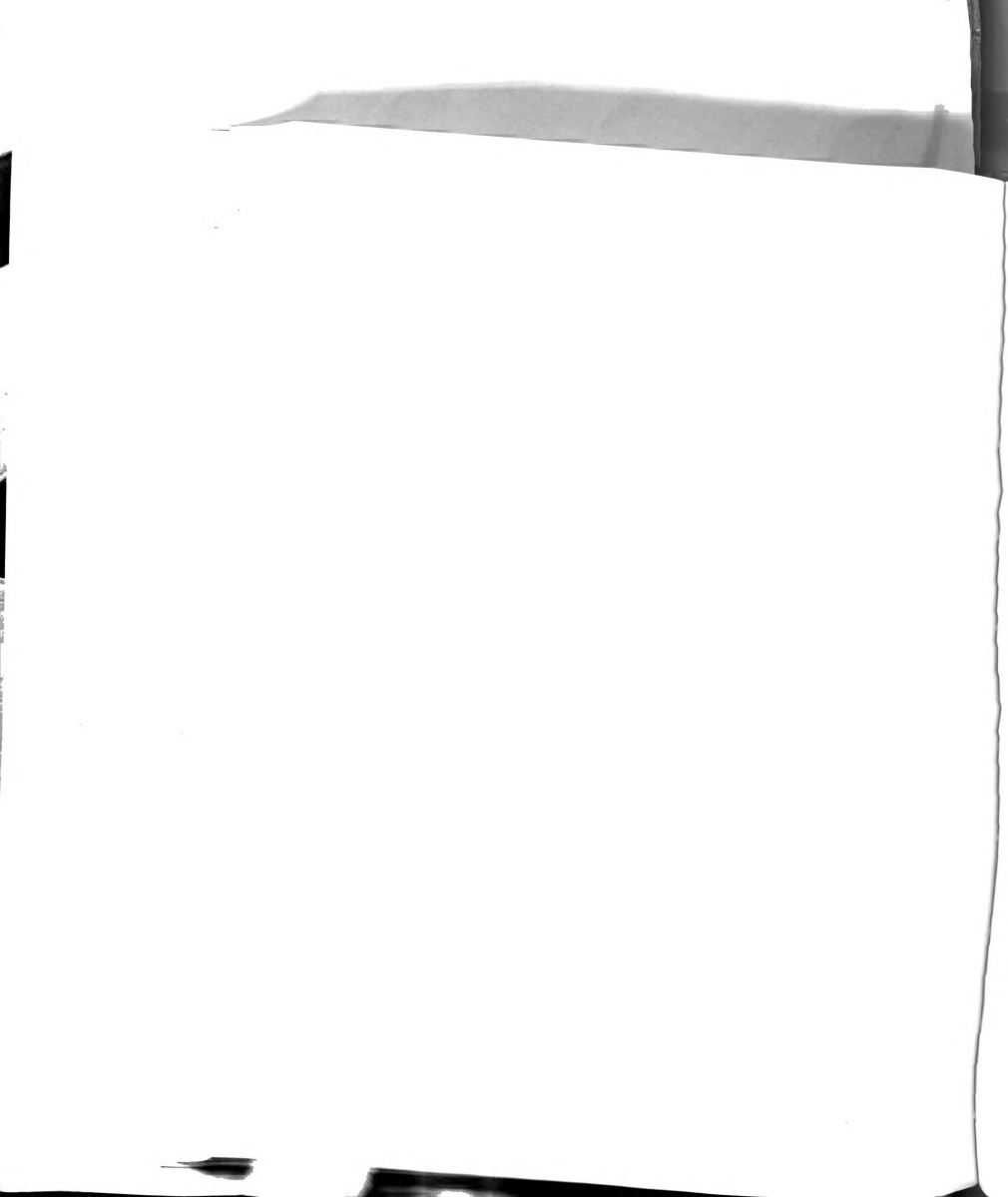
Weed species found on control plots during the course of the growing season were: Agropyron repens (L.) Beauv.

(quack grass). Amaranthus retroflexus L. (redroot pigweed). Ambrosia artemisiifolia L. (common ragweed). Capsella Bursa-pastoris (L.) Medic. (shepherd's purse). Chenopodium sp. (lamb's quarters). Digitaria sanguinalis (L.) Scop. (hairy crabgrass). Lamium amplexicaule L. (henbit). Oxalis europaea Jord. (European woodsorrel). Polygonum persicaria L. (spotted knotweed). Portulaca oleraceae L. (common purslane). and Stellaria media L. (common chickweed).

Although weed control ratings were made many times during the growing season, the results of three equally spaced ratings present a clear picture of the findings. Figure 2 gives a graphic presentation of weed control at mid-June, mid-July, and mid-August. After the middle of August all treatments had ceased to give effective control and no further weed ratings were made. Tables 3, 4, and 5 show the corresponding analysis of variance data for these three periods presented graphically in Figure 2.

The wide range of weed control obtained by the various treatments is shown in Figure 3. At six weeks after treatment most plots containing either simazine or atrazine were practically weed free. In direct contrast, the weeds in the ureaformaldehyde plots were much taller and healthier looking than those growing in the control plots.

In this experiment no appreciable weed control was obtained from Stoddard solvent, ureaformaldehyde, 3,5-dimethyltetrahydro 1,3,5 2H thiadiazine-2-thione (DMTT), sodium methyl dithiocarbamate (SMDC), trichloronitromethane (chloropicrin), ethyl N, N-di-n propylthiolcarbamate (EPTC)



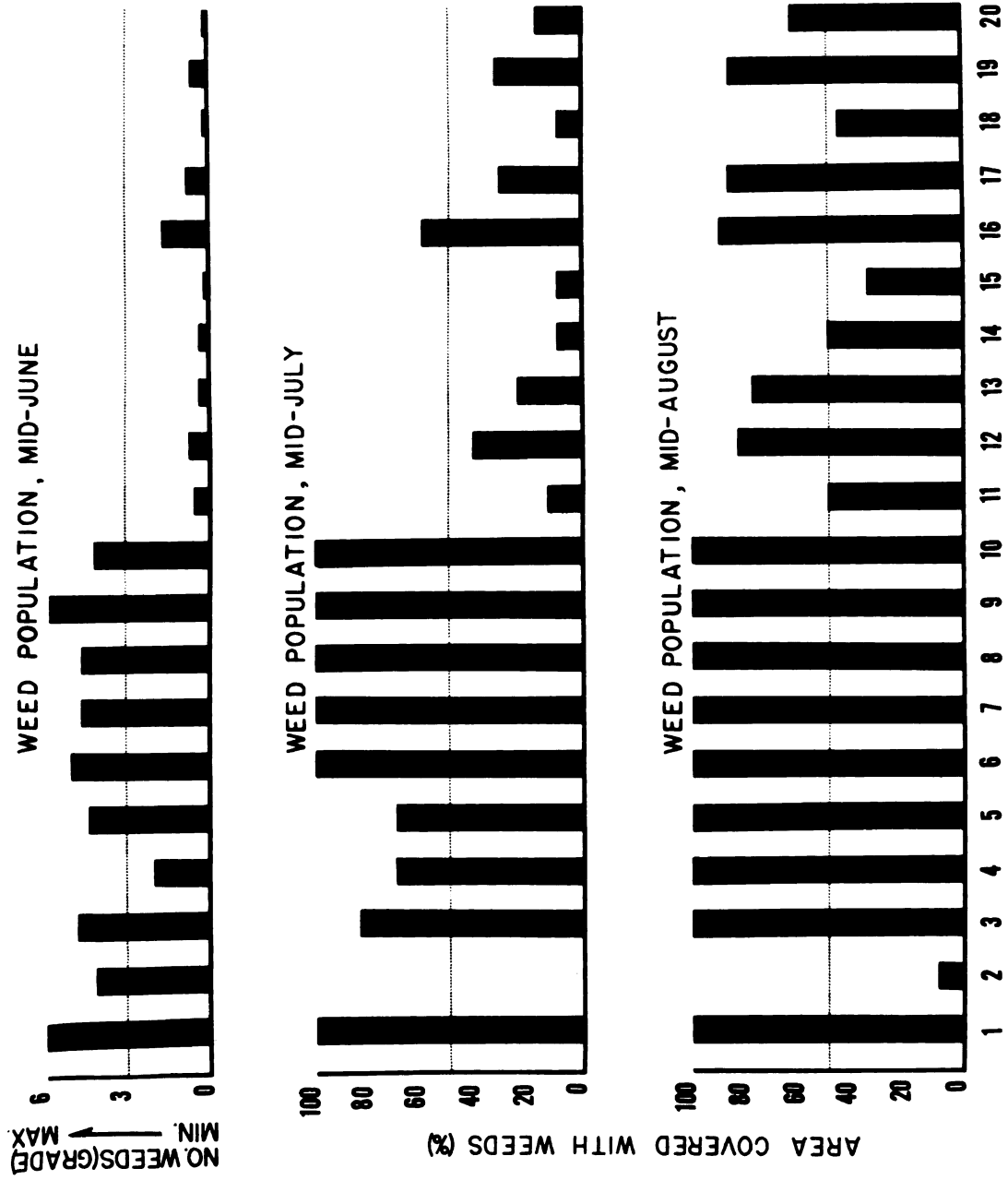


Figure 2. Weed population in nursery seedbeds.

Table 3. Mid-June weed control.

Treat. No.	Treatment	Rate/acre (active)	Mean weed population ¹	Statistical significance ²	
				5% level	1% level
1	Control	--	0.00		
9	Ureaformaldehyde	127 gal.	5.83		
6	D&T	280 lb.	5.00		
3	Stoddard solvent	25 gal.	4.83		
7	SMDC	100 gal.	4.00		
8	Chloropicrin	35 gal.	4.00		
5	EPTC	4 lb.	4.33		
2	Handweed ³	--	4.10		
10	Ureaformaldehyde plus allyl alcohol	72 gal.	4.10		
	plus ethylene dibromide	50 gal.			
4	Methyl bromide	8.4 gal.			
16	Simazine	870 lb.	2.00		
17	Simazine	2 lb. G	1.00		
12	DNBP	4 lb. G	.83		
10	Atrazine	7 lb.	.00		
11	Ureaformaldehyde plus simazine	4 lb. G	.00		
13	Simazine	127 gal.	.50		
14	Simazine	4 lb. W	.33		
15	Simazine	2 lb. W	.33		
18	Simazine	4 lb. W	.10		
20	Simazine	8 lb. W	.10		
	Atrazine	8 lb. G	.10		

¹Ratings based on number of weeds per 1.0 square feet. 0(0 weeds). 1(1-3 weeds). 2(4-8 weeds). 3(9-15 weeds). 4(16-50 weeds). 5(51-100 weeds). 6(100 plus weeds).

²All treatments not connected by a line are significantly different, as determined by Duncan's multiple range test.

³The relatively low position of handweed reflects rapid weed growth.

Table 4. Mid-July weed control.

Treat. No.	Treatment	Rate/acre (active)	Mean weed population ¹	Statistical, significance—	
				5% level	1% level
1	Control	--	10.00		
6	DMTT	280 lb.	10.00		
7	SMDC	100 gal.	10.00		
8	Chloropicrin	35 gal.	10.00		
9	Ureaformaldehyde	127 gal.	10.00		
10	Ureaformaldehyde plus allyl alcohol	72 gal.	10.00		
	plus ethylene dibromide	50 gal.			
3	Stoddard solvent	8.4 gal.	8.33		
4	Methyl bromide	25 gal.	7.00		
5	EPTC	870 lb.	7.00		
16	Simazine	4 lb. G	6.00		
12	DNBP	2 lb. G	4.16		
17	Simazine	7 lb. G	3.66		
10	Atrazine	4 lb. G	3.33		
13	Simazine	4 lb. G	2.50		
20	Atrazine	2 lb. W	1.83		
11	Ureaformaldehyde plus simazine	8 lb. G			
		127 gal.			
14	Simazine	4 lb. W	1.33		
15	Simazine	4 lb. W	1.00		
18	Simazine	8 lb. W	1.00		
2	Simazine	8 lb. G	1.00		
	Handweed	--	0.00		

¹ Ratings based on percent of plot covered by weeds: 0 equals 0% weed coverage and 10 equals 100% weed coverage.

² All treatments not connected by a line are significantly different, as determined by Duncan's multiple range test.

Table 5. Mid-August weed control.

Treat. No.	Treatment	Rate/acre (active)	Mean weed population ¹	Statistical significance ²	
				5% Level	1% level
1	Control	---	10.00		
6	DMT	286 lb.	10.00		
7	SMDC	109 gal.	10.00		
8	Chloropicrin	35 gal.	10.00		
9	Ureaformaldehyde	127 gal.	10.00		
10	Ureaformaldehyde plus allyl alcohol	72 gal.	10.00		
	plus ethylene dibromide	56 gal.			
3	Stoddard solvent	8.4 gal.	0.83		
4	Methyl bromide	25 gal.	0.83		
5	EPTC	870 lb.	0.83		
16	Simazine	4 lb. G	0.83		
17	Simazine	2 lb. G	9.00		
18	Simazine	4 lb. G	8.00		
19	Atrazine	4 lb. G	8.00		
12	DNBP	7 lb. W	8.33		
13	Simazine	2 lb. W	7.83		
20	Atrazine	8 lb. G	6.33		
11	Ureaformaldehyde plus simazine	127 gal.			
14	Simazine	4 lb. W	5.00		
18	Simazine	4 lb. W	5.00		
15	Simazine	8 lb. G	4.00		
	Simazine	8 lb. W	3.50		
2	Handweed	--	1.00		

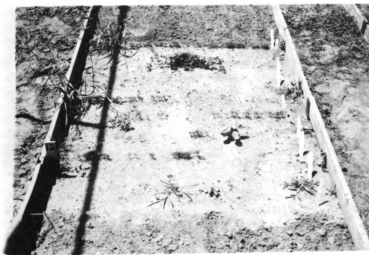
¹Ratings based on percent of plot covered by weeds: 0 equals 0% weed coverage and 10 equals 100% weed coverage.

²All treatments not connected by a line are significantly different, as determined by Duncan's multiple range test.



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Right: A 2 pound rate
of wettable simazine
(lower left in seedbed)
gives almost complete
weed eradication.



Left: Premerge (lower
left in seedbed) gives
adequate early season
weed control with less
damage to tree seedlings
than the simazine treat-
ment pictured above.

Right: Ureaformaldehyde
(center of seedbed)
stimulated weed growth.
Weeds in these plots
were more luxuriant
than those in the
control.

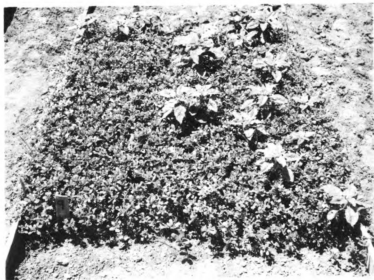


Figure 3. Extreme in weed control at six weeks after treatment.



and the combination treatment of ureaformaldehyde plus allyl alcohol and ethylene dibromide. While all of the fore-mentioned treatments, with the exception of ureaformaldehyde, were significantly different from the control at mid-June, none of them could still be considered effective. Lack of any noticeable control by the usually effective Stoddard solvent is possibly due to type of applicator used (fly sprayer) and also to poor timing.

All other treatments gave satisfactory weed control until July 1, at which time methyl bromide and the 2-pound granular simazine applications were no longer giving adequate control. By mid-July 4,6-dinitro-2-sec butylphenol, amine salt (DNBP), simazine (4# G), and atrazine (4# G) were beginning to lose their potency, although fair control was still being obtained. All of the simazine treatments applied as a wettable powder and the 8-pound granular applications of simazine and atrazine kept the plots sufficiently clean until early August.

Simazine at the 8 pound rate gave the best weed control over the longest period, followed closely by the 4 pound rate applied as wettable powder. Two pounds of wettable simazine gave equal or better control than 4 pounds of granular atrazine. Likewise, 4 pounds of wettable simazine gave equal or better control than 8 pounds of granular atrazine. This is due in part to the better coverage obtained with wettable formulations. The lower solubility of simazine (5 p.p.m.) as compared to atrazine (70 p.p.m.) is likely the

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More important factor, however, since lower solubility of simazine keeps it in the strata of weed germination for a longer period of time. As a whole, none of the s-triazines gave the long-lasting control that has been obtained in previous tests.¹ Above normal rainfall during the testing period may account for the shorter residual activity of these herbicides.

Seedling Injury

Increased tree mortality was usually directly correlated with the effectiveness of the various weed control chemicals used in these tests, although DNBP did not follow this pattern. Table 6 shows data on survival of the seven coniferous species tested under the 20 chemical treatments. No results were obtained for European larch, white fir, and balsam fir because of poor germination.

Stoddard solvent, DMTT, SMDC, and the combination treatment of ureaformaldehyde plus allyl alcohol and ethylene dibromide caused no injury to any of the tree seedlings tested. On the other hand, these same treatments gave little or no weed control. Chloropicrin, another ineffective weeding agent in this experiment, caused significant damage to jack pine, red pine, white pine, and white spruce. Ureaformaldehyde which actually stimulated weed growth, damaged Scotch pine and red pine.

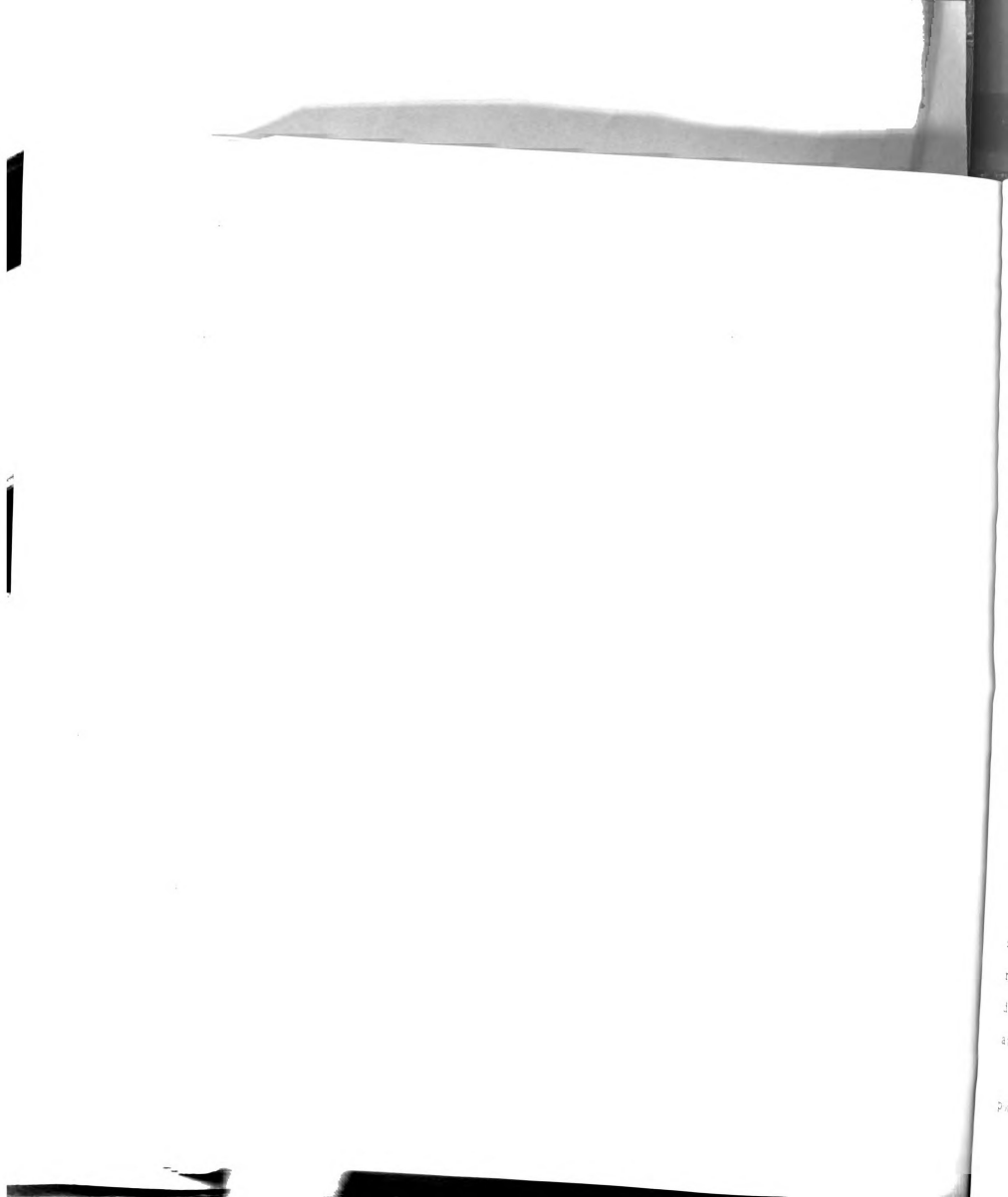
The only treatment which reduced germination was EPTC. Germination of all tree seedlings was reduced significantly

¹Freeman, F. W., 1959, Unpublished data, Michigan State University.

Table 6. Survival index of tree seedlings in the nursery weed control experiment. 1

Treat. No.	Treatment	Tree Species						
		Jack pine	Scotch pine	Red pine	White pine	Norway spruce	White spruce	Douglas- fir
1	Control	2.50	3.33	1.00	2.83	3.10	3.50	4.00
2	Handweed	3.10	3.10	2.00	2.83	3.10	3.10	3.00
3	Stoddard solvent	2.33	2.50	2.50	2.00	3.50	2.83	3.50
4	Methyl bromide	0.83	1.00	1.50	2.00	1.00	2.10	3.33
5	EPIC	1.10	1.33	1.10	1.50	2.33	1.00	0.83
6	DMTT	3.50	3.33	3.10	2.50	3.33	3.50	4.33
7	SMDC	2.50	3.00	2.83	3.00	3.00	3.50	4.00
8	Chloropicrin	2.00	2.50	1.00	2.00	2.00	2.10	3.83
9	Ureaformaldehyde	3.00	2.10	1.83	2.00	2.00	2.00	3.83
10	Ureaformaldehyde plus allyl alcohol	4.00	3.50	2.00	2.00	4.10	3.00	5.00
11	Ureaformaldehyde plus ethylene dibromide	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	Ureaformaldehyde plus simazine (4# W)	0.33	2.00	2.10	2.00	2.33	1.83	3.00
13	DNBP	0.50	0.10	0.00	1.00	1.00	0.00	1.00
14	Simazine (2# W)	0.00	0.00	0.00	0.10	0.33	0.00	0.50
15	Simazine (4# W)	0.00	0.00	0.00	0.00	0.00	0.00	0.10
16	Simazine (8# W)	0.00	0.00	0.00	0.00	0.00	0.00	0.10
17	Simazine (2# G)	0.00	0.10	0.00	1.83	1.50	0.50	2.33
18	Simazine (4# G)	0.00	0.33	0.10	1.10	0.00	0.00	1.00
19	Simazine (8# G)	0.00	0.00	0.00	0.50	0.00	0.00	0.33
20	Atrazine (4# G)	0.00	0.10	0.33	1.00	0.00	0.00	0.00
20	Atrazine (8# G)	0.00	0.00	0.00	0.33	0.00	0.00	0.00
L.S.D.	5% level	.85	.87	.72	.09	.70	.59	.71
	1% level	1.13	1.15	.90	.92	.94	.78	.94

10(0 trees), 1(1-10 trees), 2(11-30 trees), 3(31-100 trees), 4(101-200 trees), 5(200 plus trees).
 Significant different from treatment 2 at 5% level.
 Significant different from treatment 2 at 1% level.



by this treatment. In contrast to this, the combination treatment of ureaformaldehyde plus allyl alcohol and ethylene dibromide increased the number of tree seedlings over those found in the control with all species except the red and white pines. The increase in jack pine, Norway spruce, and Douglas-fir was highly significant. This increase may be due to the stimulating effect of allyl alcohol on the growth of nursery stock (Wilde et al., 1950).

All of the remaining treatments which gave better weed control (methyl bromide, DNBP, and various s-triazine treatments) also damaged the tree seedlings more severely. Methyl bromide and DNBP were not as severe on seedlings, however, as simazine and atrazine. With all species except Douglas-fir, seedling death in methyl bromide plots was significantly higher than controls.

DNBP shows definite promise as a herbicide which will give good weed control during the early part of the growing season with a minimum of damage to the germinating tree seedlings. Survival of red pine and Douglas-fir was not significantly different from the control. With the exception of jack pine, the remaining species, even though significantly different from the control, still showed fair resistance and survival. Table 7 shows the survival of the different tree seedlings under the DNBP, simazine, and atrazine treatments.

In the array of simazine and atrazine treatments, 4 pounds of wettable or granular simazine was for practical

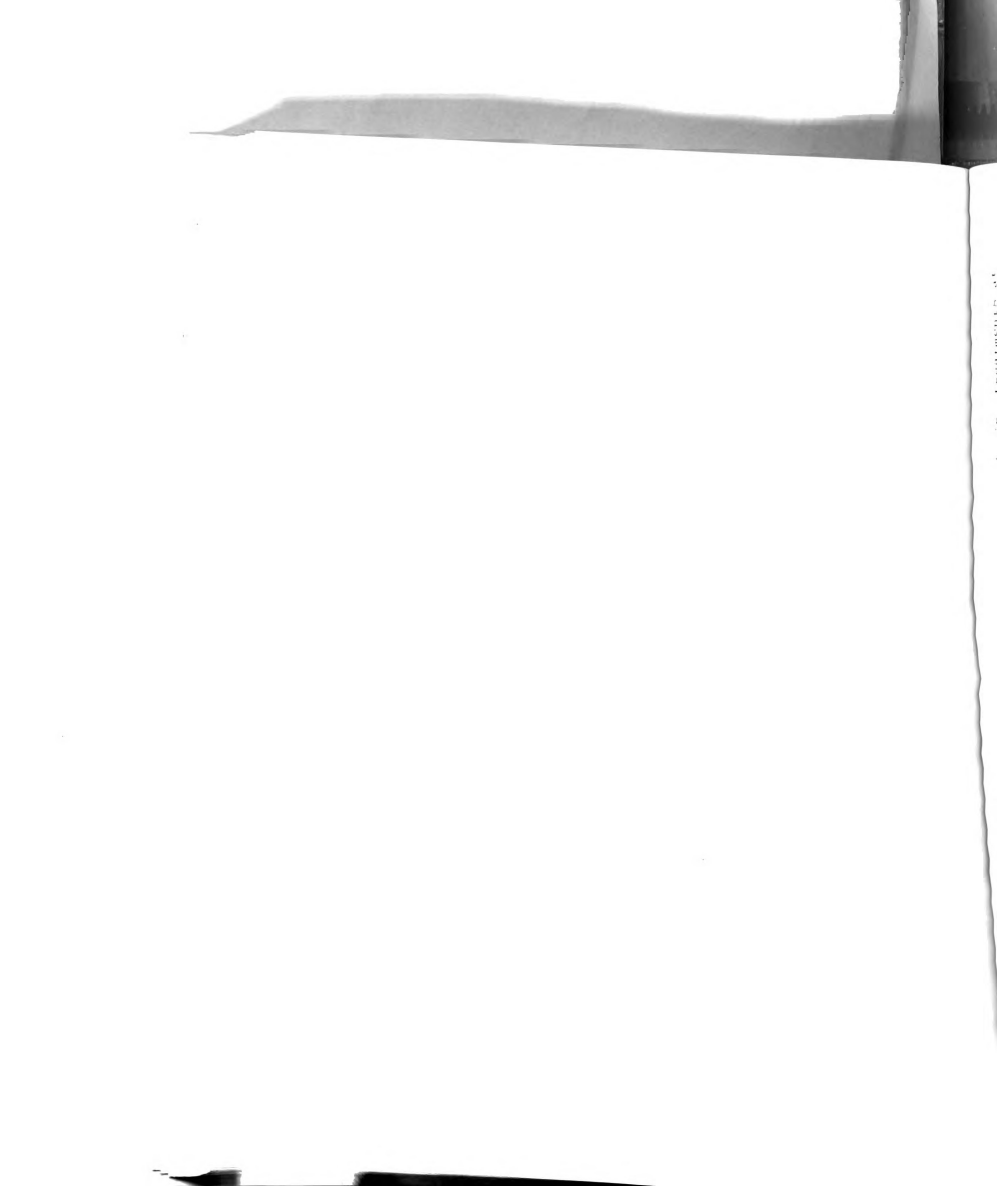


Table 7. Percentage of seedlings remaining alive under DNBP and s-triazine treatments at mid-July.

Treat. No.	Treatment	Percentage of seedlings (based on ranking of seedlings in controls)						
		Jack pine	Scotch pine	Red pine	White pine	Norway spruce	White spruce	Douglas fir
12	DNBP	10	63	81	70	73	58	82
13	Simazine (2# W)	10	5	0	58	31	0	27
14	Simazine (4# W)	0	0	0	6	10	0	14
15	Simazine (8# W)	0	0	0	0	0	0	4
16	Simazine (2# G)	21	37	25	64	47	16	64
17	Simazine (4# G)	0	10	6	41	21	0	27
18	Simazine (8# G)	0	0	0	18	0	0	6
19	Atrazine (4# G)	0	5	12	35	21	0	18
20	Atrazine (8# G)	0	0	0	11	0	0	0

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purposes as severe as 8 pounds of the same formulation. In most cases these treatments destroyed practically all seedlings of all species, as did the two atrazine treatments.

Under the 2 pound wettable and granular simazine treatments the kill of seedlings was reduced in some cases, indicating that even smaller dosages at more frequent intervals might be employed more successfully. White pine and Douglas-fir are more resistant to the s-triazines than the spruces and other pines tested. Kuntz et al. (1960b) also found that when atrazine was applied to emerging red pine and white pine seedlings, phytotoxic effects developed more rapidly and to a greater degree on red pine than on white pine, especially at higher dosages. Additional work by these same researchers, Bagley and Miyoshi (1959) and White (1962) has shown that the s-triazines can be used with lining-out size coniferous species with little or no resultant injury. Apparently chemical make up or some physical factor associated with older age increases the resistance of the young trees to these herbicides.



CHAPTER IV

TOLERANCE OF SEVERAL HARDWOOD AND CONIFEROUS SPECIES TO SIMAZINE TREATMENTS

The tolerance of eleven species of hardwoods and conifers (Table 8) to simazine was investigated. In addition to rates of application, the effect of simazine applied as surface treatments was compared with treatments in which the herbicide was mixed with the upper inch of soil.

The experiment was carried on in the greenhouse at Michigan State University's Hidden Lake Gardens, an arboretum located at Tipton, Michigan. The general layout of the experiment is shown in Figure 4. Utility grade plastic pots with a 6-inch top diameter were used. The pots were arranged on the bench in a latin square design. Prior to sowing, all seed with the exception of the oaks, was soaked for two weeks in water maintained at a 38°F. temperature. The oaks had been stratified in moist sand during the winter. Sufficient seed was sown in each pot to permit thinning of conifers to 10 seedlings per pot and hardwoods to 3 seedlings per pot at two weeks after germination. A combination of approximately 20 percent sphagnum peat and 80 percent sandy loam (by volume) was used for the potting mixture. The rates and placement of active simazine applied were:

1. Four pounds per acre surface application of simazine.



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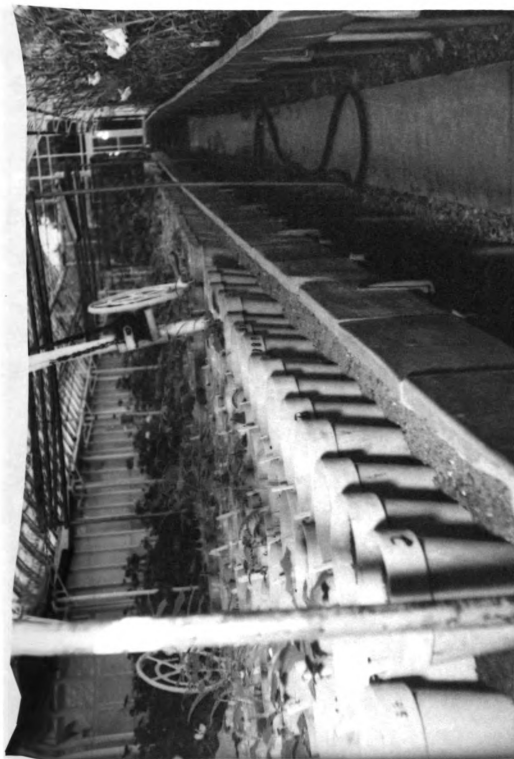


Figure 4. Layout of greenhouse experiment testing tolerance of several hardwood and coniferous species to simazine treatments.

Table 8. Tree species used in greenhouse simazine experiment.

Common name	Scientific name
Green ash	<u>Fraxinus pennsylvatica</u> var. <u>lanceolata</u> (Borkh.) Sarg.
Honeylocust	<u>Gleditsia triacanthos</u> L.
Norway spruce	<u>Picea abies</u> (L.) Karst.
White spruce	<u>Picea glauca</u> (Moench.) Voss.
White pine	<u>Pinus strobus</u> L.
Scotch pine	<u>Pinus sylvestris</u> L.
Douglas-fir	<u>Pseudotsuga menziessi</u> (Mirb.) Franco.
White oak	<u>Quercus alba</u> L.
Bur oak	<u>Quercus macrocarpa</u> Michx.
Red oak	<u>Quercus rubra</u> L.
Northern white-cedar	<u>Thuja occidentalis</u> L.

2. Four pounds per acre of simazine mixed with the upper inch of soil.
3. Eight pounds per acre surface application of simazine.
4. Eight pounds per acre of simazine mixed with the upper inch of soil.
5. Control.

Four percent granular simazine was used for all treatments and these treatments were applied at the time of sowing.

The soil was not sterilized, since this would reduce or eliminate the population of microorganisms. Captan drenches were applied during the first three weeks following

germination. The conifer seed, with the exception of northern white-cedar, had been coated with captan prior to sowing. Tap water was applied by greenhouse hose as needed.

Seedling Survival and Analysis

It became apparent that difficulties arising from soil or water borne organisms were going to complicate the procedure for recording results. Low germination percentage of some species, coupled with delayed germination of others, added to the problem. Number of weeks survival after germination was judged the system most applicable to all species and conditions. Accordingly, weekly ratings were made noting the dates of germination, condition of the seedlings, and their length of survival.

Survival of tree seedlings under these treatments was subjected to analysis of variance, using plot means as items. For each analysis the degrees of freedom were as shown below:

<u>Source of Variation</u>	<u>Degrees of Freedom</u>
Total variation	24
Among rows (blocks)	4
Among columns	4
Among treatments	4
Error	12

Root Elongation

A preliminary investigation was also set up in the greenhouse to determine whether the rate of root elongation had any effect on the tolerance of different species to simazine. Two species relatively resistant to simazine (white pine and bur oak) and two species easily killed by



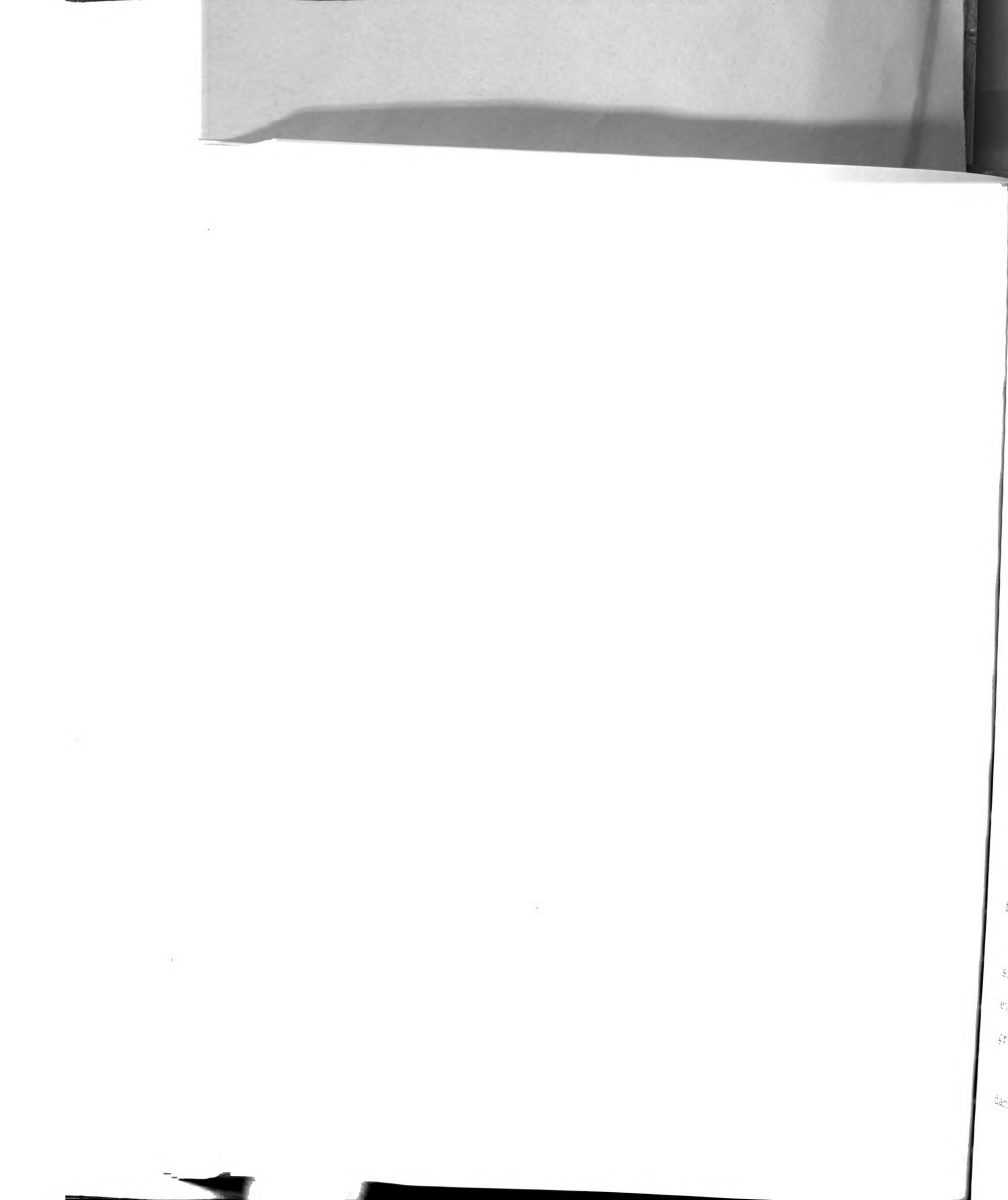
Simazine (red pine and Scotch pine) were used in this study. The seeds were germinated in steam sterilized soil contained in thin, plexiglass containers (pictured in Figures 6, 7, 8, and 9) measuring 9-1/2 inches long, 7 inches wide and 1/2 inch deep. These containers were held in a rack which tilted the bottom of the containers 30 degrees inward from the top. The rack was constructed in such a fashion that the roots were shielded from the light by black shade cloth, yet the containers could be slipped out readily for observation. In this position, the roots developed along the face of the plexiglass allowing easy observation and measurement.

An additional study compared the toxic effect of simazine on a normal rooted bur oak with one in which the root system was confined to the upper layer of soil. One 6-inch pot was fitted with a false bottom which confined root elongation to a 3-inch depth while the other pot was left with the normal 6-inch depth. This false bottom was constructed of aluminum foil covered cardboard which was sealed around the perimeter with paraffin. Four 1/4-inch drainage holes were drilled in the sides of the pot just above the false bottom. The pots were then sown to bur oak and simazine was applied as a 4 pound per acre surface treatment.

Results and Discussion

Seedling Survival

White pine, as was the case in the Bogue Nursery experiment described previously, proved to be more resistant



to simazine than the other conifers (Table 9). Scotch pine, northern white-cedar, Douglas-fir, white spruce, and Norway spruce were all killed within 5 weeks after germination under all simazine treatments. Some of the white pine seedlings not only survived the 4 and 8 pound treatments during the 12-week check after germination, but also put on second year growth the following spring. For practical purposes, the 4 pound treatments were as severe on the conifers as were the 8 pound rates. White pine survival was significantly higher under both 4 and 8 pound surface applications as compared to either the 4 or 8 pound rate mixed with the upper inch of soil. There was no significant difference, in respect to placement of simazine, with the other conifers tested.

The hardwood species, with the exception of green ash, as a whole, proved more resistant to simazine than the conifers. Green ash was killed quite readily by all simazine treatments.

Honeylocust showed considerable resistance to this herbicide. As with the white pine, surface treatments were far less damaging to this species than the mixed treatments (Figure 5). While most of the honeylocust seedlings in the simazine-soil mixed treatments and 8 pound surface treatments eventually died, many of them did survive and put on new growth during the second spring.

None of the three oak species showed any apparent damage from any of the treatments (Figure 5).

Table 9. Duration (weeks) of seedling survival under various simazine treatments¹.

Tree Species	4# simazine (surf. appl.)	4# simazine (mixed appl.) ¹	8# simazine (surf. appl.)	8# simazine (mixed appl.) ¹	Control	$\frac{\text{L.S.D.}}{5\%}$	$\frac{\text{L.S.D.}}{1\%}$
Green ash	3.2	2.2	2.2	2.0	12.0	1.07	2.35
Northern white-cedar	2.8	4.2	1.0	2.0	12.0	2.04	2.87
Douglas-fir	2.0	3.0	2.4	3.0	8.0	3.00	4.21
Honeylocust	12.0	10.8	12.0	8.2	12.0	2.37	3.33
Bur oak	12.0	12.0	12.0	12.0	12.0		
Red oak	12.0	12.0	12.0	12.0	12.0		
White oak	12.0	12.0	12.0	12.0	12.0		
Scotch pine	1.0	1.0	1.0	1.2	7.4	2.40	3.30
White pine	10.4	0.0	12.0	0.0	12.0	3.00	5.04
Norway spruce	4.0	4.4	4.0	3.0	12.0	2.01	4.00 ^a
White spruce	1.0	2.2	1.0	1.8	10.0	2.00	2.81

¹Simazine was mixed with the upper inch of soil before seeding.



A. 4-pound surface treatment, B. 4-pound treatment mixed with upper inch of soil, C. 8-pound surface treatment, D. 8-pound treatment mixed with upper inch of soil, E. control.



Figure 5. Tolerance of honeylocust (top photograph) and bur oak to various simazine treatments at 3 months after application.

Root Elongation

The root elongation of Scotch, red and white pine at three weeks of age is approximately equal (Figures 6, 7, and 8). It seems likely, therefore, that the tolerance exhibited by white pine is not due to rapid root elongation. The absorbing area of the root apparently does not move out of the zone of high herbicide concentration any quicker than the roots of the other more sensitive conifers. On the other hand, roots of bur oak moved 9 inches deep in only 15 days, although the hypocotyl had not yet appeared (Figure 9). In this case the maximum absorbing area of the root apparently moves quickly below the zone of simazine concentration.

Confining the roots of bur oak to the upper 3 inches of soil substantiated the belief that at least a good part of the tolerance observed in oaks is due to the root growth habit of the plant rather than the ability of the plant to render the herbicide harmless through some physiological process. Figure 10 shows the two bur oaks which have both been treated with 4 pounds per acre simazine. The plant on the left with the confined root system is showing the typical sequence of simazine toxicity, marginal chlorosis followed closely by necrosis and death. The plant on the right, however, is quite healthy. These symptoms appeared approximately eight weeks after treatment and eventually resulted in the death of the plant with a confined root system.

In a later experiment carried on with C¹⁴-labeled simazine, a bur oak seedling was treated with a 3 pound per

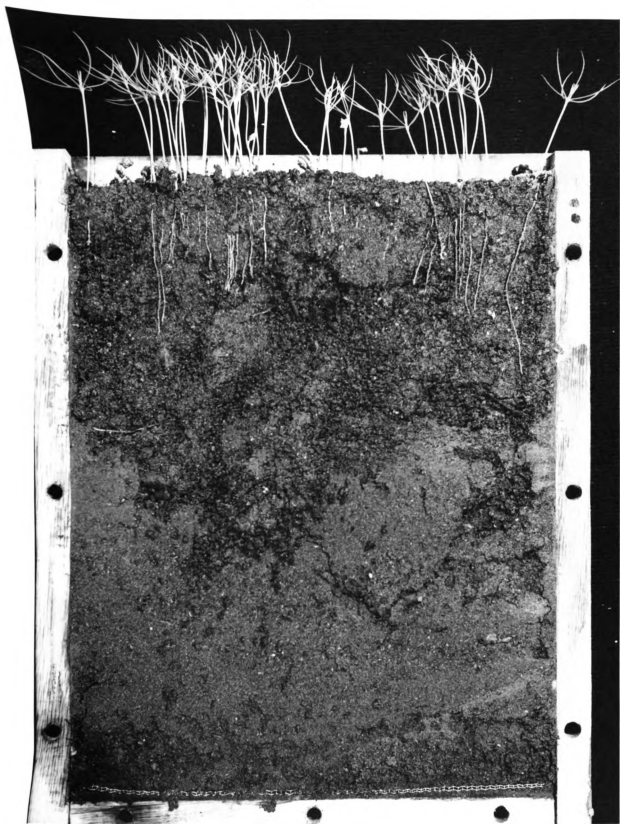


Figure 6. Root elongation of Scotch pine at 3 weeks after germination. The container is 9-1/2 inches deep.



Figure 7. Root elongation of red pine at 3 weeks after germination. The container is 9-1/2 inches deep.



Figure 8. Root elongation of white pine at 3 weeks after germination. The container is 9-1/2 inches deep.

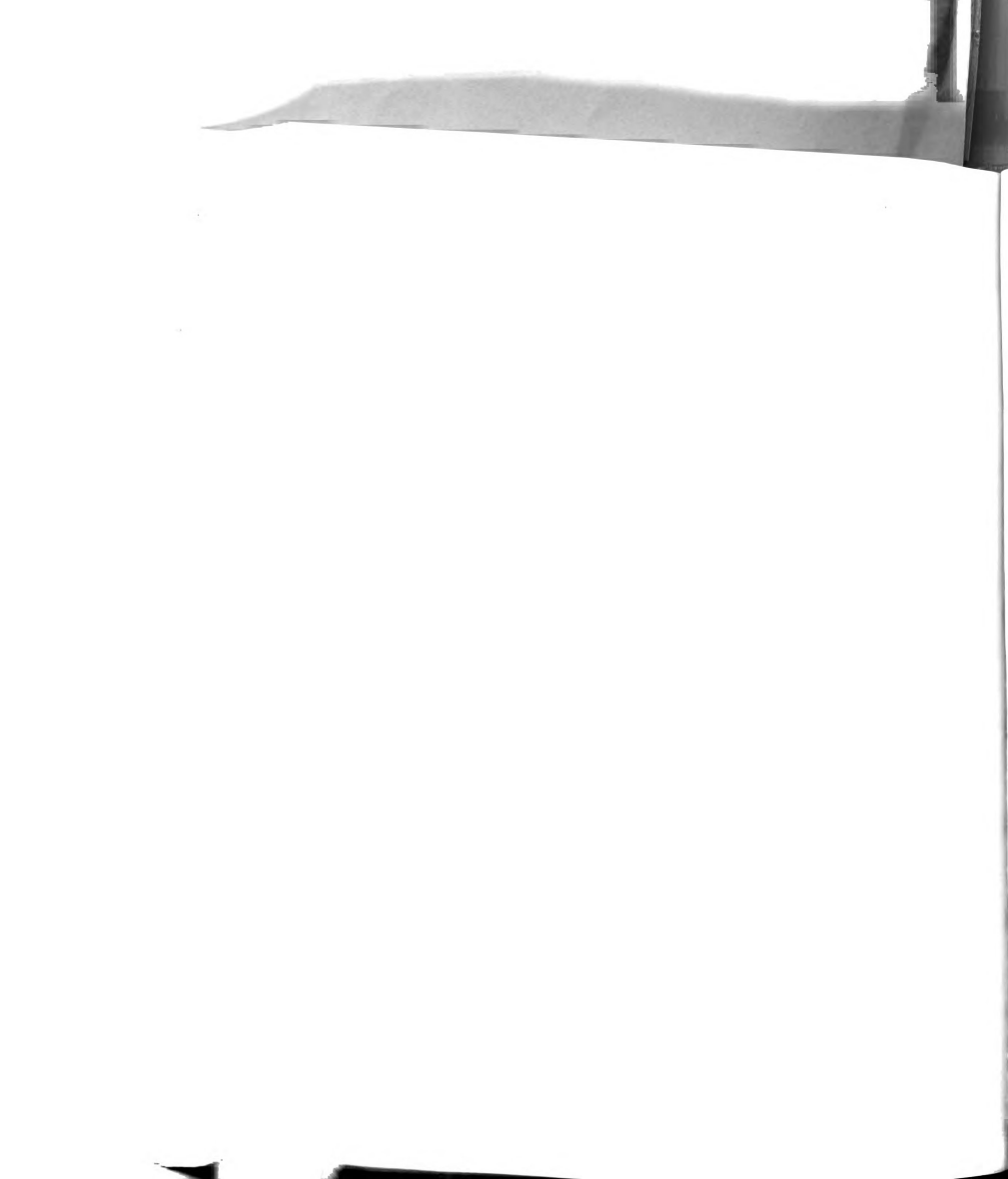


figure 9. Root elongation of bur oak at 15 days. Note that the hypocotyl has not yet appeared. The container is 9-1/2 inches deep.





Figure 10. Bur oak seedlings (above) 10 weeks after treatment with 4 pounds per acre simazine. The seedling on the left has its root system confined to the upper 3 inches of soil. Autoradiogram of bur oak (below) 5 weeks after treatment with 3 pounds per acre radioactive simazine.



acre dosage and an autoradiogram made of this plant five weeks later (Figure 10). Simazine or its degradation products seem to be fairly evenly distributed throughout the plant.



CHAPTER V

THE UPTAKE AND DISTRIBUTION OF C¹⁴-LABELED SIMAZINE OR ITS DEGRADATION PRODUCTS IN MYCORRHIZAL AND NON- MYCORRHIZAL RED AND WHITE PINE SEEDLINGS

In both the Bogue Nursery and greenhouse experiments described previously, white pine seedlings exhibited far more tolerance to simazine than red pine seedlings. On the other hand, observations of root elongation by these two conifers show no marked differences. The roots of the more simazine-tolerant white pine were not moving below the zone of high simazine concentration any more rapidly than those of red pine. This third study was set up to determine by use of C¹⁴-labeled simazine, if simazine is taken up in equal amounts by these two species. In addition, the distribution of the absorbed C¹⁴ was established by autoradiography and standard counting procedures. As a third objective, an attempt was made to determine whether the mycorrhizal relationship had any effect on the absorption and distribution of this herbicide.

Description of Growing Chamber and Growth Medium

A small polyethylene growing chamber (6' x 10') previously constructed for control of air borne organisms, was used to grow the red and white pine seedlings (Figure 11). A 12-inch exhaust fan was installed in one end of this growing

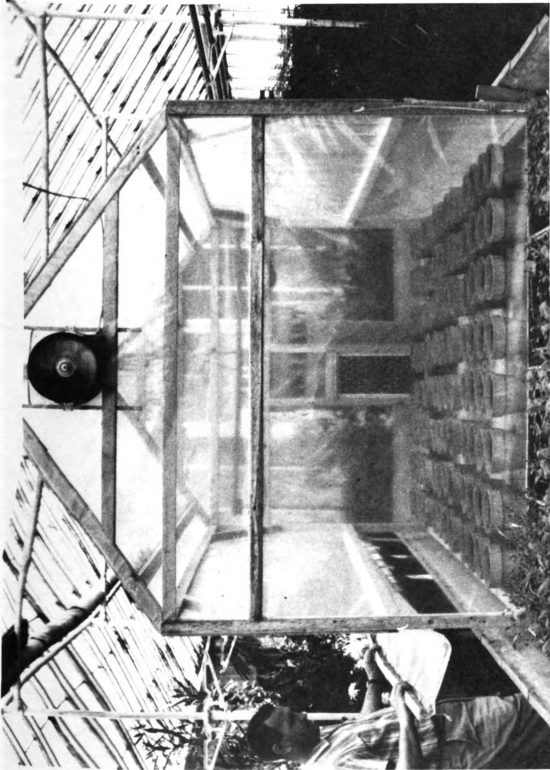


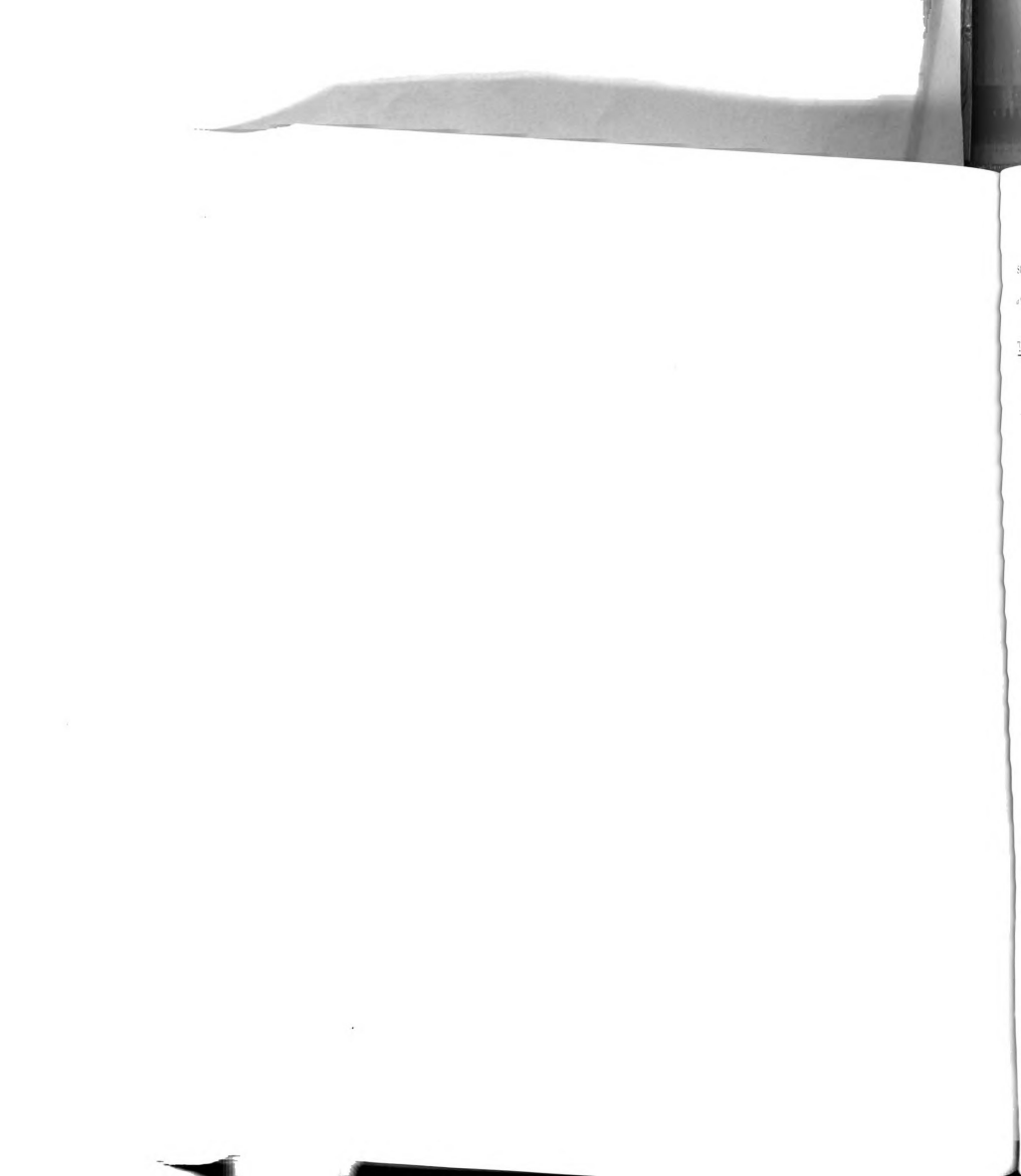
Figure 11. Polyethylene greenhouse used for growing inoculated and noninoculated red and white pine seedlings. Hidden Lake Gardens, Tipton, Michigan.

chamber and a 12 by 20 inch fiberglass filter in the other end. The lower section of either side could be lifted for watering, but was firmly secured at all other times. During the summer, the interior was kept from overheating by evaporative cooling and overhead slat shading. Evaporative cooling was obtained by dripping cold water through a thin, wire-enclosed excelsior container placed outside the greenhouse, immediately in front of the air filter. The air cooled by the damp excelsior moved into the growing chamber because of the pressure deficit created by the exhaust fan.

A Prairie soil, Fordville sandy loam, was used as the growing medium. This soil, obtained in South Dakota,¹ was free of fungi which form mycorrhizal relationships with coniferous tree roots. Six-inch plastic utility pots, fumigated with methyl bromide, were used for seed germination. It was soon evident, however, that seedling survival could not be obtained in the soil without some form of soil fumigation. The soil was then fumigated in the pots with methyl bromide. Ten days after fumigation the pots were again sown to red and white pine.

Approximately 2 months after germination, one-half of the red and white pine pots were inoculated with a nursery soil containing mycorrhizae-forming fungi. The nursery soil applied as an inoculum amounted to 1 percent of the original volume of soil in the pot. The inoculum was worked into the

¹Soil for the experiment was provided by Dr. L. O. Fine, South Dakota State College, Brookings, South Dakota.



surface layer of the soil and the seedlings were grown for an additional 5 months.

Treatment with Radioactive Simazine

Five months after inoculation the seedlings were lifted for transplanting. The trees were sorted for size and the roots washed thoroughly under tap water. Fordville sandy loam was again used as the growth medium and 9 ounce, waxed Dixie cups served as growing containers. Drainage holes were punched in the bottom of the cups and they were placed on a shallow baking tray which had been covered with a wire screen. This tray was used to confine the leachate and thus prevent contamination. The growing containers were arranged on the tray in a split plot design. Treatments were replicated five times.

Two weeks after transplanting, 20 ml. of an aqueous suspension containing 7.08 microcuries of C^{14} -labeled simazine² was applied to the soil medium of each plant (Figure 12). This quantity was equivalent to the rate of 3.10 pounds active simazine per acre. Non-treated controls of both inoculated and non inoculated plants were carried throughout the experiment.

The plants were grown for an additional 5-1/2 weeks in the soil treated with radioactive simazine. They were then lifted, the roots washed thoroughly in tap water, and divided into tops and roots. Seedlings were then mounted

²Radioactive simazine (7.84 microcuries/mg.) supplied by Geigy Agricultural Chemicals, Saw Mill River Road, Ardsley, New York.





Figure 12. Application of radioactive simazine treatments. Upper photo: Close up showing container and seedling size. Lower photo: Arrangement of seedlings on leaching tray.

on absorbent mounting paper. The mountings were separated by a hard finish construction paper and pressed inside a drying oven with an 80 pound weight. The seedlings remained in the drying oven for 2-1/2 weeks.

Autoradiograms and Counting

Two radioassay procedures were used in this study. Autoradiograms of the seedlings were made and plant parts were also counted in a gas-flow Geiger-Muller counting assembly.

After the plants were removed from the drying oven, they were placed in contact with Kodak Blue Brand X-ray film for a 4-week exposure period.

When the autoradiograms were completed, the plants were removed and prepared for counting. Approximately 1/4 inch of the stem and root was removed at the point of severance. Needles, stems, and roots of each plant were cut into fine pieces to obtain even distribution within the planchets used in the Geiger counter. The plant parts were oven dried at 70°C. for 24 hours in a gravity flow oven and placed in a silica gel desiccator prior to weighing. Immediately after weighing, the samples were counted with a gas-flow, thin window Geiger-Muller tube and Tracerlab "Versa/Matic" scaler. All counts were corrected for self-absorption before being subjected to statistical analysis (see Appendix).

Statistical Analysis

Data obtained from these treatments was tested by the

analysis of variance, using plot means as items. For each analysis the degrees of freedom were as shown below.

<u>Source of variation</u>	<u>Degrees of freedom</u>
Total	9
Replications	4
Treatments	1
Error	4

Results and Discussion

Close examination of seedling roots just prior to treatment with radioactive simazine revealed that mycorrhizae had advanced only into the initial stage of development. None of the plants had developed the typical fungal mantle normally found on young pine seedlings. Slow development of mycorrhizae is likely due to time of inoculation, since the inoculum was applied during early October. Even though the plants were given long days by incandescent lighting during the fall and winter months, top growth and apparently root growth were not very active during this period.

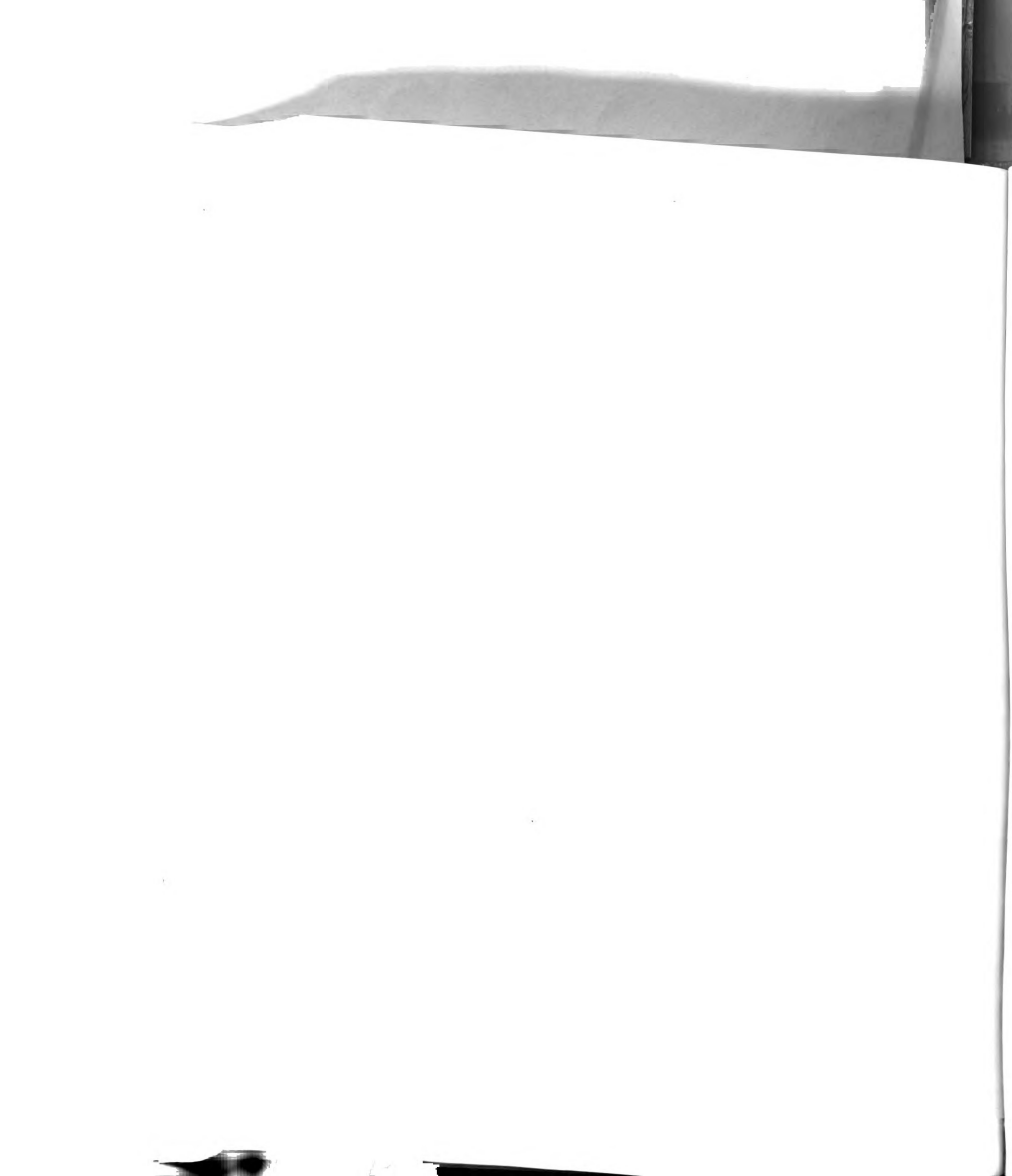
Autoradiograms

There was no apparent relationship between mycorrhizae and distribution of simazine in red pine seedlings (Figure 13). In some replications the uptake in the roots and tops appears heavier in the noninoculated treatments, while in other replications the reverse is true. As a whole, however, distribution of radioactive material appears to be more uniformly dispersed throughout the entire red pine seedling than is the case with white pine. Counts of radioactive plant





Figure 13. Autoradiograms and seedling photographs of red pine. Upper left: Autoradiogram of inoculated seedling. Lower left: Photo of this same tree. Upper right: Autoradiogram of noninoculated seedling. Lower right: Photo of this same tree.



parts, which appear later in the report, bear out the conclusion drawn from the autoradiograms.

Autoradiograms of white pine (Figure 14) in all five replications show a much heavier concentration of radioactive material in the roots of noninoculated plants. Stem and needle outlines of noninoculated plants also appear generally darker than those of inoculated plants.

Counts of Radioactive Plant Parts

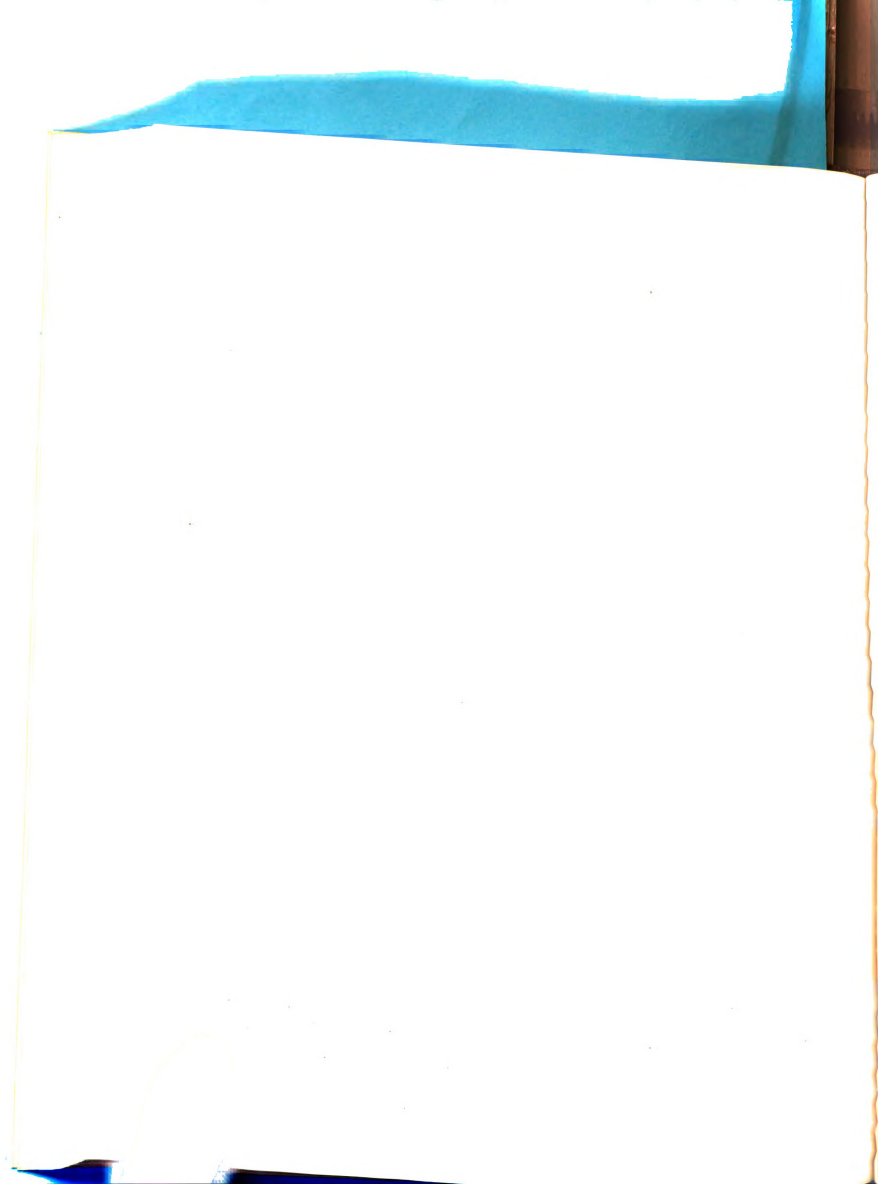
Table 10 shows total uptake of simazine to be approximately equal for both red and white pine. However, seedlings were separated into roots, stems, and needles for counting in

Table 10. Mean counts of C^{14} in red and white pine (entire plant).

Treatment	White pine	Red pine
Inoculated	1.923	3.854
Noninoculated	4.084	3.082
Total	6.007	6.936

order to pick up differences in distribution of simazine within the plants. Total counts, after being corrected for self-absorption, were compared on a weight basis for purposes of analysis (Table 11).

In white pine all of the root counts of noninoculated plants are higher than the inoculated plants. In fact, the counts of noninoculated roots were more than double those of the inoculated roots (Table 11). Stem and leaf counts follow



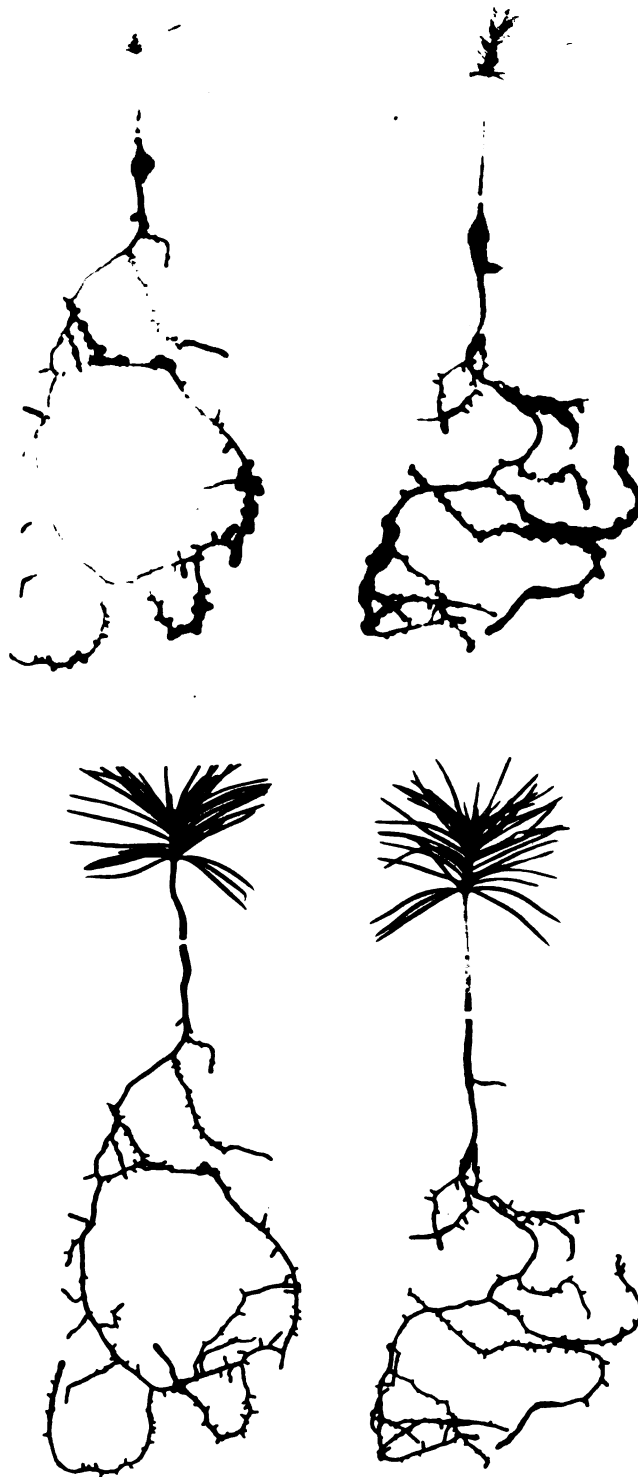


Figure 14. Autoradiogram and seedling photographs of white pine. Upper left: Autoradiogram of inoculated seedling. Lower left: Photo of this same tree. Upper right: Autoradiogram of noninoculated seedling. Lower right: Photo of this same tree.



Table 11. Counts of C^{14} in roots, stems, and leaves of red and white pine seedlings.

Replication	Counts per minute per milligram			
	Inoculated white pine	Non- inoculated white pine	Inoculated red pine	Non- inoculated red pine
Root Counts				
1	.707	2.450	1.044	.328
2	1.503	3.385	1.255	.848
3	.903	5.201	2.828	1.450
4	.578	.769	.752	.985
5	2.825	3.098	.852	3.508
Stem Counts				
1	.343	.174	.810	.628
2	.287	.480	2.050	.628
3	.707	.927	.642	.658
4	.208	.459	1.040	.818
5	.460	1.048	.480	1.627
Leaf Counts				
1	.235	.075	1.040	.307
2	.154	.308	2.430	.421
3	.202	.347	.924	.523
4	.120	.300	.184	.504
5	.202	.312	1.100	2.070

this same pattern with the exception of the first replication. Analysis of variance for the total seedling showed a significant difference in the amount of C^{14} present in inoculated and noninoculated plants (Table 12).

Table 12. Counts of C^{14} in inoculated and noninoculated white pine seedlings.

Treatment	Mean counts per milligram
Inoculated	1.924
Noninoculated	4.086

F value for differences between treatments significant at 5% level.

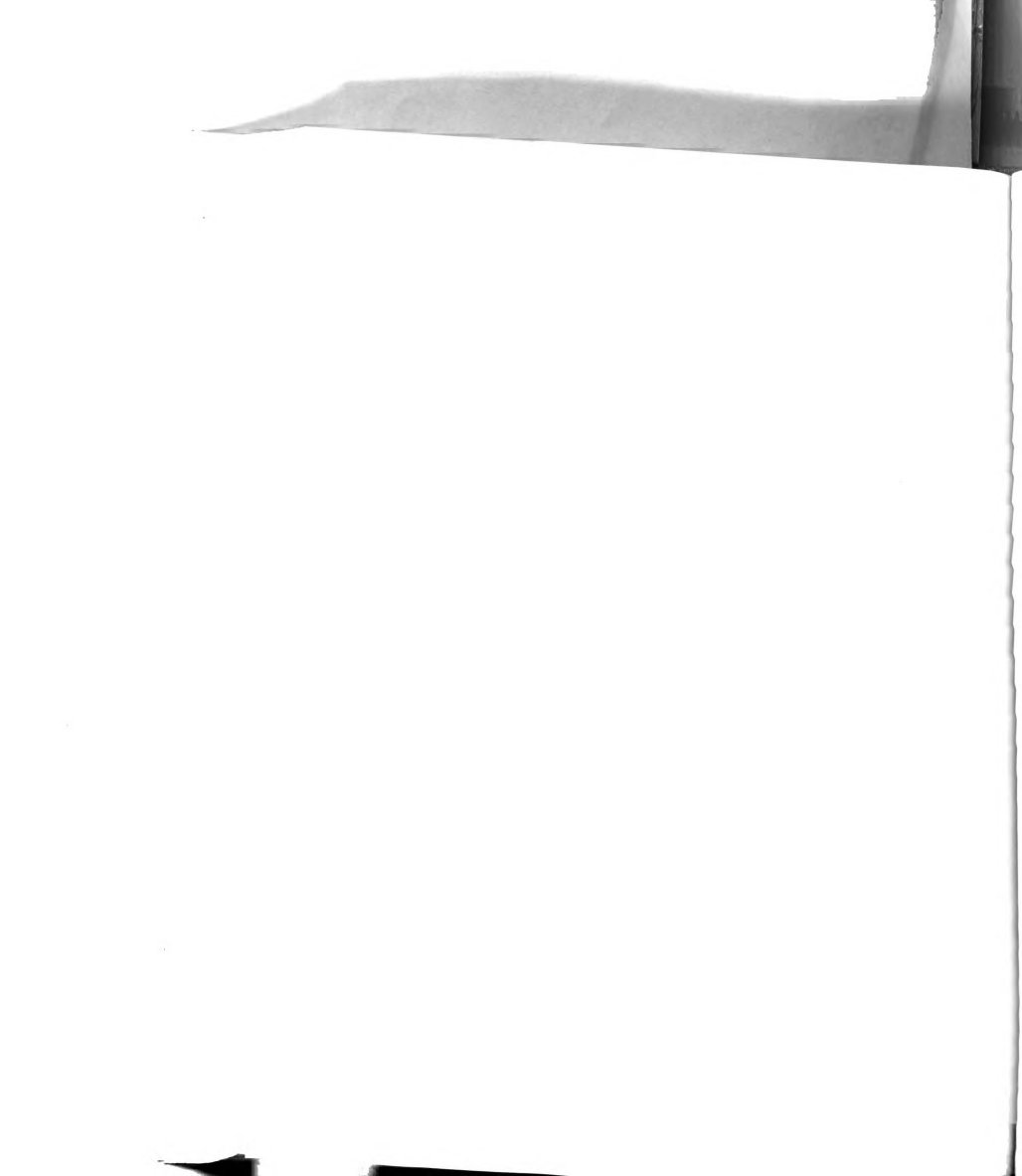
Analysis of variance showed no difference in simazine uptake between inoculated and noninoculated red pine seedlings. However, a more advanced stage of mycorrhizal development than that present in this study must be tested before its effect on simazine uptake can be substantiated.

It is evident in Table 13 that distribution of C^{14} is extremely important. In red pine the C^{14} is fairly evenly distributed throughout the plant. A much higher percentage of the C^{14} , however, is retained in the non-photosynthetic organs of the white pine.

Table 13. Distribution of C^{14} in red and white pine (percentage basis).¹

Plant part	Inoculated white pine	Non- inoculated white pine	Inoculated red pine	Non- inoculated red pine
Root	65.0	75.0	40.0	43.0
Stem	22.8	16.0	28.1	32.6
Leaf	12.2	9.0	31.9	24.4

¹Average of 5 replications.



The needles of red pine contain approximately 3 times the amount of radioactive material (computed on percentage of total uptake) as do those of white pine (Table 14). The ratio of distribution of C^{14} from top to roots of red pine is 1 to 1.5, while in white pine the ratio from tops to roots is 1 to 0.5. Sheets (1961) found this same relationship in work with oat plants (simazine susceptible) and cotton plants which show intermediate tolerance to simazine. The concentration of C^{14} from radioactive simazine treatments was approximately three times higher in the oat leaves than in the leaves of cotton.

Table 14. Percentage of C^{14} in leaves and roots of red and white pine.

Tree species	Inoculated	Non-inoculated	Tree species mean
Leaves			
White pine	12.2	9.0	10.60
Red pine	31.9	24.4	28.15
Roots			
White pine	65.0	75.0	70.0
Red pine	40.0	43.0	41.2

F value for difference between species significant at 5% level.

There was no difference between stem counts of the two species at the 5 percent level. There was a difference, however, at the 10 percent level.



Analysis of root counts (Table 14) also showed a difference between the two species.



CHAPTER VI

SUMMARY AND CONCLUSIONS

Weed control in nursery management is a time consuming, expensive, never-ending operation. Chemical control prior to 1950 was confined largely to fumigation of seedbeds with poisonous gases and treatment of conifers with petroleum and mineral spirits. Since the advent of organic herbicides after the second World War, literally hundreds of weed control agents have been placed on the market. While many of these products have been tested to some extent, the surface has only been scratched.

The first phase of this nursery weed research compared some of the newer soil fumigants and herbicides with those used in the past. Comparison of duration and thoroughness of weed control was one objective and extent of damage to seven germinating coniferous species was the other. In the wet spring of 1960 no appreciable weed control was obtained from Stoddard solvent, ureaformaldehyde, DMTT, SMDC, chloropicrin, EPTC, and a combination treatment consisting of ureaformaldehyde plus allyl alcohol and ethylene dibromide. EPTC reduced germination of most species. Chloropicrin caused significant damage to jack, red and white pines, and white spruce. Ureaformaldehyde damaged only Scotch pine and red pine. The remainder of the forementioned treatments caused no injury to any of the tree seedlings tested. The poor





weed control obtained with Stoddard solvent was attributed to poor timing of applications and the type of applicator used.

Methyl bromide and DNBP gave better weed control than the treatments discussed above, but the kill of seedlings was also far greater. Of these two treatments, DNBP gave longer-lasting weed control and was also less toxic to most tree species. DNBP shows definite promise as a herbicide which will give good control during the early part of the growing season with a minimum of damage to young seedlings. Survival of red pine and Douglas-fir was not significantly different from the control. From the results achieved with DNBP in this study, additional investigation of its full potential is definitely warranted.

Weed control with the s-triazines was superior to all other treatments. At six weeks after application most plots containing either simazine or atrazine were practically weed free. These two herbicides, when applied at 4 pound rates, continued to give adequate weed control until late July. Because of its lower solubility, simazine (5 p.p.m.) gave a longer weed free period than equal amounts of atrazine (70 p.p.m. solubility). Neither of these two s-triazines gave the season-long weed control that had been attained in previous tests by the writer. The above normal rainfall during the testing period may account for the shorter residual activity experienced in this test.

Damage to all germinating tree species by both simazine



and atrazine was quite severe, in many cases destroying all seedlings at the 8 pound rates. The 2 pound treatments were less damaging, indicating that very light dosages applied at more frequent intervals might be feasible with some species. Of the seven conifers tested, white pine proved the most tolerant to these s-triazines.

★ Further testing of simazine rates and placement was made in greenhouse experiments in which both conifers and hardwoods were used as test plants. White pine again proved to be more resistant to simazine than the other conifers tested. Northern white-cedar, Douglas-fir, Scotch pine, Norway spruce, and white spruce were all killed within five weeks after germination by all treatments. In contrast to this, red, white, and bur oaks were not damaged by any of the treatments. Honeylocust showed considerable resistance to this herbicide, and as with white pine, surface applications were far less damaging to this species than treatments in which the herbicide was mixed with the upper inch of soil.

It was postulated that some of the species more resistant to simazine, such as white pine and the oaks, extended their roots below the zone of high herbicide concentration more rapidly than the easily killed species. Root elongation studies were therefore set up to determine whether rate of root elongation had any effect on tolerance. It was found that the roots of white pine did not elongate any faster than those of simazine susceptible Scotch and red pines. On the





other hand, roots of bur oak moved 9 inches deep in only 15 days. In this case the maximum absorbing area of the root apparently moves quickly below the zone of high simazine concentration. Further work in which the roots of bur oak were confined to the upper three inches of simazine-treated soil, substantiate the belief that at least a good part of the tolerance observed in oaks is due to the root growth habit of the plant rather than the ability of the plant to break this herbicide down into harmless chemical products through some physiological process. The plant with the confined root system began to show typical simazine toxicity symptoms and eventually died, while those with normal root development remained healthy. Oak seedbeds can apparently be treated with up to 4 pounds active simazine per acre without damaging seedlings.

Since there is such a wide difference in the tolerance of red and white pines to simazine, a third study was set up to determine, by use of C^{14} -labeled simazine, the uptake and distribution of this herbicide in these two species. In addition, the effect of the mycorrhizal relationship associated with these two pines was incorporated into the study. Two radioassay procedures, autoradiography and counting of plant parts, were used.

Results of this study showed the total uptake of simazine to be approximately equal for both red and white pine. C^{14} is fairly uniformly distributed throughout red pine, but is confined more to non-photosynthetic organs in



white pine. The ratio of distribution of C^{14} from top to roots of red pine is 1 to 1.5, while in white pine the ratio from tops to roots is 1 to 0.5. Since simazine kills by blocking the Hill reaction during photosynthesis, the reason for the greater tolerance exhibited by white pine seems evident.

At the time of radioactive treatment, mycorrhizae had advanced only into the initial stage of development on the inoculated seedlings. Even at this early stage of development, however, the inoculated white pine seedlings contained significantly less C^{14} than the noninoculated white pines. In fact, counts in noninoculated plants were more than double those of inoculated plants.

No apparent differences were noted in simazine uptake between inoculated and noninoculated red pine. The mycorrhizal relationship either had no effect on simazine uptake with this species or perhaps develops slower on red pine and therefore had not reached a stage where it could influence uptake. Certainly a more advanced stage of mycorrhizal development than that present in this study must be tested before its effect on simazine uptake in red pine can be substantiated.



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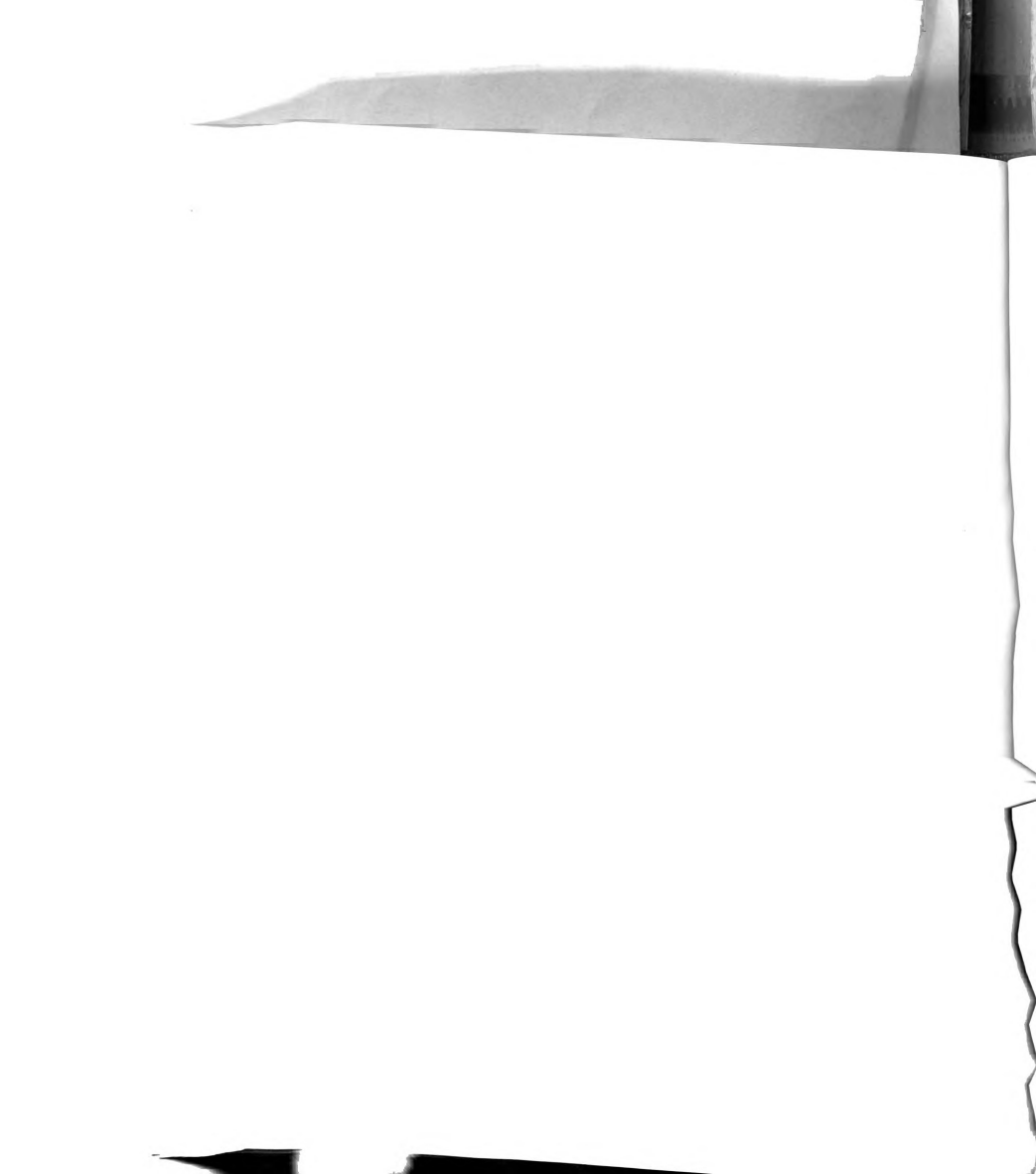
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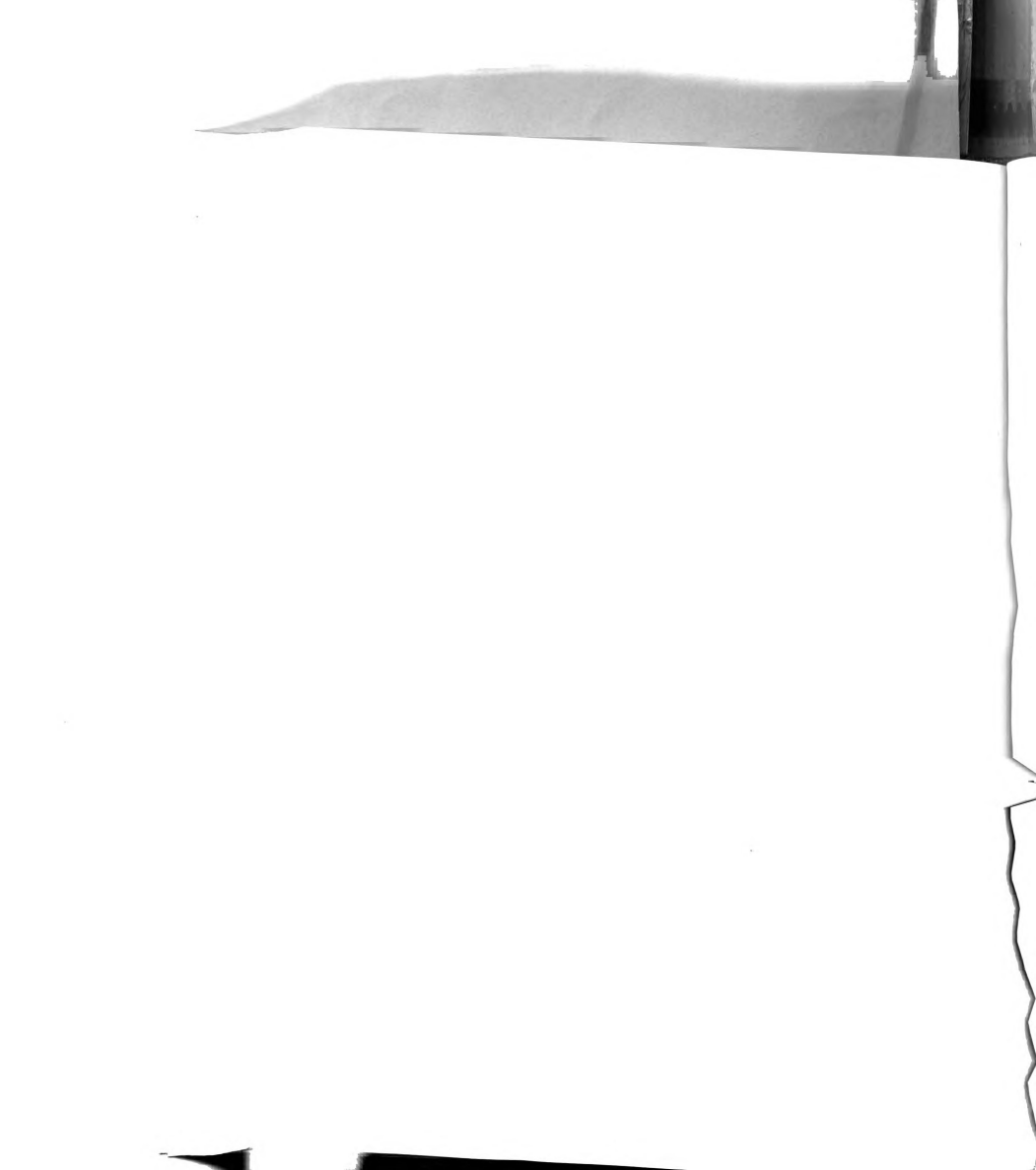
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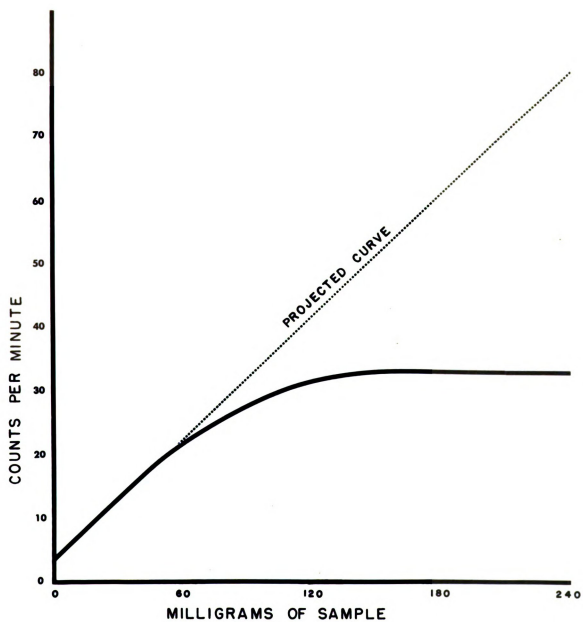




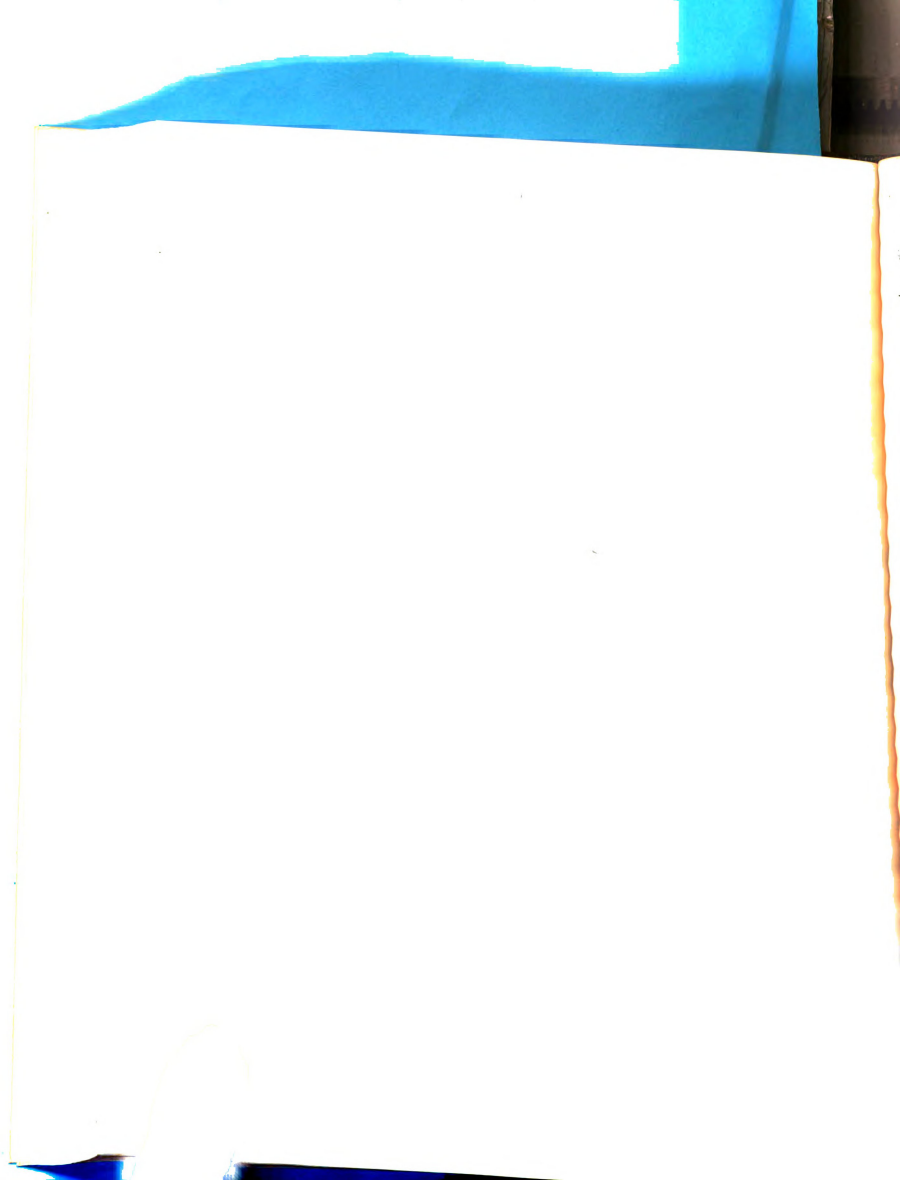
APPENDIX



79

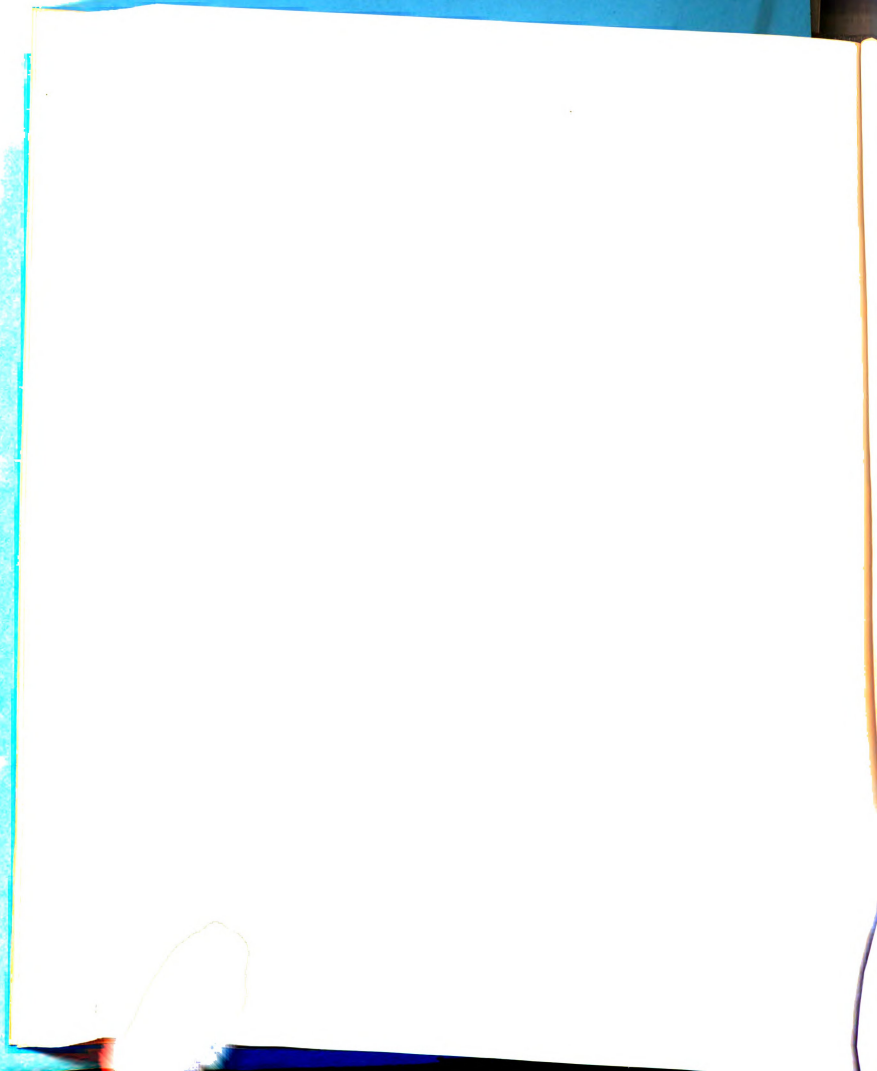


Appendix 1. Self-absorption curve.



Appendix 2. Listing of common and chemical names of herbicides and soil fumigants mentioned in the text.

Common name	Chemical name
Allyl alcohol	allyl alcohol
Atraton	2-methoxy-4-ethylamino-o-isopropyl-amino-s-triazine
Atrazine	2-chloro-4-ethylamino-o-isopropylamino-s-triazine
Chlorazine	2-chloro-4,o-bis (diethylamino)-s-triazine
Chloropicrin	trichloronitromethane
Copper sulfate	copper sulfate
Dalapon	2,2-dichloropropionic acid
Ethylene dibromide	ethylene dibromide
EPTC	ethyl N, N-di-n-propylthiolcarbamate
G-3002o	2-chloro-4-methylamino-o-isopropyl-amino-s-triazine
Ipazine	2-chloro-4-diethylamino-o-isopropyl-amino-s-triazine
Maleic hydrazide	maleic hydrazide
Methyl bromide	methyl bromide
Monuron	3-(p-chlorophenyl)-1, 1-dimethylurea
DMTT	3,5-dimethyltetrahydro 1,3,5 2 H thiadiazine-2-thione
DNBP	4,o-dinitro-o-sec-butylphenol (amine salt)
Prometone	2-methoxy-4,o-bis(isopropylamino)-s-triazine
Propazine	2-chloro-4,o-bis(isopropylamino)-s-triazine
Sesone	sodium, 2,4-dichlorophenoxyethyl sulfate
Simazine	2-chloro-4,o-bis(ethylamino)-s-triazine
Simetone	2-methoxy-4,o-bis(ethylamino)-s-triazine
Sodium arsenite	sodium arsenite
Stoddard solvent	stoddard solvent
TCA	trichloroacetic acid
Trietazine	2-chloro-4-diethylamino-o-ethylamino-s-triazine
Ureaformaldehyde 85	ureaformaldehyde
SMDC	sodium methyl dithiocarbamate
2,4-D	2,4-dichlorophenoxyacetic acid



Appendix 3. Total weight, total counts per minute, background counts, per minute, corrected counts per minute and adjusted counts per minute for inoculated white pine.

Replication	Total weight (milligrams)	Background counts/min.	Total counts/min.	Corrected counts/min.	Adjusted counts/min.
Leaf					
1	87.3	11.05	30.30	18.35	20.55
2	58.8	11.00	20.83	8.93	9.10
3	47.0	11.00	25.04	13.74	13.74
4	66.2	13.00	20.66	7.66	8.04
5	81.4	13.00	28.16	15.16	16.52
Stem					
1	21.3	11.05	19.26	7.31	7.31
2	20.4	11.05	17.82	5.87	5.87
3	18.0	11.00	24.63	12.73	12.73
4	16.0	13.00	19.33	3.33	3.33
5	14.0	13.00	19.53	6.53	6.53
Root					
1	75.8	11.05	62.11	50.16	53.67
2	80.2	11.00	138.88	126.98	142.21
3	54.7	11.00	61.34	49.44	49.44
4	71.6	11.00	51.02	39.12	41.46
5	66.9	13.00	256.41	243.41	282.35

¹Total counts minus background counts equals corrected counts per minute.

²This column obtained after correction from self-absorption curve.

Appendix 3 (continued). Total weight, total counts per minute, background counts per minute¹, corrected counts per minute² and adjusted counts per minute² for noninoculated white pine.

Replication	Total weight (milligrams)	Background counts/min.	Total counts/min.	Corrected counts/min.	Adjusted counts/min.
Leaf					
1	83.0	11.95	17.73	5.78	6.30
2	80.8	11.95	34.84	22.89	24.95
3	107.3	11.90	43.29	31.39	37.35
4	74.2	13.00	40.81	27.81	29.01
5	63.0	12.89	31.84	18.95	19.70
Stem					
1	15.8	11.95	14.70	2.75	2.75
2	24.7	11.95	24.03	12.08	12.08
3	26.8	11.90	36.79	24.89	24.89
4	23.4	13.00	23.75	10.75	10.75
5	13.1	13.00	26.73	13.73	13.73
Root					
1	80.2	11.95	192.30	180.35	190.58
2	111.0	11.95	322.58	310.63	375.80
3	90.3	11.90	434.78	422.88	477.85
4	103.7	13.00	80.64	67.64	79.81
5	62.7	13.00	256.41	243.41	250.71

¹Total counts minus background counts equals corrected counts per minute.

²This column obtained after correction from self-absorption curve.

Appendix 3 (continued). Total weight, total counts per minute, background counts per minute, corrected counts per minute, and adjusted counts per minute for inoculated red pine.

Replication	Total weight (milligrams)	Background counts/min.	Total counts/min.	Corrected counts/min.	Adjusted ¹ counts/min.
Leaf					
1	30.3	11.95	70.92	58.97	58.97
2	31.4	11.90	88.40	76.50	76.59
3	46.9	11.90	55.24	43.34	43.34
4	40.9	13.00	22.17	9.17	9.17
5	34.8	13.00	51.28	38.28	38.28
Stem					
1	12.0	11.95	21.78	9.83	9.83
2	10.3	11.90	33.11	21.21	21.21
3	14.3	11.90	21.09	9.19	9.19
4	9.7	13.00	23.09	10.09	10.09
5	12.8	12.89	19.12	6.23	6.23
Root					
1	25.8	11.95	62.11	50.16	50.16
2	20.2	11.95	37.31	25.36	25.36
3	23.2	11.90	77.51	65.61	65.61
4	31.4	13.00	36.63	23.63	23.63
5	25.2	13.00	34.48	21.48	21.48

¹Total counts minus background counts equals corrected counts per minute.

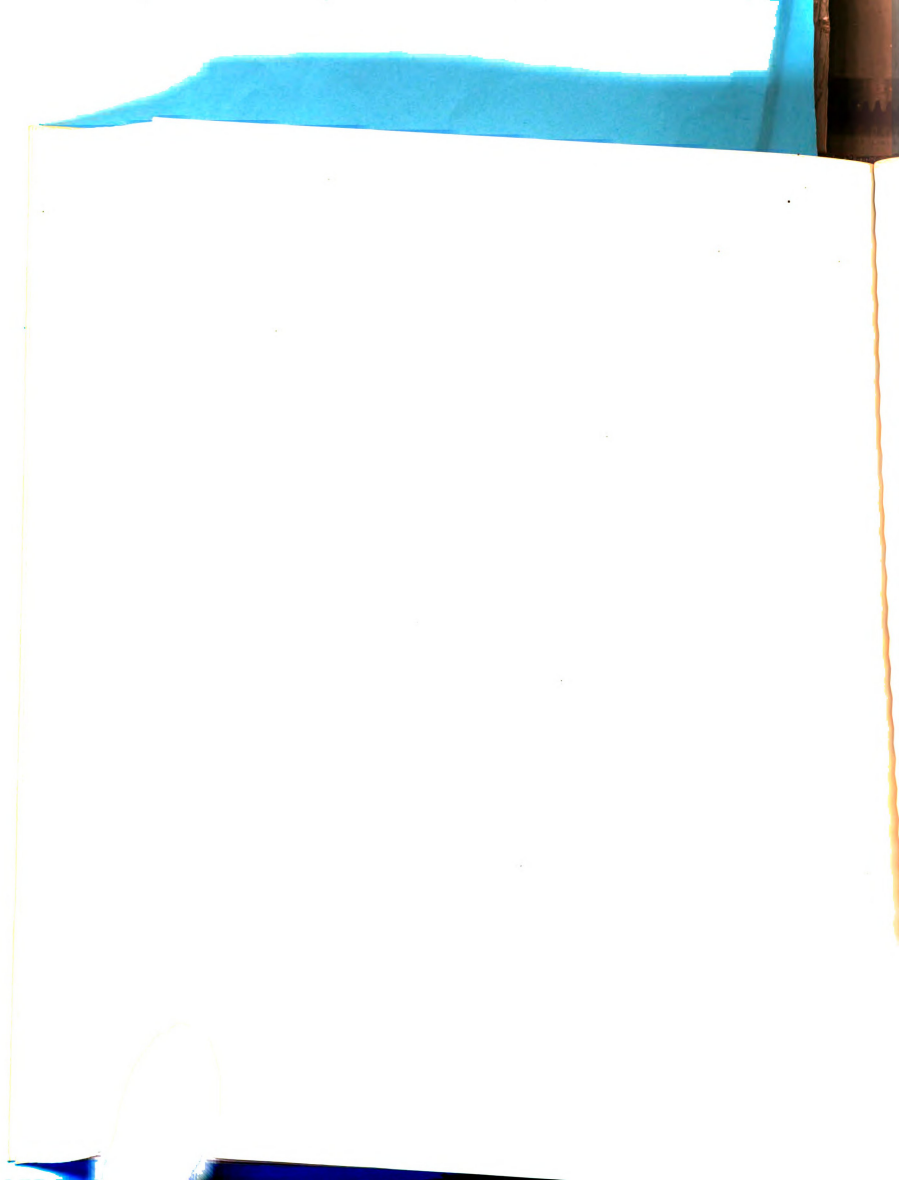
²This column obtained after correction from self-absorption curve.

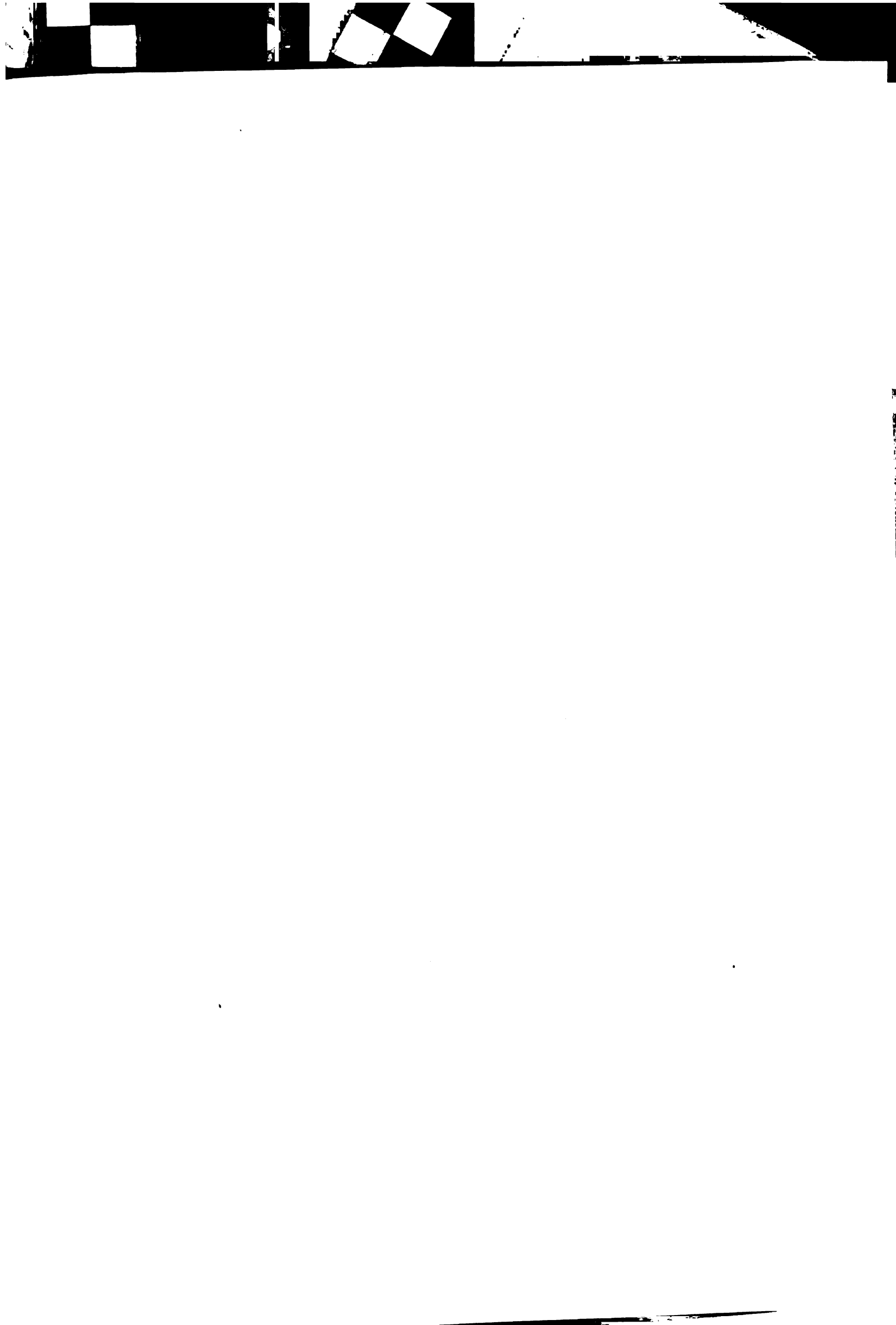
Appendix 3 (continued). Total weight, total counts per minute, background counts per minute, corrected counts per minute¹ and adjusted counts per minute² for noninoculated red pine.

Replication	Total weight (milligrams)	Background counts/min.	Total counts/min.	Corrected counts/min.	Adjusted counts/min.
Leaf					
1	53.8	11.05	33.33	21.38	21.38
2	40.8	11.00	31.04	19.74	19.74
3	47.2	11.00	30.63	24.73	24.73
4	47.9	13.00	37.17	24.17	24.17
5	50.2	13.00	120.87	110.87	110.87
Stem					
1	14.4	11.05	21.00	9.05	9.05
2	23.2	11.05	20.52	14.57	14.57
3	15.0	11.00	21.78	9.88	9.88
4	14.8	13.00	25.12	12.12	12.12
5	10.1	13.00	30.21	26.21	26.21
Root					
1	53.0	11.05	20.07	17.72	17.72
2	58.0	11.05	00.97	40.02	50.00
3	48.1	11.00	81.90	70.00	70.00
4	53.1	13.00	05.35	52.35	52.35
5	71.0	13.00	250.00	237.00	251.22

¹Total counts minus background counts equals corrected counts per minute.

²This column obtained after correction from self-absorption curve.







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