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Title: SOME PHYSIOLOGICAL EFFECTS OF ATRAZINE ON DOUGLAS-FIR SEEDLINGS

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This study was undertaken to ascertain some of the physiological effects of the herbicide atrazine on Douglas-fir seedlings. Photosynthet c and respirational rates were measured on seedlings grown in a controlled environment chamber. Height and dry weight increases, shoot/root ratios and percent total nitrogen were obtained from treated and control seedlings growing on field plots.

Treatment of Douglas-fir seedlings with subtoxic levels of atrazine caused an initial repression of photosynthesis and respiration, with a subsequent recovery. Seedlings grown in sand and treated with one fourth pound per acre (0.275 ppmw) had rates of both physiological functions which exceeded the controls significantly 36 days after chemical application. Application of one half pound per acre (0.55 ppmw) supressed both photosynthesis and respiration to a great extent, with full recovery occurring only after 36 days.

Seedlings grown on forest soils had similar, but less pronounced, responses when treated with much higher concentrations of herbicide. Eight pounds per acre (11.6 ppmw) inhibited photosynthesis for a few days with recovery complete after nine days. Neither photosynthesis nor respiration were markedly altered by lower application rates of atrazine.

Atrazine slightly inhibited height growth and dry weight increase the year of application in field plots where seedlings were grown under weed-free conditions. The year following treatment the seedlings exhibited increased growth which compensated for the inhibition noted the first year. The shoot/root ratios were not altered by atrazine either year. Percent total nitrogen was increased by the application of the herbicide the year of treatment but there were no significant differences the second year.

# SOME PHYSIOLOGICAL EFFECTS OF ATRAZINE ON DOUGLAS-FIR SEEDLINGS

by

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# SOME PHYSIOLOGICAL EFFECTS OF ATRAZINE ON DOUGLAS-FIR SEEDLINGS

## INTRODUCTION

Herbicides are becoming increasingly important as silvicultural tools in establishing, thinning and even harvesting of trees as more intensive management practices are applied to our forest lands. Herbicides are especially important in reforestation for reducing the risk of planting failure and protecting the landowner's investment in seedling procurement and planting . Landowners are actively seeking any treatment which will prevent the demise of seedlings, speed their growth, or both.

Drought causes a high percentage of failures in conifer plantations. Competing herbaceous vegetation intensifies dry conditions on sites with a potential for commercial timber production. Herbicides, especially atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), have proven effective in reducing the competing vegetation and are now being used extensively in Christmas tree plantations and in conifer reforestation, especially on droughty Douglas-fir sites.

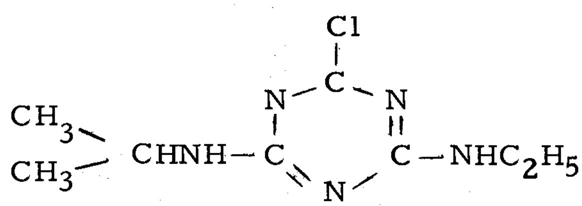
The striking success of chemical weed control has now focused attention on the performance of the young tree. Has the herbicide simply reduced the competition the tree faces for water, sunlight and nutrients or might it also have some physiological effects on the tree itself? Such a question can only be answered by physiological studies.

This exploratory study was undertaken to investigate some of the effects of atrazine on the physiological functions of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings. The changes in photosynthetic and respirational rates, height and weight increases and mineral uptake were studied in the laboratory and in the field. The measurements of photosynthesis and respiration were made on young seedlings grown in a controlled environment chamber; measurements of growth and mineral uptake were made on two-year-old trees growing on field plots.

# REVIEW OF LITERATURE

Atrazine is a member of the triazine family of herbicides which were first synthesized and tested by Gysin and Knusli (23) in 1952 at Geigy laboratories in Basle, Switzerland. The triazines have in common a six membered heterocyclic ring with alternating carbon-nitrogen atoms and alkyl-amino substitution on two of the carbon atoms. The third carbon atom has a chloro, mercapto or methoxy substitution. Atrazine is the analog in most common use in forestry; simazine, the chlorinated diethylamino triazine, is also in wide use.

The structural formula of atrazine is:




It is a white crystalline powder with a melting point of 173-175°C and a molecular weight of 215.7. Atrazine is soluble in water (70 ppmw at 27°C), chloroform (52,000 ppmw), methanol (18,000 ppmw) and petroleum ether (12,000 ppmw). It has very low toxicity to wildlife and fish with acute oral $LD_{50}$ for rats of 3080 mg/kg. There have been no substantiated reports of skin irritations resulting from applications of atrazine (23). Basically, this compound is safe to handle, easy to use, and effective in controlling weeds. It is thus likely to receive wide usage.

## Mode of Action

It has been well established that triazines interfere with the carbohydrate balance of the plants. Gast (17) and Moreland et.al. (35) showed that, although the herbicide blocked sucrose synthesis, a supply of exogenous sucrose overcame the toxicity symptoms in the plant. These studies indicate that the triazines interfere with the formation of the monosaccharides, not with their subsequent incorporation into starch and other polysaccharides. Many studies have shown that triazines are potent inhibitors of the Hill reaction (19, 20, 28, 36), causing 50% inhibition at concentrations as low as $7 \times 10^{-7}$ molar. Investigations suggest that the herbicidal action of these compounds is related to their ability to prevent oxygen evolution and noncyclic photophosphorylation associated with light reaction II (5, 6, 28). Good (19, 27) proposes that the triazines interfere with the function of a catalytic center which is essential for the oxygen production of chloroplasts by electrostatic attraction between the inhibitor and the active site of an enzyme.

Ashton and coworkers (2) have investigated the structural changes wrought by atrazine on the cells and chloroplasts of bean leaves. They noted precocious development of vacuoles, cessation of cambial activity and chloroplast destruction. Damage to chloroplasts started with disruption of frets, swelling of compartments and finally the breakdown of the limiting membrane allowing the cytoplasm and stroma material to escape into the vacuole (3). Ashton hypothesized that this damage was not caused by the herbicide per se but rather by a toxic substance that was formed by the interaction of the chemical and light, possibly a "free radical" (1).

Ashton (4), Sasaki (42), and van Oorschot (48) have conducted investigations of $CO_2$ uptake of plants treated with triazines and all indicate that the chemicals drastically inhibit $CO_2$ fixation in the light. Bean plants exposed to low concentrations of simazine (0.25 - 4 ppm) by Ashton (4), had $CO_2$ fixation blocked almost completely by concentrations above one ppm.

Sasaki and Kozlowski (42) treated red pine seedlings with 20 pounds of atrazine per acre, incorporated into loamy soil. They reported a rapid decrease in photosynthesis and in three to four weeks $CO_2$ uptake was negligible. Two year old needles became flaccid and chlorotic within two weeks and death occurred shortly after four weeks. No mention was made of the triazine concentration in the seedlings.

The same initial suppression of photosynthesis was found by van Ooschot (48) but some plants, such as maize and, to a lesser extent, French bean, had the ability to recover their $CO_2$-fixing capacity following removal of the triazines from the root environment.

He attributed the ability to regain photosynthetic efficiency to physiological inactivation of the absorbed herbicide by the plant.

Numerous studies have been made of the detoxication of triazines by plants and the role this ability might play in the selectivity shown by the herbicides, Montgomery and Freed (33) state that the ability of a plant to detoxify the chlorotriazines by rapidly converting them to the hydroxy analog is the key to resistance. An earlier investigation (51) indicated that the hydroxy derivatives of simazine and atrazine demonstrated no activity as photosynthetic inhibitors of Chlorella. Hamilton and Moreland (24) identified the compound that converted simazine to hydroxy-simazine in corn as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. Other authors have shown that there may be an alternate mode of detoxication which is found in some of the less resistant species. Pea plants dealkylated atrazine to 2-chloro-4-amino-6-isopropylamino-s-triazine, a compound less toxic than atrazine (44).

## Effects on Growth and Nitrogen Content

Kozlowski and coworkers (9, 29, 49) at the University of Wisconsin have conducted numerous studies of the effects of several triazines on conifer seedlings. In general, they found that when the herbicide was soil-incorporated, in a sandy medium, the chemicals were lethal to germinating pine seedlings with application rates as low as one pound per acre. Surface and foliage application of triazines on the other hand, when applied to seedlings 18 weeks old were not harmful. The moisture content of needles on four year old spruce seedlings was lowered considerably by 16 pounds of atrazine per acre. de Vries' (13) study with Monterey pine showed that simazine, soil incorporated at a rate of 6#/A, caused a 12% reduction in dry weight and a slight increase in top-root ratio. Grand and noble fir were not damaged and Douglas-fir and ponderosa pine were only moderately damaged when they were germinated in soil which had been treated with three pounds of atrazine per acre (50).

It has been noted by many experimenters that the chlorotriazines increase the growth and nitrogen content of plant species which tolerate them. Bartley (7) reported that corn plants, treated with simazine, were larger in size, greener in color, and produced greater yields than untreated corn growing in adjacent plots. Ries, Larsen and Kenworthy (40) found that peach and apple trees, treated with simazine and amitrole-T, had higher leaf nitrogen and greater terminal shoot growth than similar trees which had mechanical weed treatment. Goren (21) and Gast (18) reported similar responses for citrus trees and grape vines. Subsequent experiments (15, 22, 38) with corn showed that simazine, especially in low concentrations, substantially increased total plant nitrogen and dry matter yield. Freney (15) found that it was not an effect on the nitrogen in the soil nor on the microorganisms, but rather a direct effect on plant metabolism. This increased efficiency of nitrate utilization is apparent only when the plants are grown under sub-optimal growing conditions of low nitrate availability and low temperature (46). Ries et al. (38) have shown that this enhancement occurs only when the plants are grown with nitrate, not ammonia, as the source of nitrogen. Study of this response to simazine now centers around the alterations induced in nitrate reductase activity. The increased levels of nitrate reductase, up to eight times greater in plants grown on simazine, lead Ries et al. (38) to propose that simazine brought about the increase in enzyme activity by: (a) increasing the uptake of nitrate, (b) increasing the rate of nitrate reductase synthesis or (c) stimulating protein synthesis with a consequential rise in enzyme activity, or some combination of the three.

# PART I. CONTROLLED ENVIRONMENT STUDY

Any chemical which, when introduced into the plant, causes an alteration in the reactions involved in photosynthesis or changes the rate of these reactions, will profoundly affect the metabolic activities of the plant. Similarly, a chemical which would affect respiration, would also have a marked effect on the well-being of the plant. It follows, then, that changes in these two processes could be an indication of the integrated physiological response of the plant to a herbicide.

The photosynthetic and respirational rates of Douglas-fir seedlings grown in a controlled environment chamber were measured periodically to give an indication of the rate at which the chemicals affected the seedlings and the duration of these effects.

Two growing media were used in this study to help ascertain the role soil particle size plays in the phytotoxicity of the herbicide. Numerous authors (12, 25, 43, 47) cite differential physiological responses obtained when the plants were grown on various soils.

# MATERIALS AND METHODS

The seedlings for these experiments were grown from seed collected in the Coast Range near Eddyville, Oregon. All seed came from a single tree, making the trees all half-sibs, in hopes of reducing genetic variability. The seeds were germinated in petri dishes and when the hypocotyl had attained a length of at least one cm, they were planted in pint plastic pots. The growing media were washed concrete sand, for Experiment I and Wren silt loam $A_1$-horizon, for Experiment II. The estimated organic matter content of the forest soil was 6 percent. Four seeds were planted per pot; then thinned before treatment with atrazine, so the two seedlings remaining would not shade each other.

The seedlings were grown in a controlled environment chamber under the following conditions:

| | Day | Night |
|---|---|---|
| Photoperiod | 16 hrs. | 8 hrs. |
| Temperature | 30°C | 20°C |
| Relative Humidity | 50-60% | 80-90% |

The light intensity was 900 f. c., emitted by a bank of cool white fluorescent tubes augmented by eleven 100 watt incandescent bulbs.

The seedlings were watered regularly and Hoagland's nutrient solution applied once weekly to those grown in sand.

The photosynthetic and respiration rates were measured as changes in $CO_2$ concentration in a closed 2.77 liter system using a Beckman L/B 15 infrared gas analyser. The appartus used was similar to the one described by Krueger (31). A new cuvette made of lucite designed by Dr. Kenneth Krueger of Pacific Northwest Forest and Range Experiment Station, Corvallis, Oregon used a molded rubber stopper as a seal at the base of the seedling stems. A circulating water bath seven cm deep was placed above the cuvette to shield the seedlings from the heat emitted by the 1200 watt light source.

Light energy in the cuvette at the seedling level was 0.369 ly/min ($\sim$3,000 foot-candles), as meaaured with a Kipp Actinometer, and was kept constant for all measurements. This was found by Krueger (31) to be saturation light intensity for Douglas-fir seedlings of a comparable age. The air temperature in the cuvette was a constant $30^{\circ}C$ and the relative humidity $45\% \pm 5$. The measurements were made in the carbon dioxide concentration range of 330 to 370 ppm. Dark respiration was measured immediately following the photosynthetic determinations with the same system by turning off the light and covering the cuvette with a black cloth. The rates of photosynthesis and respiration were computed from the changes in $CO_2$ concentration and expressed in mg $CO_2$ absorbed or evolved per gram dry weight of needles per hour.

The atrazine was applied in varying concentrations as pounds per acre, active ingredient, based on surface area of the pot. A concentrated stock solution was prepared and the appropriate dose was formulated for each pot by diluting the stock solution with distilled water to make 50 ml aliquots.

Concentrations of atrazine used were 0, 1/4, and 1/2 pounds per acre (0, 0.275, and 0.55 ppmw), active ingredient, in Experiment I, and 0, 2, 4, and 8 pounds per acre (0, 2.9, 5.8, and 11.6 ppmw) in Experiment II. The highest concentration in each experiment was found in screening trials to be near the lethal dosage for Douglas-fir seedlings growing in sand and soil respectively. Treatments were assigned pots at random.

The seedlings were 90 days old when treated with atrazine in Experiment I, and 75 days old when treated in Experiment II.

Photosynthetic and respirational measurements were made 0, 3, 9, and 36 days after treatment on those plants in Experiment I and after 0, 3, 9, 27, and 90 days on those plants in Experiment II. The first experiment was designed as a 3 (treatments) x 4 (time after treatment) x 4 (replications) factorial. The second was a 4 x 5 x 4 factorial experiment. Three measurements, each a day apart, were made on the pots in Experiment I, while each pot in Experiment II was measured twice.

# RESULTS - LABORATORY STUDIES

## Experiment I

Atrazine had a marked effect on the photosynthesis and respiration of Douglas-fir seedlings grown in sand. Within a short time after application of the herbicide, the total photosynthetic rate was depressed, with the depression related to the concentration of the chemical applied (Figure 1). The rate of carbon dioxide fixation four days after treatment with 1/4 pound of atrazine per acre was 18.3% below the rate of the untreated control. One half pound of the herbicide per acre reduced the total photosynthetic rate by 56.1% after the same period of time.

After the initial inhibition the seedlings began to regain their photosynthetic efficiency. Ten days after application of atrazine, those seedlings treated with 1/4 pound per acre had rates equivalent to those of the control plants. After ten days those seedlings treated with 1/2 pound per acre showed some recovery when compared to the rates of $CO_2$ uptake four days after the start of the experiment. Thirty-six days after treatment with 1/4 pound per acre the seedlings had photosynthetic rates which exceeded that of the controls by 68.2%. Those seedlings receiving the higher concentration of atrazine had recovered and had photosynthetic rates nearly equal to those of the control plants.

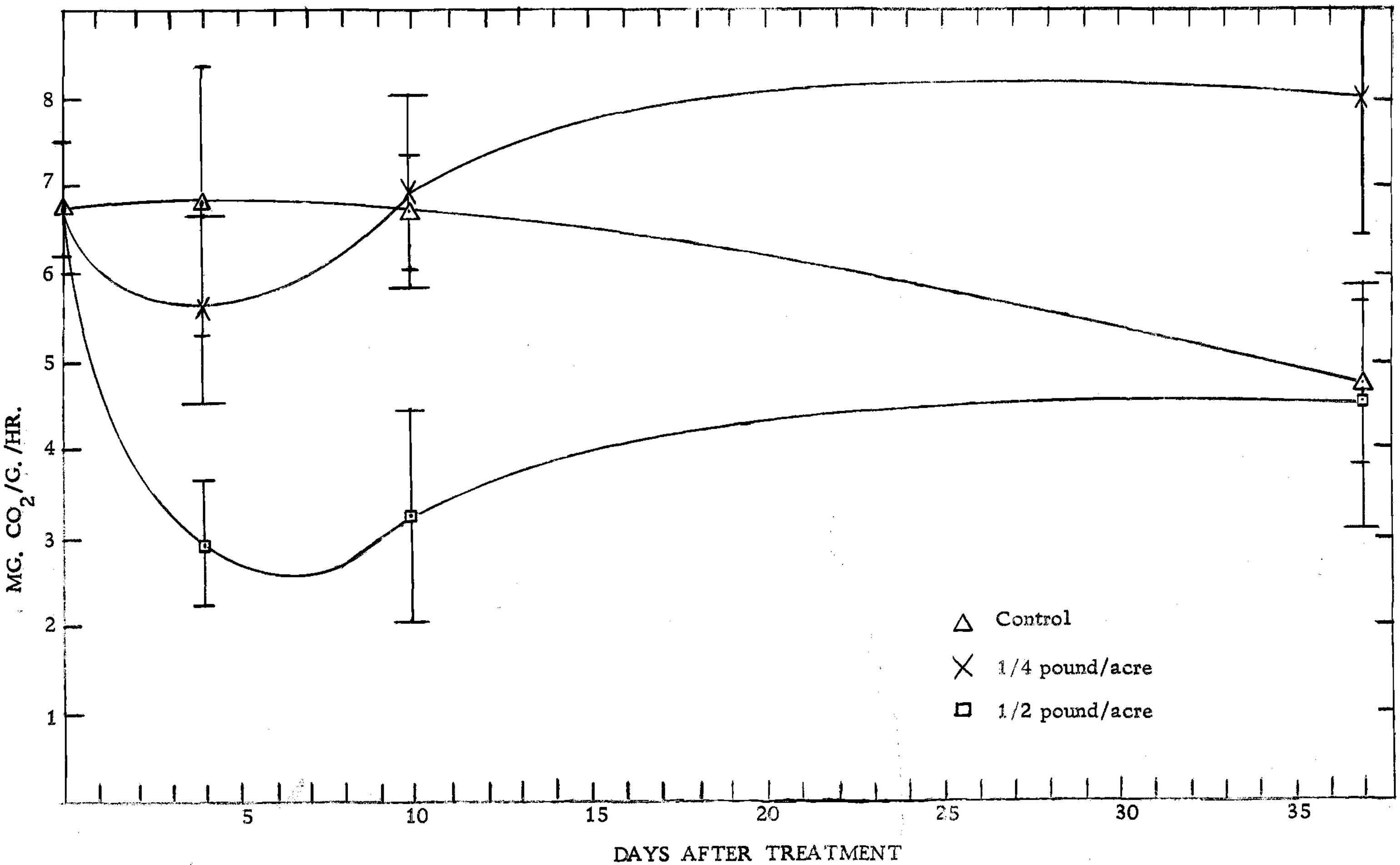



Figure 1. Rates of total photosynthesis of Douglas-fir seedlings grown in sand, after application of two concentrations of atrazine (95% confidence limits plotted).

Dark respiration was affected by atrazine in a way similar to photosynthesis, but to a lesser extent (Figure 2). Four days after treatment with 1/4 or 1/2 pound per acre of the herbicide, the respirational rates of the seedlings were about 20% below the rates of the control plants. All treatments showed a subsequent recovery, with those plants which received 1/4 pound per acre having rates of respiration which exceeded those of the control seedlings by 77.7% after 36 days.

Net photosynthesis was increased significantly by the 1/4 pound per acre treatment. After 36 days, the plants to which atrazine had been applied fixed 7.060 mg $CO_2$ per hour per gram dry weight of needles, whereas the mean rate for the control plants was 4.226.

## Experiment II

Douglas-fir seedlings grown in a clay loam forest soil were resistant to much higher application rates of atrazine than the seedlings grown on washed sand. The only significant differences in photosynthetic rates were observed three days after treatment with the herbicide. The highest concentration used, eight pounds per acre, reduced total photosynthesis for only a few days, with subsequent recovery (Figure 3). Treatment with two pounds of atrazine per acre caused a short-lived photosynthetic enhancement which was evident three days after treatment. At the 9 and 27 day measurement periods

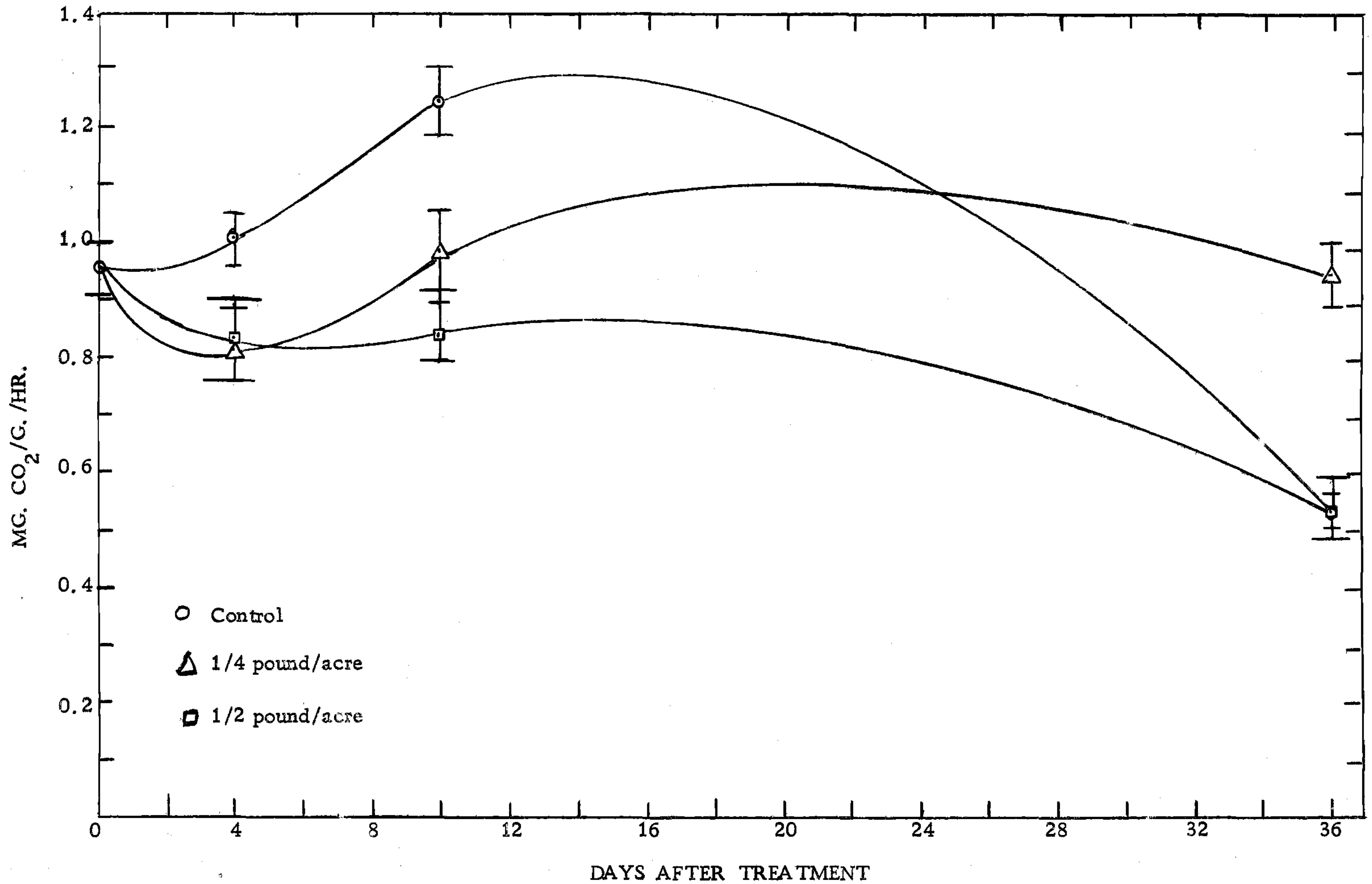



Figure 2. Rates of dark respiration of Douglas-fir seedlings grown in sand, after application of two concentrations of atrazine (95% confidence limits plotted).

the photosynthetic rate of all treatments were below that of the control but not significantly. Ninety days after treatment the measurements were so widely dispersed for all concentrations that no conclusions could be drawn.

There were no significant differences in respiration rates observed with any treatment at any age (Figure 4).

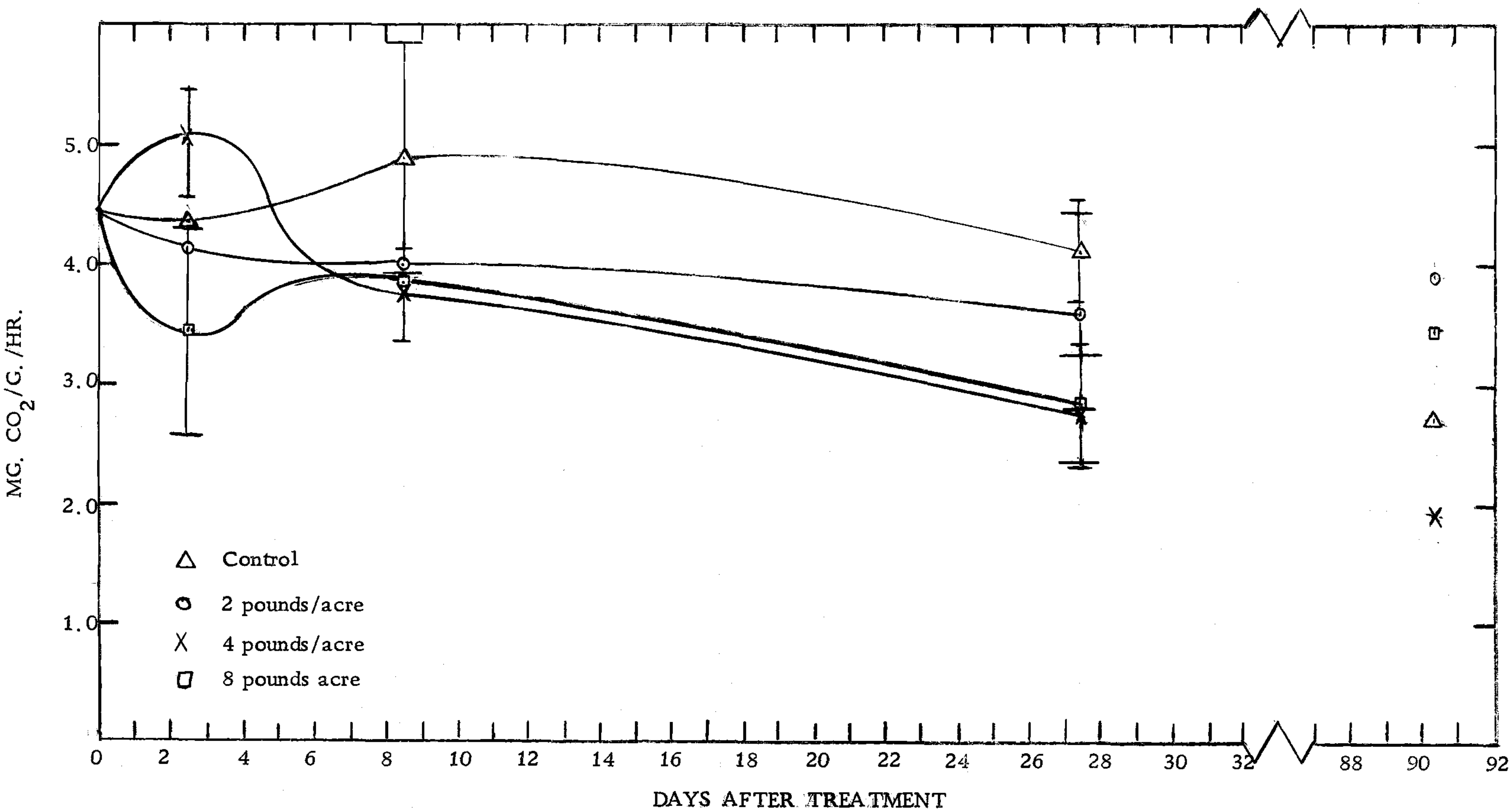



Figure 3. Rates of total photosynthesis of Douglas-fir seedlings grown in soil, after application ot three concentrations of atrazine. (95% confidence limits plotted).

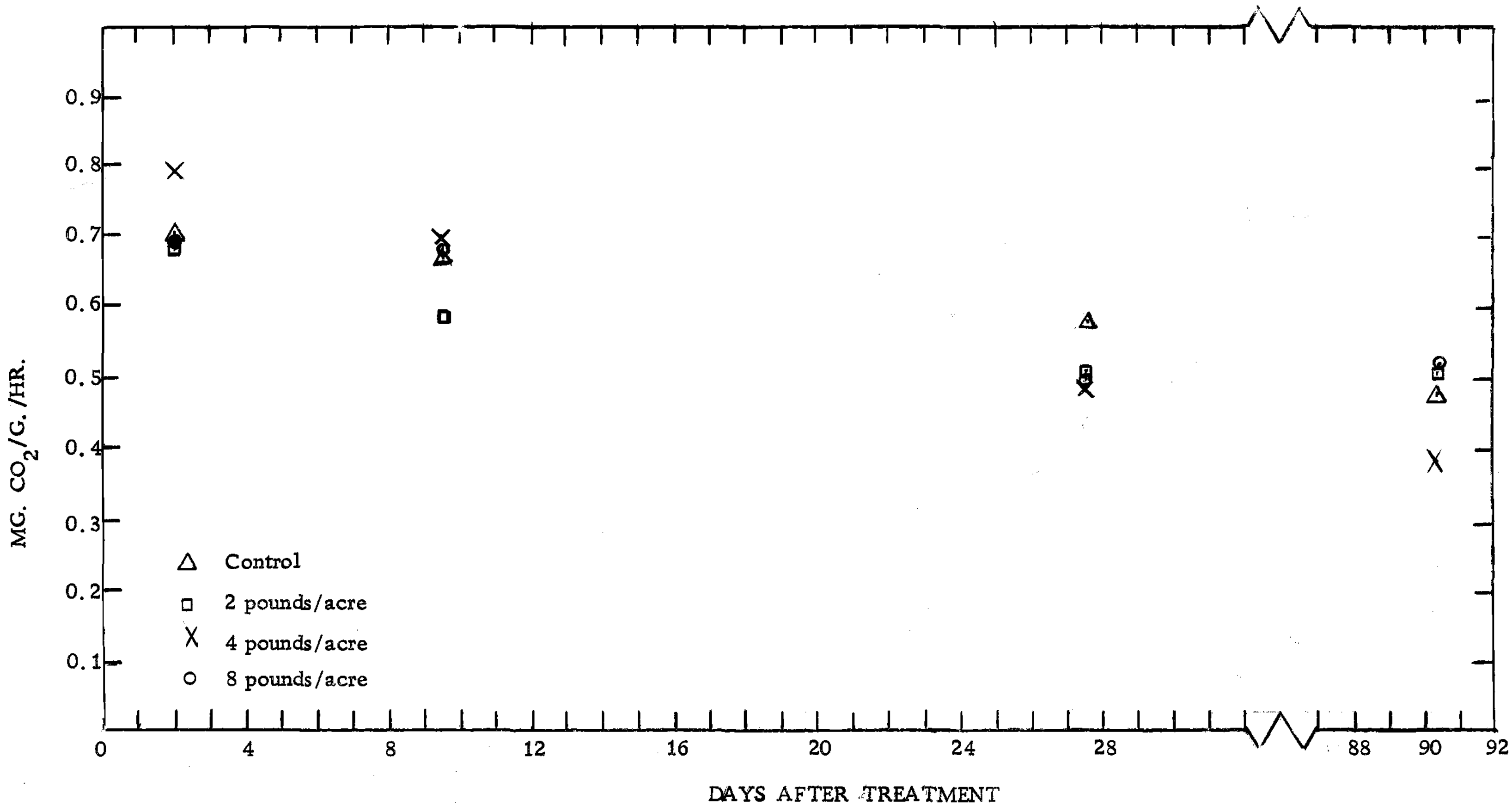



Figure 4. Rates of dark respiration of Douglas-fir seedlings grown in soil, after application of three concentrations of atrazine. No significant differences.

# DISCUSSION - LABORATORY STUDIES

Douglas-fir seedlings do not appear to be biochemically resistant to atrazine. Initial screening tests showed that rates higher than 1/2 pound per acre were lethal to a high percentage of the seedlings grown in washed sand. The great genetic variation of Douglas-fir was quite evident, both in screening trials and in the various experiments. The two seedlings in a pot often had very different morphological and physiological responses to treatment, even when the plants were the same age and size. When treated with high concentrations of atrazine, one seedling occasionally exhibited toxicity symptoms and died in about two weeks, while the other seedling remained a healthy appearing, dark green color. This inherent variation contributed considerably to the wide range of values obtained in these experiments as is evidenced by the large confidence limits plotted on Figures 1, 2, and 3, and the dispersion of points on Figures 5, 6, and 7 when the photosynthetic and respiration rates were plotted for individual pots.

The untreated (control) plants of both experiments exhibited declining rates for both photosynthesis and respiration over the measurement periods of the investigation (Figures 5, 6, and 7). Krueger (31) noted a similar decline in both physiological functions in his experiments with Douglas-fir seedlings of comparable age. The decline in photosynthesis may be attributed to different needle area to

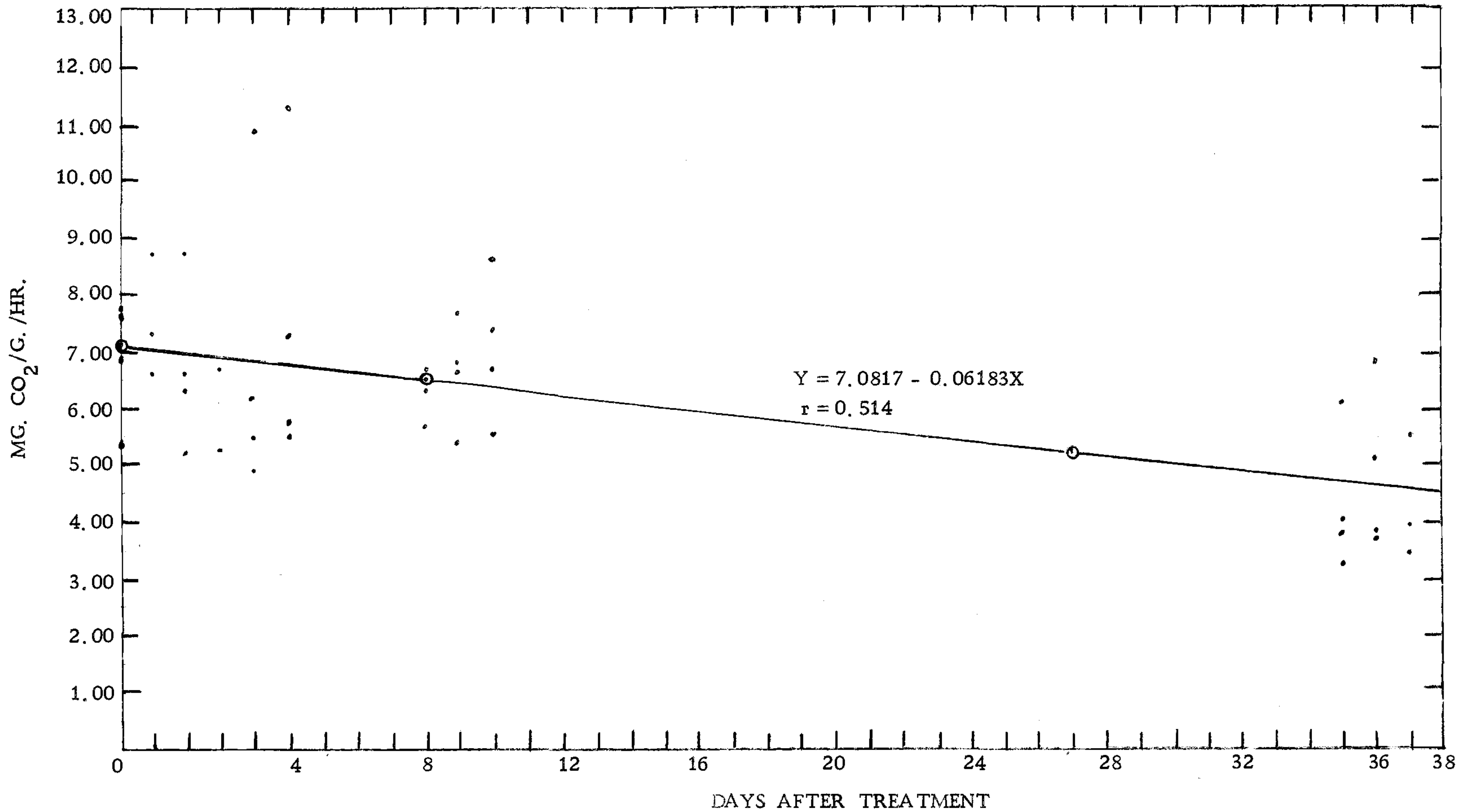



Figure 5. Change in photosynthetic rate of control seedlings grown in sand with increasing age. Regression line significant at 1% level.

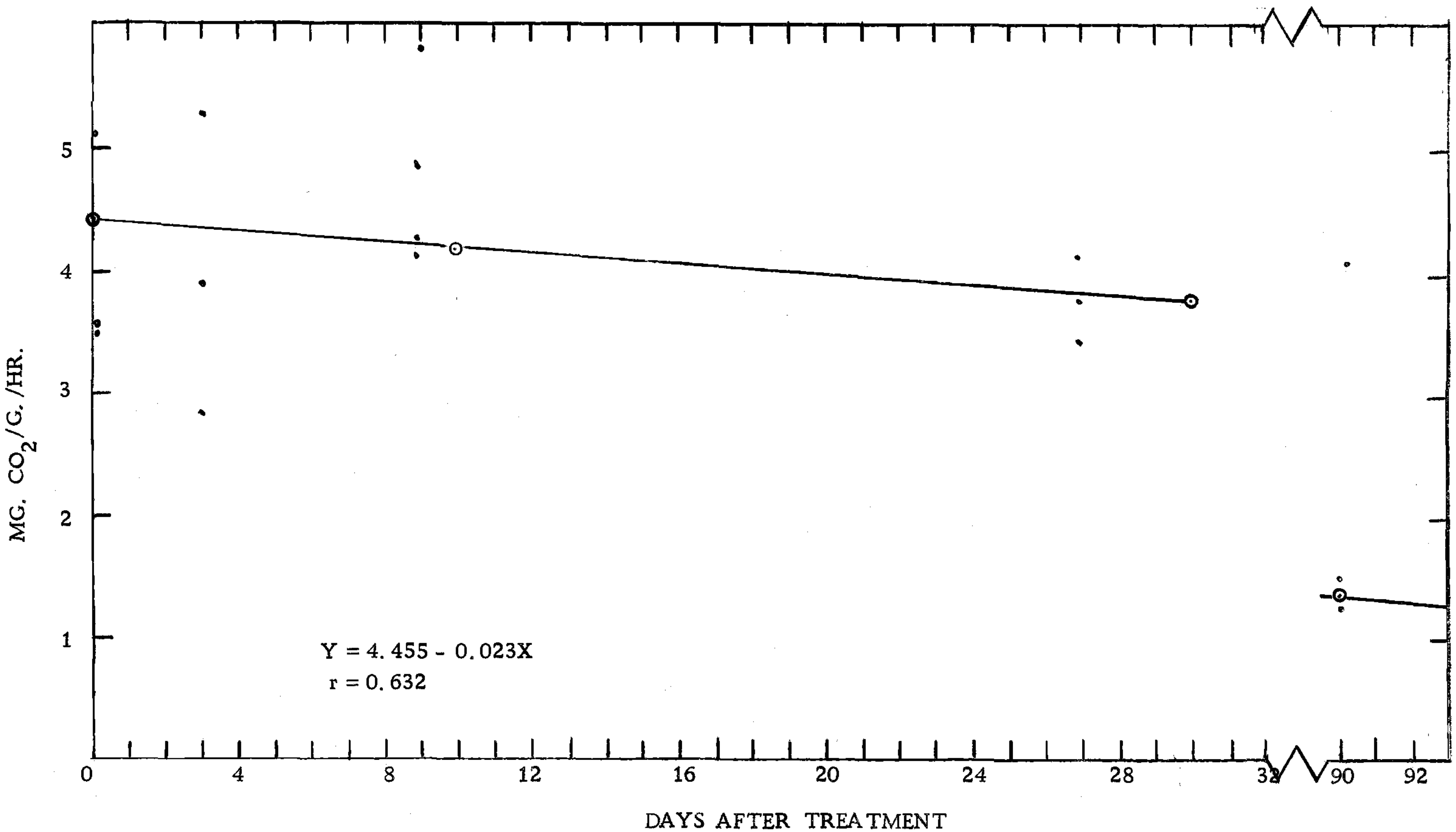



Figure 6. Change in photosynthetic rate of control seedlings grown in soil with increasing age. Regression line significant at 1% level.

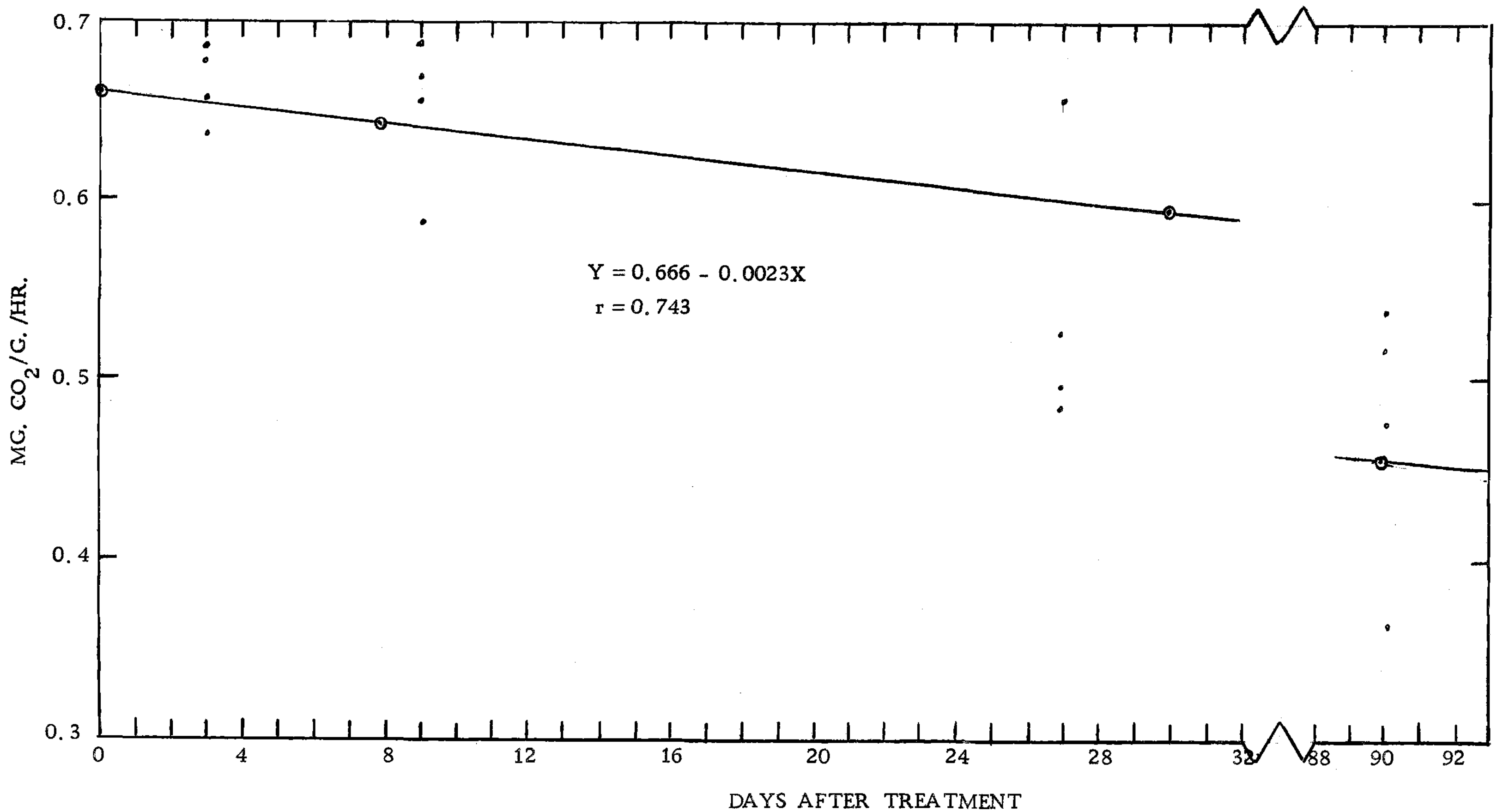



Figure 7. Change in respiration rate of all seedlings grown in soil with increasing age. Regression line significant at 1% level.

weight relationship, increased self-shading, or reduced efficiency of the chloroplasts. A similar reduction in mitochondrial efficiency or number may explain the decrease in respiration.

The inhibitory effects of atrazine on the physiological functions of the seedlings is evidenced by the reduced photosynthetic and respirational rates noted three and four days after treatment in both experiments. This is in agreement with other studies of the effects of triazines on plants (4, 16, 42, 48). The inhibitions recorded were likely magnified by the high temperature (30°C) under which the plants were grown in the chamber and under which the measurements were made in the cuvette. Kozlowski _et al_. (30) found that pine seedlings grown in a chamber kept at 30°C were much more susceptible to atrazine than were seedlings germinated in cooler growth rooms.

The less pronounced inhibition that occurred in Experiment II is probably related to the increased adsorption of the herbicide exhibited by the clay loam soil as compared to the washed river sand. Thirty-two times as much atrazine was required to cause a similar inhibition in the soil (8#/A) as in the sand (1/4#/A). Many studies have shown that pH of the soil, clay content, and organic matter content have a marked influence on the adsorptive characteristics of a growing medium (14, 27). Concentration of the chemical in the soil solution is a function of time and initial concentration as well as being related to the soil factors mentioned above.

Several authors have noted beneficial effects such as increases in shoot growth, dry weight and protein content when resistant or partially resistant plants have been exposed to triazines (7, 18, 21, 38, 40). The seedlings in Experiment I, in response to low levels of atrazine, had photosynthetic rates which significantly exceeded the rates of the control plants after the initial period of inhibition described above. Those seedlings which were treated with 1/4 pound per acre had photosynthetic rates which surpassed the controls by nearly 70 percent 36 days after treatment. The dry weight of the treated seedlings did not exceed that of the controls despite the increased photosynthesis, probably because of the initial inhibition of photosynthesis and the short period of increased growth (Appendix Table A). In Experiment II, where the last measurements were made 90 days after treatment, there were significant gains in dry weight with the increase related to the concentration of atrazine applied (Figure 8 and Appendix Table B). In the second experiment the atrazine adsorbed by the soil may have acted as a reservoir capable of supplying the herbicide to the soil solution, and consequently to the seedlings, in low concentrations over an extended period of time.

The respiration rates recorded for the seedlings treated with atrazine may help to explain some of the apparent incongruities reported in the literature relating to the effects of triazines on dark

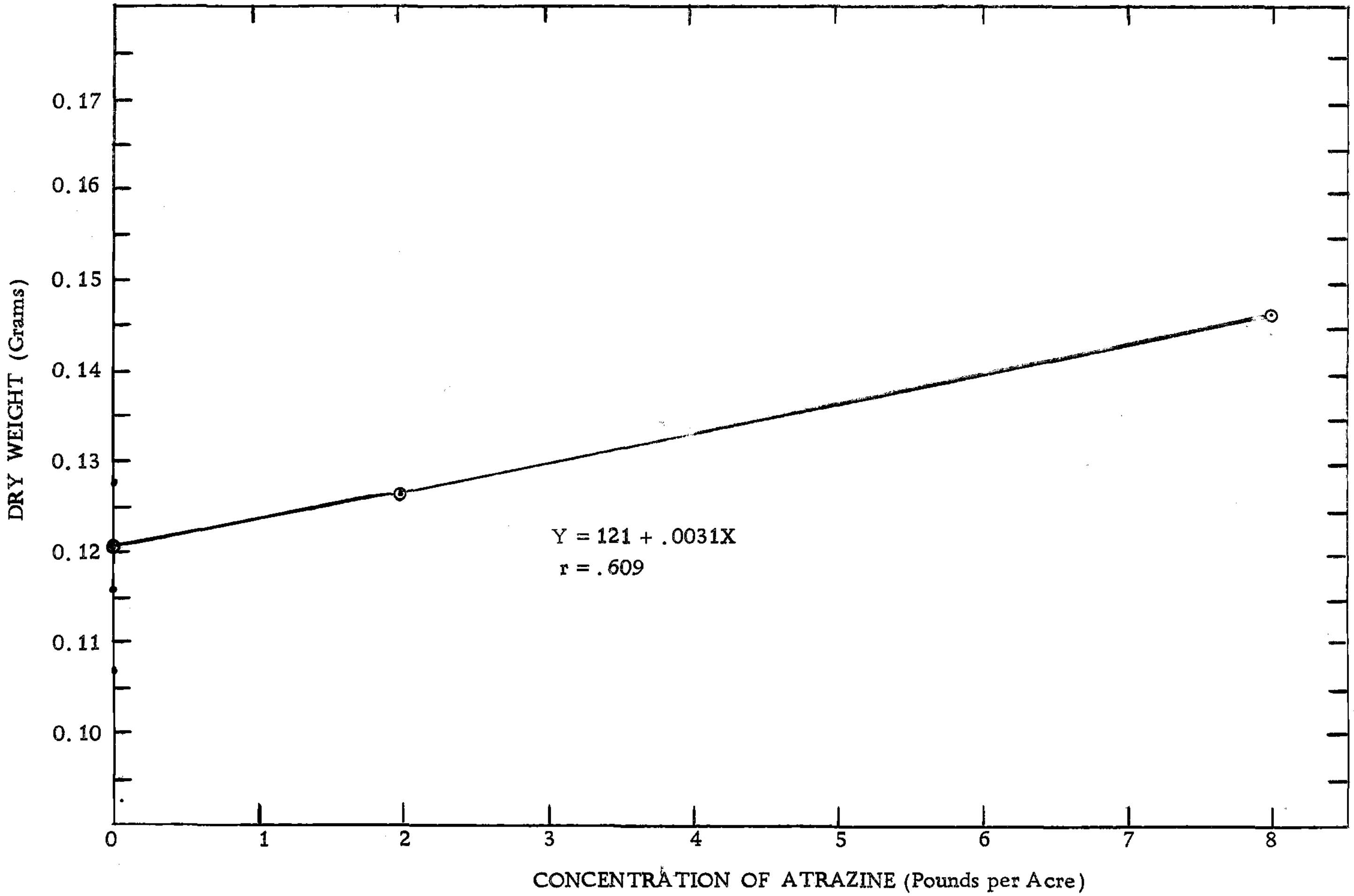



Figure 8. Increase in dry weight of seedlings grown in soil with increasing concentrations of atrazine 90 days after application. Regression line significant at 5% level.

respiration. Funderbuck and Davis (16) report that atrazine decreased respirational rates of all plants, both resistant and sensitive, tested 7 and 11 days after treatment. Ries et al. (38), on the other hand, measured respirational rates that were significantly higher than the control on 21-day-old rye plants exposed to low concentrations of simazine. The rate of respiration in this study appeared to be a function of the time elapsed after application of the herbicide. A decrease in the respirational rate was noted four and ten days after treatment with a low concentration of atrazine in Experiment I. In the same experiment, 36 days after the herbicide was added to the sand, the seedlings were respiring at a rate considerably higher than the control plants. The initial repression, possibly related to reduced growth caused by photosynthetic inhibition, was followed by an increase in respiration which could be related to increased growth, accelerated nitrogen uptake, and/or stepped up production of amino acids and proteins (8, p. 177-197).

Atrazine markedly altered the fresh weight/dry weight ratio of the seedlings grown in soil. There was a significant increase in the ratio as the concentration of the herbicide increased (Figure 9 and Appendix Table B). Ries et al. (39) reported similar results for rye grown in nutrient solution in which a low concentration of simazine had been added. Clausen and Kozlowski (9) noted an increase in the

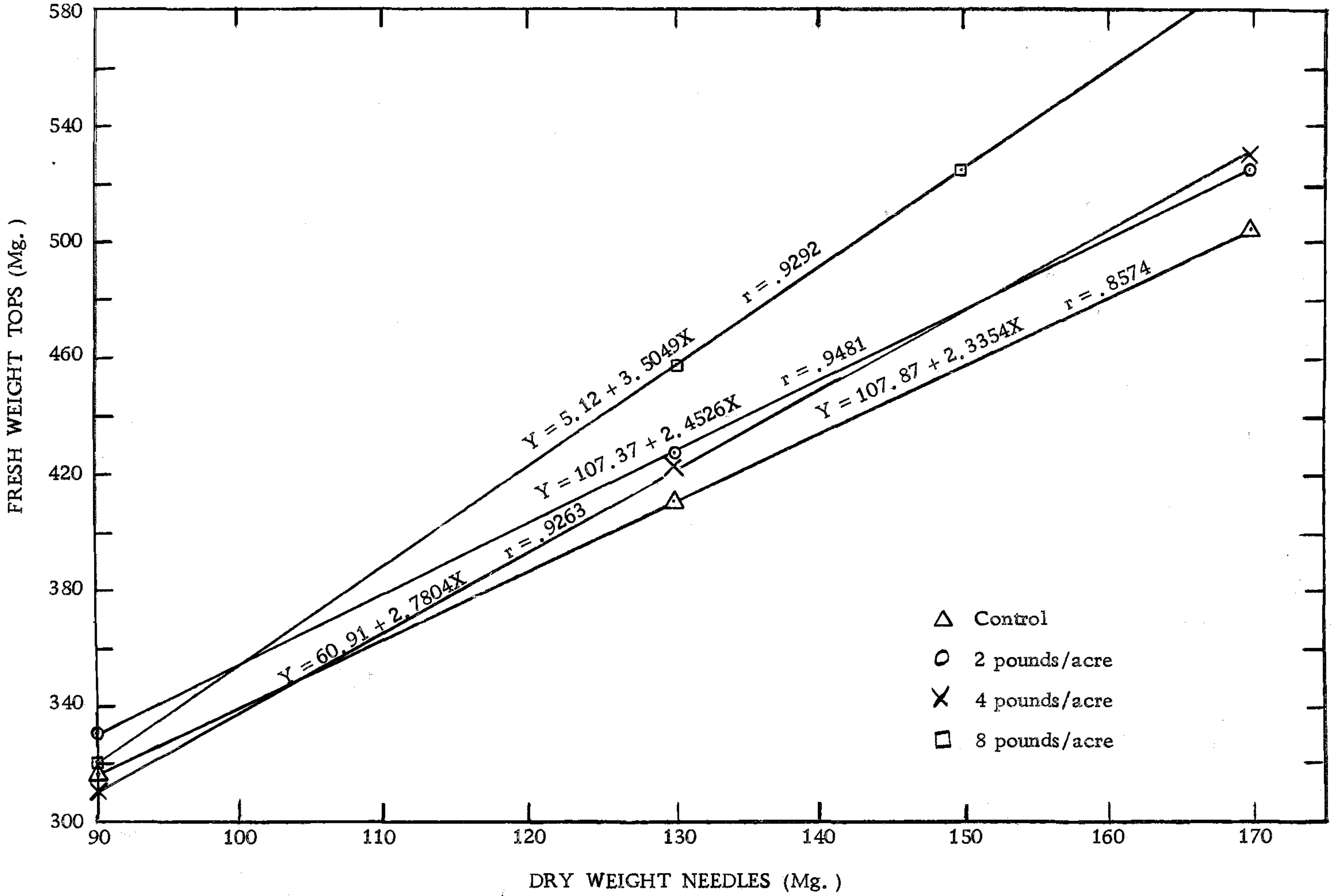



Figure 9: Linear regressions of fresh weight tops and dry weight needles of seedlings grown in soil for 90 days after treatment.

moisture content of 3-0 spruce seedlings when treated with four pounds of atrazine per acre but significant decrease when higher concentrations of the herbicide were applied.

# PART II. FIELD STUDY

The first part of this study was conducted on seedlings grown in a growth chamber where the environment was controlled. In the second part the effects of atrazine on Douglas-fir seedlings grown under field conditions were investigated.

An earlier study indicated atrazine had a beneficial effect on growth of conifers but it could not be determined if this response was attributable to reduced competition alone or if the chemical has a stimulatory effect on the seedlings. The entire study area was kept weed free by periodic hoeing in an attempt to separate the physiological influence of the herbicide from environmental effects of reduced competition.

# METHODS AND MATERIALS

The field study was conducted on a one-fourth-acre plot of ground near Philomath, Oregon. The area had a bentgrass cover before plowing and discing to prepare a suitable planting site. The soil is a reddish clay loam of the Wren series, well drained, with a northwest aspect and slope of five to ten percent.

The planting stock were 2-0 Douglas-fir seedlings. They had been grown in the Oregon State University nursery on the McDonald Forest at Corvallis from seed collected from a low elevation in the Willamette valley. The plots were laid out in a randomized block design with three treatments and four blocks. Atrazine was applied with a MAT-OSU plot sprayer borrowed from the Farm Crops Department. Three application rates of atrazine were tested: 0, 1 1/3, and 4 pounds per acre, active ingredients. All treatments were applied in water, at a total spray volume of 20 gallons per acre. The plots were sprayed March 31, 1967, three days prior to transplanting the seedlings.

The plots were four feet wide and 100 feet long. Fifty trees were planted on each atrazine treated plot and 75 trees on each control plot. The trees were planted two feet apart in the rows, with the additional 25 trees in the control plots being interplanted between alternate seedlings.

The plots were hoed periodically throughout the summers of 1967 and 1968 to eliminate all competing vegetation.

## Collection of Data

Measurements of current year's leader growth were made on July 16, 1967 and September 24, 1968. The trees had ceased elongation and had set buds by these dates.

In November, 1967, 30 trees, and in September, 1968, five trees were carefully dug up from each row and the number of actively growing root tips counted. Entire trees were then dried in an oven at 80°C for five days before dry weight measurements were made. The plant material was then ground in a Wiley mill and analysis made for total nitrogen using the micro-Kjeldhal process. Entire stems were used for analysis.

# RESULTS

The physiological effects of atrazine on the growth of field grown Douglas-fir seedlings were determined by measuring differences in height growth, dry weight increase, shoot/root ratio and percent total nitrogen in the aerial portion of the plants.

Height growth was adversely affected by the chemical the year of treatment, but the year following application, the growth on the treated plots exceeded, but not significantly, the elongation shown on control plots (Table 1).

Treatment with atrazine also inhibited dry weight increase the year of treatment. The weight of seedlings grown on plots treated with the herbicide at the four pound per acre rate weighed significantly less than the control (Table 2). The second year saw complete recovery with no significant differences between treated and control plants, in fresh or dry weight.

The shoot/root ratio changed considerably between 1967 and 1968, but there were no significant differences between treatments either year (Table 3).

Seedlings grown on plots which were treated with atrazine had higher levels of total nitrogen the first year than did plants grown on control plots (Table 4). As was found with the growth measurements, the differences were not observed the second year.

Table 1. Mean terminal shoot elongation by years (cm).

| Year | Atrazine Applied (lb/acre) | | |
|---|---|---|---|
| | 0 | 1 1/3 | 4 |
| 1967 | 5.9 | 5.3** | 5.4** |
| 1968 | 22.9 | 25.1 | 25.7 |

**Mean significantly less than control at 99% confidence level.

Table 2. Mean dry weight of entire seedlings by years (g).

| Year | Atrazine Applied (lb/acre) | | |
|---|---|---|---|
| | 0 | 1 1/3 | 4 |
| 1967 | 17.17 | 16.56 | 13.65** |
| 1968 | 64.68 | 55.36 | 64.70 |

**Mean significantly less than control and 1 1/3 acre at 99% confidence level.

Table 3. Mean shoot/root ratio by years.

| Year | Atrazine Applied (lb/acre) | | |
|---|---|---|---|
| | 0 | 1 1/3 | 4 |
| 1967 | 0.849 | 0.883 | 0.876 |
| 1968 | 1.934 | 1.769 | 1.972 |

No significant differences.

Table 4. Mean percent total nitrogen of seedling needles and twigs.

| Year | Atrazine Applied (lb/acre) | | |
|---|---|---|---|
| | 0 | 1 1/3 | 4 |
| 1967 | 2.02 | 2.10* | 2.08* |
| 1968 | 2.01 | 1.96 | 2.00 |

*Means significantly higher than control at 95% confidence level.

# DISCUSSION

The effects of atrazine on the growth of field grown Douglas-fir seedlings is similar to the results noted in the laboratory study, only the time required for recovery was longer in the field. Growth was inhibited the first year as shown by significantly less height growth and weight increase on those seedlings which were grown on treated plots. The higher concentration had the greatest repressive effect on dry weight increase while both treatments reduced height growth similarly.

The second year growth on the treated plots exceeded growth on the controls. The height growth was significantly greater at the 90 percent confidence level for the trees grown on the higher concentration of atrazine. Dry weight of all seedlings was nearly the same at the end of the second year, showing full recovery from the depressed condition noted after the first year.

The repression of growth observed the first year in this experiment is quite similar to the results obtained with Monterey (13), loblolly and slash pine (10) and rye (41). It may be explained by a reduction in photosynthesis for a considerable period of time without a similar retardation of respiration as was found in Experiment II of this study. Growth of the aerial portion of the seedlings had ceased by mid-June, 1967, two and a half months after treatment. This early cessation of growth, probably a result of the droughty spring and summer, would not have given the plants time to recover photosynthetic efficiency the first growing season. The increased growth the second year may be a result of an enhanced photosynthetic rate similar to that observed in Experiment I. Other experimentors (18, 41) have found that low concentrations of triazine herbicides have increased dry matter production. The residual concentration of atrazine the year following treatment would be expected to be very low because of leaching, adsorption or degradation by micro-organisms.

Contrary to the results of Conner and White (10) the herbicide did not alter the shoot/root ratio of the seedlings. This may be a difference due to species of seedlings, age of the seedlings, or growing media.

The effects of atrazine on the total nitrogen content of the treated seedlings follows closely the findings of other investigators who used such dissimilar plants as rye, corn, rice, beans, slash pine, loblolly pine, peach, and apple (10, 39, 40, 41). All have observed a significant increase in percent nitrogen after treatment with subtoxic levels of triazines.

# SUMMARY

The beneficial effects of herbicides as an aid in seedling establishment by reducing competition for moisture, sunlight and available nutrients have been shown. Interest has turned now to the effects of sublethal concentrations of the herbicide on the growth of the seedlings. This study investigated some of the physiological effects of atrazine on Douglas-fir seedlings grown both in a controlled environment chamber and in the field under natural conditions.

Rates of photosynthesis and respiration were affected similarly, but to a different degree, by atrazine. These functions of the plant were inhibited by the chemical for a few days, with subsequent recovery. In the case of seedlings grown in sand and treated with atrazine at a rate of one-fourth pound per acre, the rates of both physiological functions were enhanced appreciably. A higher rate of application suppressed both photosynthesis and respiration to a greater extent, with full recovery obtained after 36 days.

Similar but less pronounced effects were observed when seedlings grown on forest soil were treated with much higher concentrations of the herbicide. Eight pounds per acre inhibited photosynthesis for a few days with recovery complete after nine days. Lower concentrations did not markedly alter the rates of either photosynthesis or respiration.

Atrazine inhibited growth of field grown seedlings the first year following treatment. Terminal elongation was reduced slightly and the dry weight of treated seedlings was significantly lower than the weight of control seedlings. In both cases the greater inhibition occurred with the higher application rate of herbicide. The second year following application the treated plants recovered and the losses noted the first year were negated by increased growth as shown by greater terminal elongation and an increase in dry weight.

The shoot/root ratios were not altered by the treatment with the herbicide either the first or the second year after application of the chemical.

Measurements of nitrogen in the aerial portion of the seedlings indicated that treatment with atrazine increased the ratio of total nitrogen to dry weight the year following treatment. There were no significant differences in percent nitrogen the second year.

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# APPENDIX

Appendix Table A. Mean dry weight and fresh weight of seedlings grown in sand and treated with two concentrations of atrazine.

| Days after treatment | Atrazine applied (lb/acre) 0 | | 1/4 | | 1/2 | |
|---|---|---|---|---|---|---|
| | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) |
| 0 | .112 | .344 | .111 | .351 | .119 | .382 |
| 3 | .099 | .323 | .113 | .330 | .108 | .362 |
| 9 | .123 | .363 | .096 | .337 | .094 | .354 |
| 36 | .155 | .460 | .125* | .435 | .102* | .386 |

*Means significantly less than control at 95% confidence level.

Appendix Table B. Mean dry and fresh weights of seedlings grown in forest soil and treated with two concentrations of atrazine.

| Days after treatment | Atrazine applied (lb/acre) 0 | | 2 | | 4 | | 8 | |
|---|---|---|---|---|---|---|---|---|
| | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) |
| 0 | .103 | .356 | | | | | | |
| 3 | .102 | .352 | .099 | .345 | .109 | .371 | .103 | .347 |
| 9 | .109 | .357 | .129 | .413 | .123 | .411 | .103 | .365 |
| 27 | .115 | .348 | .116 | .378 | .112 | .395 | .114 | .391 |
| 90 | .118 | .380 | .124 | .410 | .145* | .463 | .141* | .476 |

*Means significantly greater than control at 95% confidence level.