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Title: THE METABOLIC PATHWAYS AND PHYSIOLOGICAL EFFECTS OF ATRAZINE IN DOUGLAS-FIR SEEDLINGS

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Herbicides are being used as a tool by forest managers to speed the restocking of sites where tree seedlings face competition from grasses for limited moisture, light and nutrients. Retarded growth or, more commonly, death of the seedlings would occur if a selective herbicide was not used to control competing vegetation on these sites. The chemical used must be selective so that it provides release from competition yet does not damage the crop species.

This study investigates the physiological effects of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) on Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings and the metabolism of the herbicide by the young trees. Measurements of photosynthesis, respiration and transpiration were made over a 30 day period to elucidate the effects of various levels of the chemical on these processes. The uptake and the metabolism of the herbicide were monitored and this made it possible to correlate the physiological responses noted with the foliar concentration of the herbicide.

The rates of photosynthesis were altered drastically by atrazine. Exposure to 1.16×10^{-6} molar atrazine reduced photosynthesis by 63 percent while treatment with the lowest concentration (1.45×10^{-7} molar) resulted in rates which were more than 30 percent greater than the controls. Internal levels of atrazine ranged from 10 to 60 nmoles per gram dry weight needles. There was a close negative correlation ($R = 0.873$) between the photosynthetic rate and the foliar levels of the herbicide.

Respiration was not markedly affected by atrazine except when the photosynthetic rates were greatly altered. This indicated that the substance was not directly regulating respiration, but that the initial drop and subsequent increase in rates were a reflection of the well-being of the plants.

Both time after treatment and concentration of atrazine affected the transpirational rates of the seedlings. Transpirational rates paralleled but lagged behind those of photosynthesis when the seedlings were first exposed to the highest concentration of the herbicide. After 30 days exposure to the compound the transpirational rates of the treated seedlings exceeded that of the controls by as much as 80 percent, indicating that the low levels of herbicide were stimulating water uptake.

The study of the uptake of atrazine by the tree seedlings indicated that it was a passive process, that is, the rate of herbicide

uptake paralleled the rate of water uptake from the growing medium.

The investigation of metabolism showed that the partial resistance to atrazine attributed to Douglas-fir is most likely a result of the plant's ability to detoxify a portion of the herbicide taken up. The rate of metabolism was such that at the end of the 30 day experiment only three percent of the herbicide taken up by the plants was in the undegraded form. The plants appear to detoxify atrazine by hydroxylation and peptide conjugation of the parent compound.

A proposed mode of action for atrazine was developed from data gathered in this study. It appears that the effects of atrazine on these plants, and probably others, is concentration dependent and that the chemical is affecting two separate metabolic processes. This would account for the often opposite responses of the plant to different levels of the herbicide.

At low concentration the chemical appears to act as a growth promoting substance which exhibits cytokinin-like properties. The evidence for this effect was the increased rates of photosynthesis, transpiration and metabolism observed.

When internal levels of the herbicide exceeded 30 nmoles per gram dry weight needles the deleterious effects of the compound were observed. Atrazine at these elevated concentrations is a potent inhibitor of photosynthesis. Death of the plant was the ultimate effect of exposure to atrazine concentrations greater than 1.16×10^{-6} molar.

The Metabolic Pathways and Physiological Effects of Atrazine in Douglas-fir Seedlings

by

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INTRODUCTION

The ever increasing demand for forest products makes more intensive management of our timber producing lands imperative. One way productivity can be increased is to insure that regeneration of denuded areas occurs with a minimum delay. The use of selective herbicides is gaining favor with forest managers as an aid in establishing coniferous seedlings on sites where there is competition from other vegetation for water, nutrients or sunlight.

The introduction of such a biologically active compound into an ecosystem can result in marked changes in the floral and faunal components of the community. Changes in plant species composition after application of selective herbicides makes them highly effective as tools in vegetation management. Indeed, through selectivity, they can be used to favor a desired species, trees in the case of a forest, over competing vegetation.

In both physical and biochemical ways plants exhibit different, and often opposite, reactions to herbicides introduced into their environment. In order for a plant community to be treated constructively, the desired species must be able to evade the phytotoxic chemical introduced or it must have the ability to metabolize the

compound or otherwise render it inactive. The vegetation which competes with the trees, on the other hand, must be susceptible to the herbicide in order for the chemical to eradicate, or at least, repress these plants.

This study was undertaken to investigate the mode of action of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) on a conifer. This compound is characterized as an inhibitor of photosynthesis and is used to reduce the herbaceous vegetation competing with coniferous tree seedlings in new plantations.

The specific approach taken to elucidate the effects of the herbicide involved measurements of the physiological responses of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings growing in nutrient solution with four concentrations of the herbicide. Photosynthesis, dark respiration and transpiration were measured over a period of 30 days and these rates were correlated with the internal concentration of the herbicide. The rates of uptake and metabolism of the herbicide were monitored to obtain the concentration of atrazine in the leaves of the seedlings.

From this study a model was developed which would outline the mode of action of atrazine and explain the selectivity noted for this herbicide.

REVIEW OF LITERATURE

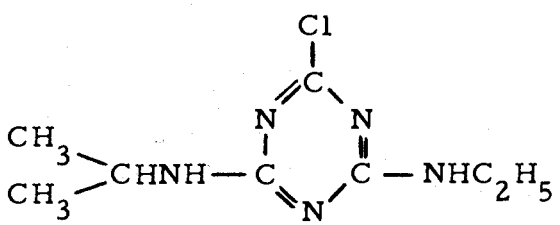
Since man first engaged in cultivation of plants he has probably been plagued with weeds (unwanted vegetation) which diverted some of the limited resources present on the site from the intended crop species. Forest managers are aware that control of competing vegetation is often necessary to insure survival of newly planted seedlings (47, 48) and that subsequent growth rates are influenced greatly by both the quantity and the growth habits of the competing vegetation present (46).

Several studies have shown that the survival of coniferous seedlings is closely correlated with weed control and its effect on soil moisture. Recent studies by Preest (54) and Bentley (5) have shown that the beneficial effects of weed control are seen in the growth rates of Douglas-fir seedlings, as well as increased survival rates. Preest (54) reported height growth and stem volume were increased 60 percent and 217 percent, respectively, by treatment with atrazine for three consecutive years. He attributed the enhanced growth observed to increased soil moisture which was available to the trees as a result of chemical weed control.

Weed control on coniferous plantations can be effected using mechanical means, such as scalping (34), mulching (20) or by using herbicides (46). The use of selective herbicides for weed control has

two obvious advantages, they can give very good control of the competing vegetation with a minimum expenditure and their use does not result in soil disturbance.

Atrazine is one such herbicide commonly used in both new field plantations and in Christmas tree orchards, as an aid in the control of competing vegetation, especially grasses. It is a member of the triazine family of herbicides which was first synthesized and tested in 1952 by Gysin and Kusli (24) in the laboratories of Geigy Chemical Company in Basle, Switzerland. The triazines have in common a molecular structure involving a six-membered heterocyclic ring with alternating carbon and nitrogen atoms and with various alkylamino substitutions on two of the carbon atoms. The third carbon atom has a chloro-, mercapto-, or methoxy- substitution. Atrazine and simazine are the most common analogs in use, especially in forestry. The structural formula of atrazine is:



Atrazine is a white crystalline powder with a melting point of 173°C and a molecular weight of 215.7. It has limited solubility in water (35 parts per million at 27°C) but is highly soluble in chloroform, methanol and petroleum ether. It has a very low toxicity to wildlife and fish with acute oral LD_{50} for rats of 3080 mg/kg (24).

This compound is safe to handle, there being no substantiated reports of skin irritations resulting from its use. Atrazine is used commercially for weed control among conifers at registered rates of three to four pounds per acre, active ingredient.

Physiological Effects

The introduction of any biologically active compound into a plant results in changes in many of the physiological processes. The effects of atrazine on several of these systems will be reviewed below.

Photosynthesis

Atrazine and the related triazines have been shown to have a marked effect on photosynthesis. Many studies have shown that triazines are potent inhibitors of the Hill reaction, causing 50 percent inhibition in concentrations as low as 7×10^{-7} molar (3, 4, 21). Investigations suggest that the herbicidal action of these compounds is related to their ability to inhibit oxygen evolution and noncyclic photophosphorylation associated with light reaction II. Numerous researchers (11, 21) 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. Good (21) proposes that the triazines interfere with the function of a catalytic center, which is essential for oxygen production in chloroplasts, by electrostatic attraction between the inhibitor and the active site of an enzyme.

Structural changes wrought by atrazine on the cells and chloroplasts of several plants have also been noted. Ashton and co-workers (1, 2) noted precocious development of vacuoles, cessation of meristematic activity and destruction of chloroplasts in treated bean seedlings. Damage to chloroplasts started with disruption of frets, swelling of compartments and finally the breakdown of the limiting membrane which allowed the cytoplasm and stroma material to escape into the vacuole. Similar destruction of chloroplasts was noted in barnyard grass exposed to 5 ppm atrazine in nutrient solution (29). Wheeler and Hamilton (86) stated the toxicity of atrazine was closely related to chlorophyll degradation. Susceptible plants exposed to concentrations as low as 1 ppm in solution had 50 percent chloroplast destruction after five days, whereas more tolerant plants such as corn showed no damage (67). Damage to chloroplasts of plants treated with atrazine can be reduced by increasing the concentration of manganese in the cells (76). Elevated manganese levels helped maintain cellular membrane integrity.

Only a few studies have reported the effects of triazine herbicides on coniferous seedlings. Red pine seedlings treated with 20 pounds per acre of soil-incorporated atrazine were found to have a rapid decrease in photosynthesis and after three to four weeks CO_2 uptake was negligible (64). Peterson (50) treated Douglas-fir seedlings grown in washed sand and in forest soil with sub-lethal levels of

atrazine and observed a decrease, followed after about 30 days by an increase in the photosynthetic rate. A stimulation of CO_2 fixation in Norway spruce was seen when seedlings were treated with low concentrations of simazine (40). Neither author suggested a mechanism which would account for the elevated photosynthetic rates reported.

Respiration

Atrazine also effects the respirational rate of treated plants. Both a decrease (19) and an increase (57) in respiration have been observed when plants were grown in the presence of triazines. Peterson (50) noted both a decrease and increase in respiration when tree seedlings were treated with low concentrations of atrazine. He reported a significant decrease initially, but observed that after 30 days the respirational rates of treated seedlings exceeded those of the untreated controls. He attributed the conflicting reports in earlier papers to measurements being made at different periods of time after treatment.

Transpiration

There are several reports of atrazine altering the rates of transpiration of treated plants (10, 23, 87). Graham and Buckholtz (23) reported that the suppression of transpiration resulted in a significantly increased moisture content in the plant. This

observation has led Wills et al. (87) to propose that the reduced transpiration is a result of the closing of the stomata triggered by photosynthetic inhibition. Researchers experimenting with soybeans, found a 50 percent reduction in transpiration after a four-hour exposure to 9 μmoles of atrazine per liter in nutrient solution (87). Peas were even more sensitive to atrazine; Humburg and Kust (31) indicated 1 μmolar concentration of atrazine in nutrient solution decidedly suppressed transpiration.

Herbicide Uptake

The rate of herbicide uptake by plants is influenced by several factors, including: (1) concentration of the herbicide in the growing medium, (2) air and soil temperature, (3) relative humidity, (4) light intensity, (5) soil moisture content and (6) partitioning of the herbicide between soil and soil solution. Roeth and Lavy (61) reported that atrazine uptake by all species tested was a function of the atrazine concentration in the soil. The studies of Kozlowski et al. (36) and van Oorschot (83) illustrate the influence of temperature on herbicide uptake. Their studies suggested that marked increases in phytotoxicity are linked with increasing temperature. The role relative humidity plays in the adsorption and translocation of herbicides was shown by Smith (75), who found a negative correlation between relative humidity and the rate of uptake. A later study by van Oorschot (83)

demonstrated the effects of light, temperature and relative humidity on transpiration, and through transpiration, the effects on the uptake of the herbicide. He showed that with increases in light intensity, temperature and relative humidity there was a concomitant increase in herbicide uptake. The link between herbicide uptake and soil moisture content has been suggested (36) but the correlation does not appear to be as strong as that reported for the other influences noted above. The authors reported a possible increase in uptake of the herbicide with increasing soil moisture.

Much of the above discussion indicates that herbicide uptake is influenced by many of the same environmental factors that affect transpiration, and there have been suggestions that these two functions are linked (60). Vostral et al. (82) showed that atrazine uptake paralleled transpiration; i.e., increases in water and herbicide uptake were proportional. This observation suggests that the uptake of atrazine by plants is a passive process.

The distribution of triazine herbicides in plants has been studied using ^{14}C-labeled compounds. One-month-old red pine seedlings grown in agar medium with various concentrations of simazine were found to have the compound distributed throughout the plant, with especially high concentrations in the roots (13). Similar results were found by Lund-Hoie (40) while investigating the uptake of simazine in Norway spruce (Picea abies). Freeman et al. (16) found

white pine (Pinus strobus) and red pine (Pinus resinosa) seedlings had about equal uptake of labeled herbicide, but distribution within the plants differed considerably. The needles of red pine had three times as much labeled material as those of white pine.

Metabolism

The metabolism of triazines by plants has been investigated recently because it appears as though the ability to detoxify the herbicide is the key to resistance of the plants exposed to the herbicide (86). Equivalent inhibition of the Hill reaction has been reported in isolated chloroplasts from both tolerant and susceptible species (43). Negi et al. (45) found that there was a strong correlation between the amount of undegraded atrazine present and the plant's susceptibility to atrazine incorporated in the soil.

Three modes of detoxification of the chlorotriazines have been detected in the species investigated. Corn, and other plants which have benzoxazinone derivatives, have been found to replace the chlorine atom with a hydroxyl group (42). The hydroxy-triazines formed are completely nonphytotoxic (42). Other resistant or partially resistant species which do not contain benzoxazinone have been found to dealkylate the chlorotriazines to less toxic compounds by cleaving one or the other of the side chains, forming 2-chloro-4-amino-6-ethylamino-s-triazine or 2-chloro-4-isopropylamino-6-amino-s-triazine (68). A recent report by Lamoureux et al. (39)

indicates a third detoxification mechanism exists. They isolated two nontoxic amino acid-triazine conjugates from sorghum.

There have been few studies conducted of triazine metabolism in conifers. Dhillon et al. (13) detected two unknown metabolites, neither of which was hydroxy-simazine, from red pine seedlings. Norway spruce, on the other hand, has been reported to have benzoxazinone in the roots of the seedlings and to have detoxified simazine to the hydroxy analog (40).

Effects of Soil

There has been considerable research into the fate of herbicides once they have been applied to soils. This aspect is of importance because the herbicide becomes compartmentalized once it has been applied. There is that portion which is available to the plants in the soil solution and that which is rendered unavailable to the plants because of adsorption, leaching, degradation or uptake by plants. A general review of the fate of atrazine when applied to soils has been presented in Residue Reviews (65).

There have been numerous studies of the adsorption of triazines in various soils. The one soil factor which is most closely related to both percent adsorption and growth reduction of test species is percent organic matter (12). Cation exchange capacity and exchangeable calcium were also strongly correlated with adsorption,

but these soil properties were highly correlated with organic matter, and among themselves (85).

Degradation of the herbicide by chemical and biological processes also contributes to a loss of the compound from the soil solution. Several studies of the chemical breakdown of triazines in soil have been reported (6, 65). Research has shown that low pH plays an important role in hydrolysis of the herbicide (6). Kaufman and Kearney (33) reported that microbial degradation of atrazine can also be an important contributor to herbicide dissipation.

Leaching of the herbicide from the root zone of the target plants renders the substance unavailable. The factors which affect leaching closely parallel those associated with adsorption (27), especially percent organic matter and clay content of the soil. The amount and rate of rainfall also affect leaching of the dissolved compound from the root zone (7).

Uptake of the herbicide by plants will reduce the amount of the chemical in the soil solution. Best and Weber (6) reported that as much as four percent of the triazines applied to the soil was found in the plants growing on the site.

All of these factors combine to regulate the amount of the herbicide which will be available to the target plants on the treated area. Some, like adsorption, are reversible, while the others limit the quantities of the active compound found in the root zone.

METHODS AND MATERIALS

This study was undertaken to determine the effects of atrazine on some physiological processes of Douglas-fir seedlings. To this end, the rates of photosynthesis, dark respiration and transpiration were measured on seedlings at several periods of time after initiation of treatment with four concentrations of herbicide. It was necessary to determine the amount of uptake and metabolism so that a continuous estimate of internal concentration of atrazine could be determined. A correlation could then be made between the physiological responses noted and the amount of undegraded herbicide within the plant.

Douglas-fir seedlings are partially resistant to atrazine. The study of the uptake and metabolism of the compound was undertaken to determine if this resistance could be explained by the ability of the plant to detoxify the herbicide.

When studying the physiological processes of an organism, the interpretation of results is clearer if environmental variability is restricted. A growth chamber was used in this study as a convenient and dependable way of assuring environmental stability.

The administration of uniform and known amounts of atrazine to seedlings presented special problems. Atrazine is adsorbed by soil, and by organic matter so that the concentrations of atrazine in soil solution are neither stable nor easily controlled. For these

reasons the seedlings used in the present investigation were grown in nutrient solution (see Appendix for details of solution used) to which the atrazine was added, to insure exposure of the plants to constant and known concentrations of the herbicide. The use of ^{14}C-labeled atrazine made possible rapid and accurate monitoring of herbicide concentrations in the growing media, and also facilitated the study of the distribution and metabolism of atrazine in the plants.

Seedlings for these experiments were grown from seeds collected in the Coast Range near Valsetz, Oregon. All seeds were obtained from cones of a single tree in hopes of reducing genetic variability.

The seeds were germinated in petri dishes. When the hypocotyl had attained a length of at least one centimeter, they were planted in plastic pots, filled with Sponge-Rok, a form of inert, expanded mica. The seedlings were watered regularly and additional nutrient solution applied at weekly intervals. The seedlings were grown in a controlled environment chamber under the following conditions:

	Day	Night
Photoperiod	16 hours	8 hours
Temperature	25°C	20°C
Relative humidity	50-60%	80-90%

The light intensity was approximately 0.08 ly/min. (900 foot candles) emitted by a bank of cool white, fluorescent tubes augmented by

eleven 100 watt incandescent bulbs. When the seedlings were 60 days old a total of 35 seedlings per treatment was removed from the pots and placed in a 12 x 24 x 3 inch aluminum pan which held four liters of the nutrient solution. The seedlings were allowed to equilibrate one week in the new growing medium before the herbicide was added to the solution. The solution was aerated continuously by bubbling a small stream of air through each pan.

The nutrient solutions to which the various concentrations of atrazine had been added were changed every fourth day to limit changes in the concentration of the herbicide caused by evaporation. Sequential sampling of the concentration of labeled material in the pans during preliminary trials showed that atrazine levels remained constant during the four day period.

The day before measurements were to be made, the seedlings were removed from the pan and placed individually in pint jars filled with nutrient solution containing the concentration of atrazine to be tested. These seedlings were placed in slotted rubber stoppers and sealed with stopcock grease, making a gas-tight seal between the jar with the nutrient solution and the aerial portion of the plant. Photosynthetic and respirational rates were measured as changes in CO_2 concentration in a closed system using a Mine Safety Appliance infrared gas analyzer. The apparatus used was similar to one described by Krueger (37). The lucite cuvette had a circulating water

jacket on the side, which served to maintain a constant internal temperature. A water bath five centimeters deep was placed above the cuvette to shield the seedlings from the heat emitted from the 1200 watt incandescent light source.

Light energy in the cuvette at the seedling level was 0.369 ly/min (approximately 3,000 foot candles) as measured with a Kipp actinometer, and was kept constant for all measurements. Studies have shown this to be saturation light intensity for Douglas-fir seedlings of a comparable age (37). The air temperature in the cuvette was a constant 25°C and the relative humidity held at 50 percent. The measurements were made while the carbon dioxide concentration was in the range of 330 to 370 ppm. Dark respiration was measured immediately following the photosynthetic determination with the same apparatus 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 adsorbed or evolved per gram dry weight of needles per hour.

The labeled atrazine was supplied by Geigy Chemical Corporation, Ardlsey, New York. The ^{14}C-ring-labeled material had a specific activity of 10.1 μC/mg. A concentrated stock solution was prepared and the appropriate dose was formulated for each treatment by diluting the stock solution with distilled water. Mole concentrations of atrazine were 0, 1.16×10^{-6} (1/4 ppm), 5.80×10^{-7}

(1/8 ppm), 2.90×10^{-7} (1/16 ppm), and 1.45×10^{-7} (1/32 ppm) active ingredient. The highest concentration used was found in screening trials to be near the lethal dosage for Douglas-fir seedlings grown in nutrient solution. (See Appendix for discussion of the preliminary study of photosynthesis.) Photosynthetic, respirational and transpirational measurements were made 2, 5, 10, and 30 days after treatment. The experiment was designed as a 5 (treatments) x 4 (time after treatment) x 7 (replications), factorial. Transpiration was measured by weighing the bottles daily to determine weight loss.

The treated seedlings were assayed for total ^{14}C content using the dry oxidation method described by Gupta (25). The seedlings to be assayed were dried and ground with a Wiley mill to pass through a 20 mesh screen. Three to seven milligrams of the ground material was analyzed in duplicate. The CO_2 released upon combustion was captured in 0.2 ml ethanolamine which had been added prior to combustion. To prepare for scintillation counting, 15 ml of counting solution composed of a 5:2 mixture of toluene and 2-methoxyethanol, 5 g per liter of PPO and 300 mg per liter POPOP, were added one hour after combustion. The vials were shaken well and counted for a total of 10,000 counts or 20 minutes in a Packard Tri Carb model 3300 liquid scintillation counter.

The rate at which atrazine was metabolized in the seedlings was quantified by sampling plant material at periodic intervals after

initiation of treatment, i.e., 3, 6, 12, and 30 days. The extraction process used was similar to that described by Shimabukuro et al. (68). The plant material was oven dried at 70°C for a day, then ground in a Wiley mill to pass a 20 mesh screen. Atrazine and its metabolites were extracted from the ground material in a Waring blender with methanol. The extract was concentrated to 5 ml and a 0.2 ml aliquot spotted on a Baker-flex silica gel 1-B thin layer chromatograph sheet. The sheets were developed to a 15 cm front in a solution of ethanol-water-acetic acid 26:57:7.5 (v/v/v). This solvent solution was selected because it gave the best separation between the various labeled compounds found in the extract from the treated seedlings. The sheets were scanned using a Packard Model 7200 Radiochromatogram scanner. The areas under the various peaks were determined using a planimeter; and the counts per minute in each peak were calculated. The concentrations of the various labeled compounds in the seedlings were obtained from these data.

The various metabolites found could not be identified using the techniques outlined above because there were no reference compounds available to compare with those isolated in this experiment.

RESULTS AND DISCUSSION

Atrazine quickly and markedly affects the physiological processes of young Douglas-fir seedlings exposed to sub-toxic levels of the herbicide. Photosynthesis was affected most dramatically, but the rates of transpiration and respiration also were altered. Douglas-fir seedlings appear to metabolize atrazine at a rate which enables the plants to grow in a solution which exposes them to a constant, low concentration of the herbicide.

Photosynthetic Response

Atrazine both reduced and increased the photosynthetic rate of Douglas-fir seedlings growing in a nutrient solution to which dissolved atrazine had been added. The rate observed was a function of both the concentration of atrazine to which the plants were exposed and the period of time after the treatment was initiated.

The photosynthetic rates were corrected for differences in dry weight using an analysis of covariance. Krueger (37) and Peterson (50), both working with Douglas-fir seedlings of a similar age and size, found that carbon fixation per gram dry weight needles decreased with age, but more importantly, with size of the seedlings. Self-shading of the needles is probably the largest factor which brings

about this decline. The regression for the covariant, dry weight of needles, was found to be significant at the 0.005 level.

An analysis of variance showed that both time after treatment and the herbicide concentration had highly significant effects on the photosynthetic rates observed. Time after treatment was significant at the 0.01 level and concentration was significant at the 0.005 level.

The highest concentration of atrazine used (1.16×10^{-6} molar) reduced CO_2 uptake greatly at each measurement period except 30 days after treatment began. The deleterious effects of atrazine at this level were observed immediately, there being a 50 percent reduction in CO_2 uptake the first day. The maximum reduction in photosynthesis was observed after five days, at which time the rate for treated plants was about one-third of the rate for the control seedlings. These seedlings showed a slow, but continuous recovery over the remainder of the test period, with the rate after 30 days not statistically different from that of the control plants.

Atrazine in low concentrations was found to stimulate photosynthesis in Douglas-fir seedlings. The lowest concentration used (1.45×10^{-7} molar) stimulated photosynthesis at all measurement intervals following treatment. The increased CO_2 fixation was observed within a day, and the plants continued to photosynthesize at an elevated level over the entire time period tested. The seedlings exposed to this low concentration of atrazine were photosynthesizing

at a rate one-third greater than the controls at the 5, 10 and 30 day measurement periods.

Between these extremes were the two intermediate herbicide levels. The plants exposed to the 2.9×10^{-7} molar solution showed no significant deviation from the control plants. The next higher level (5.8×10^{-7} molar) showed a small increase in photosynthesis followed by a slow but steady decline over the study period. These results are summarized in Table 1 and shown graphically in Figure 1.

Table 1. Mean photosynthetic rate of Douglas-fir seedlings treated with atrazine (mg CO_2 taken up/g dry weight needles/hr).

Atrazine level (molar)	Time after treatment (days)			
	2	5	10	30
Control	11.04 bc	11.17 b	10.48 b	9.11 b
1.16×10^{-6}	4.76 d	4.11 c	5.00 c	8.46 b
5.80×10^{-7}	13.58 a	12.50 ab	7.19 c	5.49 c
2.90×10^{-7}	9.18 c	9.34 b	11.04 b	9.20 b
1.45×10^{-7}	12.81 ab	14.81 a	13.47 a	12.39 a

Letters indicate means at each time period which are significantly different at the 0.05 level using the Tukey test of significance.

Photosynthetic Rate and Internal Concentration of Atrazine

One of the hypotheses to be tested in this study was that the observed physiological response would be a function of the concentration of the herbicide present in the leaves of the plant. To this

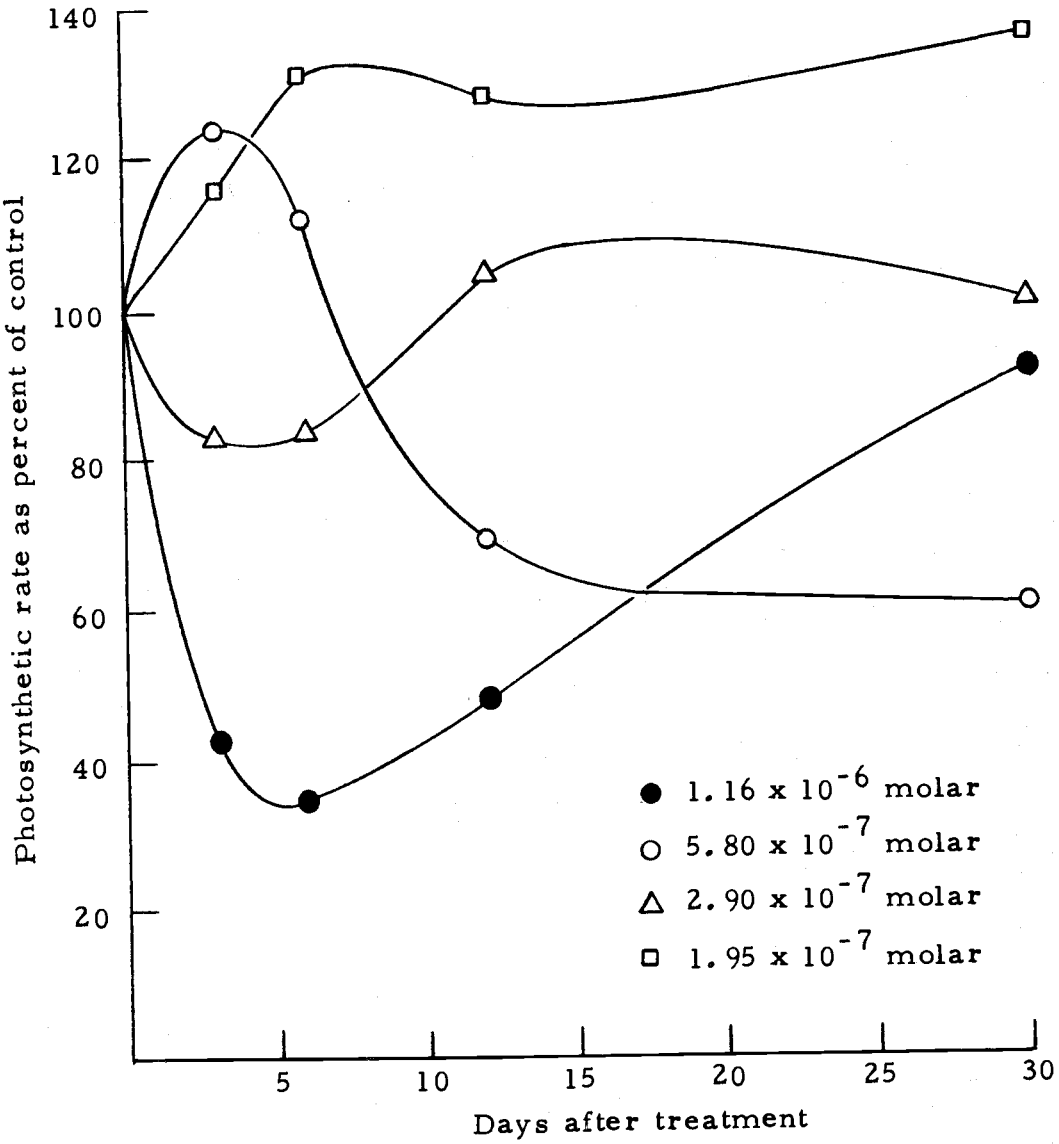


Figure 1. Photosynthesis of Douglas-fir seedlings as a function of time after treatment.

end, the levels of undegraded atrazine present in the plants were determined and a regression run between photosynthetic rate and concentration of atrazine present in the needles. (See section on Metabolism for details of assay for atrazine.) The relationship was found to be highly significant with a correlation coefficient (r) of -0.944. The linear regression equation calculated was Y = 135 - 0.991X, where Y is the expected photosynthetic rate as a percent of control and X is the ng of atrazine present per mg dry weight needles (Table 2). The linear equation calculated is only valid over the ranges of atrazine used and extrapolation to other concentrations would be subject to error (see Figure 2).

Multiple regression analysis provides a means of further assessing the individual and combined relationships of herbicide concentration and time on the photosynthetic rates of the seedlings tested in this study. In the analysis of factors influencing photosynthesis, the regression equation assumes the form:

$$Y = 173 + 1.09X_1 - 75X_2 + 0.05X_1^2 + 22.1X_2^2 - 0.56X_1X_2 - 0.04X_1^2X_2 - 1.80X_1X_2^2 + 0.007X_1^2X_2^2$$

where Y is the expected photosynthetic rate, X_1 is time after treatment and X_2 is atrazine concentration. The multiple correlation coefficient (R) was calculated after each successive addition of a variable as shown in Table 3. The variables which contributed

Table 2. The photosynthetic rate and amount of undegraded atrazine present in needles of treated Douglas-fir seedlings.

Treatment (molar)	Days after treatment	Photosynthetic rate as percent of control	Atrazine present in needles*
1.16×10^{-6}	3	43	84.76
	6	37	77.11
	12	48	85.99
	30	93	49.27
5.80×10^{-7}	3	124	14.38
	6	112	14.08
	12	69	59.98
	30	60	61.81
2.90×10^{-7}	3	83	48.35
	6	84	68.85
	12	105	14.38
	30	101	29.68
1.45×10^{-7}	3	116	7.96
	6	133	16.52
	12	128	8.57
	30	136	11.32

*Atrazine concentration expressed as ng of atrazine per mg dry weight needles.

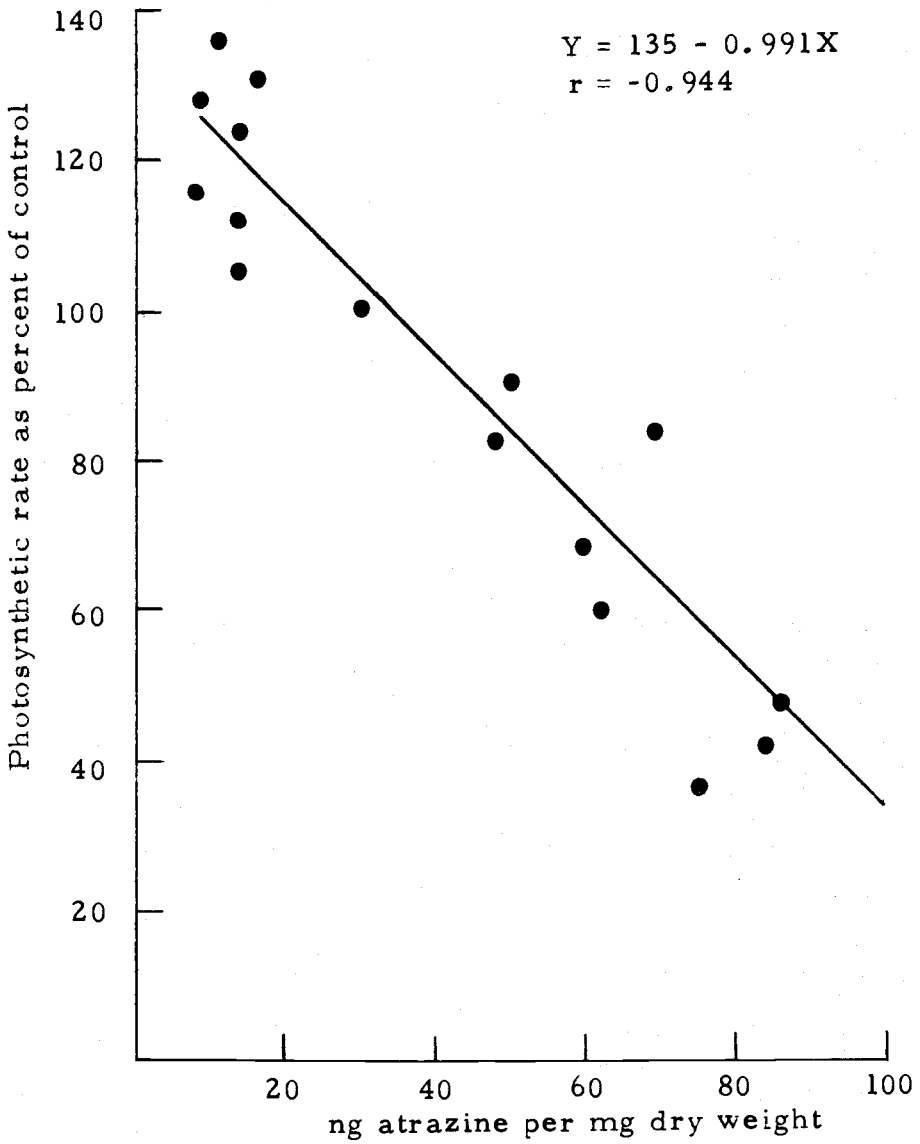


Figure 2. Photosynthesis of Douglas-fir seedlings as a function of foliar atrazine concentration.

Table 3. The correlation of photosynthesis (Y) with atrazine concentration and time.

Factors in equation	Factor entering	R	Difference in R due to factor entering
$Y = f(X_2)$	X_2	0.572	0.572*
$Y = f(X_2, X_1^2X_2^2)$	$X_1^2X_2^2$	0.646	0.074**
$Y = f(X_2, X_1^2X_2^2, X_1X_2)$	X_1X_2	0.716	0.070**
$Y = f(X_2, X_1^2X_2^2, X_1X_2, X_1)$	X_1	0.750	0.034
$Y = f(X_2, X_1^2X_2^2, X_1X_2, X_1, X_1^2X_2)$	$X_1^2X_2$	0.776	0.026
$Y = f(X_2, X_1^2X_2^2, X_1X_2, X_1, X_1^2X_2, X_1X_2^2)$	$X_1X_2^2$	0.803	0.027
$Y = f(X_2, X_1^2X_2^2, X_1X_2, X_1, X_1^2X_2, X_1X_2^2, X_2^2)$	X_2^2	0.869	0.066*
$Y = f(X_2, X_1^2X_2^2, X_1X_2, X_1, X_1^2X_2, X_1X_2^2, X_2^2, X_1^2)$	X_1^2	0.873	0.004

*Factor significant at 0.05 or less

**Factor significant at 0.10

X_1 is time after treatment; X_2 is atrazine concentration

significantly to the predictive value of the equation were atrazine concentration, the square of atrazine concentration and the two interaction terms.

Figure 3 is a response surface graph depicting the effects of time and atrazine concentration on net photosynthesis. It was plotted using the points generated by the regression equation above. It illustrates the dual properties of atrazine, i.e., the increased photosynthetic rates at low levels and inhibition when the plants are exposed to higher amounts.

Discussion of Photosynthetic Effects

Two things are seen from the measurements of photosynthesis: 1) very low concentrations of atrazine markedly stimulate photosynthetic activity in Douglas-fir seedlings, and 2) the plants, by metabolizing the compound, can regain photosynthetic efficiency after exposure to levels of the herbicide which inhibited photosynthesis at first.

There have been several earlier reports of increased growth or photosynthesis caused by low rates of triazine herbicides. Ries (58, 59) reported increased yields of corn and wheat when the plants were grown in nutrient solution with low levels of simazine. Peterson (50) found that Douglas-fir seedlings grown in sand were

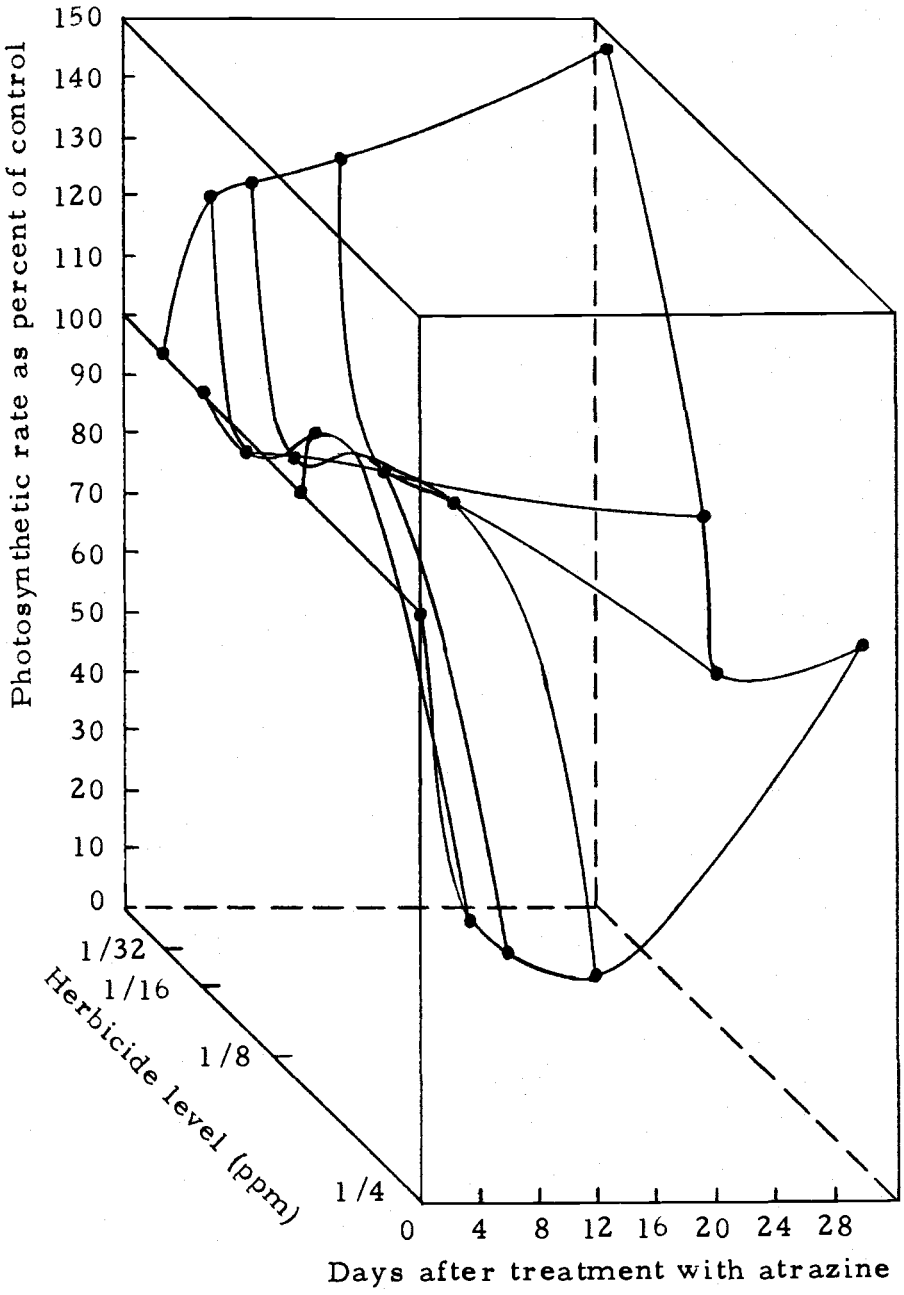


Figure 3. Response surface graph of net photosynthesis (from regression).

fixing carbon at an elevated rate after exposure to low concentrations of atrazine.

Reports of atrazine inhibiting photosynthesis are common (50, 83) and there are several reports of first a reduction and then a recovery of photosynthetic rate after exposure to moderate levels of triazines. Shimabukuro and Swanson (70) showed a recovery in sorghum, and Sutton et al. (77) reported several aquatic plants regained photosynthetic efficiency one or two days after treatment with simazine.

This study made it possible to follow the concentration of atrazine within the leaves of the seedlings, and to calculate the correlation between the observed photosynthetic rate and the level of undegraded chemical. As seen earlier this correlation was very close (significant at the 0.005 level). This appears to verify the conclusion of researchers, such as Shimabukuro and Swanson (69), who reported that recovery of photosynthetic activity is related to metabolism of atrazine.

Figure 4 depicts the outcome of the measurement of photosynthesis under varying light intensity before and after treatment with atrazine. The plot of these rates gives curves which are typical of a noncompetitive inhibitor of an enzyme mediated reaction. A Lineweaver-Burk plot of these curves yields identical values for K_m

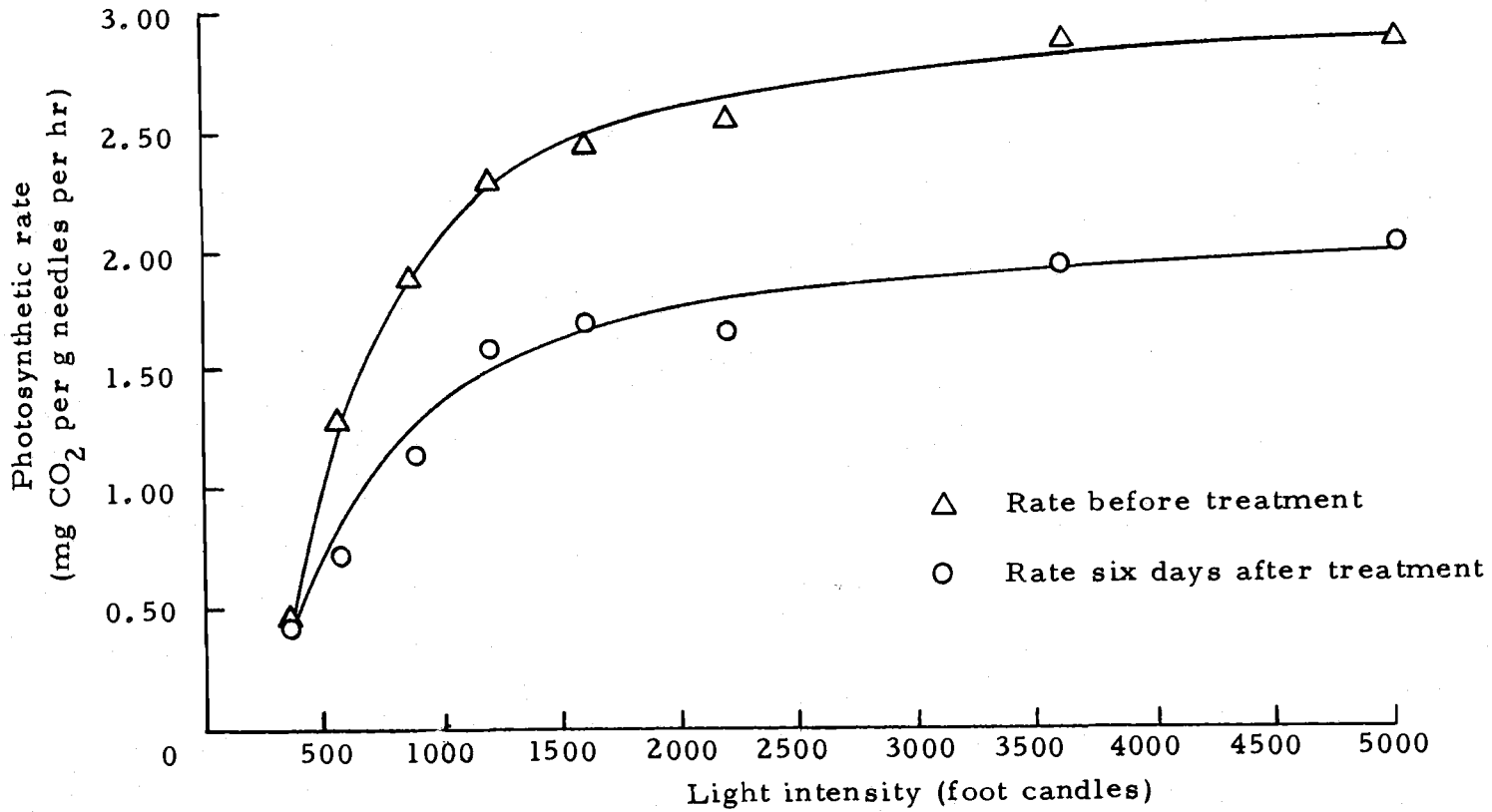


Figure 4. Photosynthetic rate of Douglas-fir seedlings before and after treatment with 1/4 ppm atrazine.

(Michaelis constant) but those for V_{max} (maximum velocity) were different, again indicating a noncompetitive inhibitor.

Respirational Response

The rates of dark respiration for seedlings treated with atrazine were not appreciably different from the control plants except when the photosynthetic rates were significantly altered. Respirational rates markedly lower than the control plants were found for seedlings growing in the solution with 1.16×10^{-6} molar concentration of atrazine at the first three measurement periods, and the seedlings exposed to the next lower level (5.8×10^{-7} molar) were depressed at the last measurements. Elevated respirational rates were found with seedlings growing in the two lowest concentrations ten days after treatment (Table 4).

Table 4. Mean respiration rates of Douglas-fir seedlings treated with atrazine (mg CO_2 evolved/g dry weight needles/hour).

Atrazine level (molar)	Time after treatment (days)			
	2	5	10	30
Control	1.95 ab	1.75 b	1.49 b	1.62 a
1.16×10^{-6}	1.21 c	0.86 c	0.89 c	1.65 a
5.80×10^{-7}	1.86 ab	2.00 a	1.67 ab	1.07 b
2.90×10^{-7}	2.44 a	1.95 ab	2.14 a	1.53 a
1.45×10^{-7}	1.81 b	1.94 ab	1.99 a	1.34 a

Letters indicate means at each time period which are significantly different at the 0.05 level using the Tukey test of significance.

Discussion of Respiration

It appears that rates of respiration closely parallel the rates of growth of the seedlings. When photosynthesis was greatly inhibited, and the growth rate therefore retarded, we find the rates of respiration were significantly lower. The initial repression followed by a recovery of respirational rate closely parallels that noted by Peterson (50) for Douglas-fir seedlings grown in sand. There are reports in the literature of both a reduction and an increase in respiration caused by exposure to triazines. Funderbuck and Davis (19) report that atrazine decreased respirational rates of both resistant and sensitive plants. Ries et al. (57), on the other hand, measured respirational rates that were significantly higher than the control on rye plants exposed to low concentrations of simazine. It appears that the rate of respiration is both a function of herbicide concentration and time after treatment. The reduction is most likely associated with reduced growth caused by the inhibition of photosynthesis, and the accelerated rate occurs when the growth rate of the plant is increasing.

Transpiration Response

An analysis of variance showed that atrazine concentration had a significant effect (0.025 level) on transpiration. The rates of

transpiration of the seedlings showed an initial depression after exposure to all four concentrations of the chemical. The inhibition of water uptake was greatest for the highest atrazine level (1.16×10^{-6} molar) after five days, where the treated plants were transpiring at a rate which was 27 percent of the control plants. The depression was not as pronounced when the seedlings were exposed to the lower concentrations. In marked contrast to the initial response to atrazine exposure were the significantly elevated rates of transpiration noted after 30 days. The rates for all time periods and concentrations are summarized in Table 5.

Table 5. Transpiration of Douglas-fir seedlings treated with atrazine (mg water/g dry weight needles/hour).

Atrazine level (molar)	Time after treatment (days)			
	2	5	10	30
Control	0.65 a	0.68 a	0.55 a	0.40 c
1.16×10^{-6}	0.34 b	0.18 b	0.37 b	0.56 b
5.80×10^{-7}	0.64 a	0.71 a	0.49 a	0.67 a
2.90×10^{-7}	0.35 b	0.58 a	0.50 a	0.46 b
1.45×10^{-7}	0.48 a	0.59 a	0.52 a	0.72 a

Letters indicate means at each time period which are significantly different at the 0.05 level using the Tukey test of significance.

Discussion of Transpiration

There are numerous reports of triazine herbicides inhibiting transpiration of plants to which the chemicals were applied. Vostral

et al. (82) reported a 67 percent reduction in transpiration at 25°C when atrazine had been added to the nutrient solution. Humburg and Kust (31) reported markedly suppressed transpiration when pea plants were grown in nutrient solution containing one μmolar concentration of atrazine. Graham and Buchholtz (23), after exposing soybean plants to 9 μmolar levels of atrazine, found a 50 percent reduction in transpiration. One researcher (63) suggested using water uptake as a bioassay for triazines in soil solution. Wills et al. (87) attributed the reduced transpiration, noted when cotton was grown in nutrient solution with atrazine, to the closing of the stomata, caused by photosynthetic inhibition. This agrees with the research of Raschke (56) who found that a compound which reduces the photosynthetic rate also caused a reduction in the rate of transpiration.

Changes in stomatal aperture have been shown (55) to respond rapidly to changes in carbon dioxide concentration in the mesophyll. In this study the depressed transpirational rates observed could be attributed to closing of the stomata caused by the increased CO_2 concentration in the leaves which occurred when photosynthesis was inhibited by atrazine.

Very low levels of triazines have been shown to increase transpiration. Ries and Wert (59) treated plants with 10^{-9} molar simazine solution, and found there was a 25 percent increase in water uptake. Plants exposed to higher levels, 10^{-7} molar, showed no

appreciable difference in water uptake when compared to untreated controls. The accelerated transpirational rates observed 30 days after treatment could be a reflection of the recovery and, in some cases, the increased photosynthetic rates noted. If stomata respond inversely to CO_2 concentration in the leaf, an increased transpirational rate would be expected when exposure to atrazine resulted in elevated photosynthetic rates, and consequently, a reduction in CO_2 levels in the mesophyll. Another possible explanation for the increased water uptake is that atrazine, in low concentrations, affects transpiration in a manner similar to kinetin. Pallas and Box (49) reported that treatment with kinetin appreciably increased transpiration in leaves both in the dark and in the light. Foy and Hiranpradit (18) reported low levels of atrazine exhibited cytokinin-like activity.

Figure 5 indicates that transpiration and photosynthesis are not affected to the same degree by exposure to atrazine, indicating that the chemical probably affects these two processes separately. If the compound were simply controlling stomatal aperture the rates of photosynthesis and transpiration should be nearly parallel.

The measurements of transpiration were particularly important because as reported below, this made it possible to determine the amount of herbicide taken up by the seedlings.

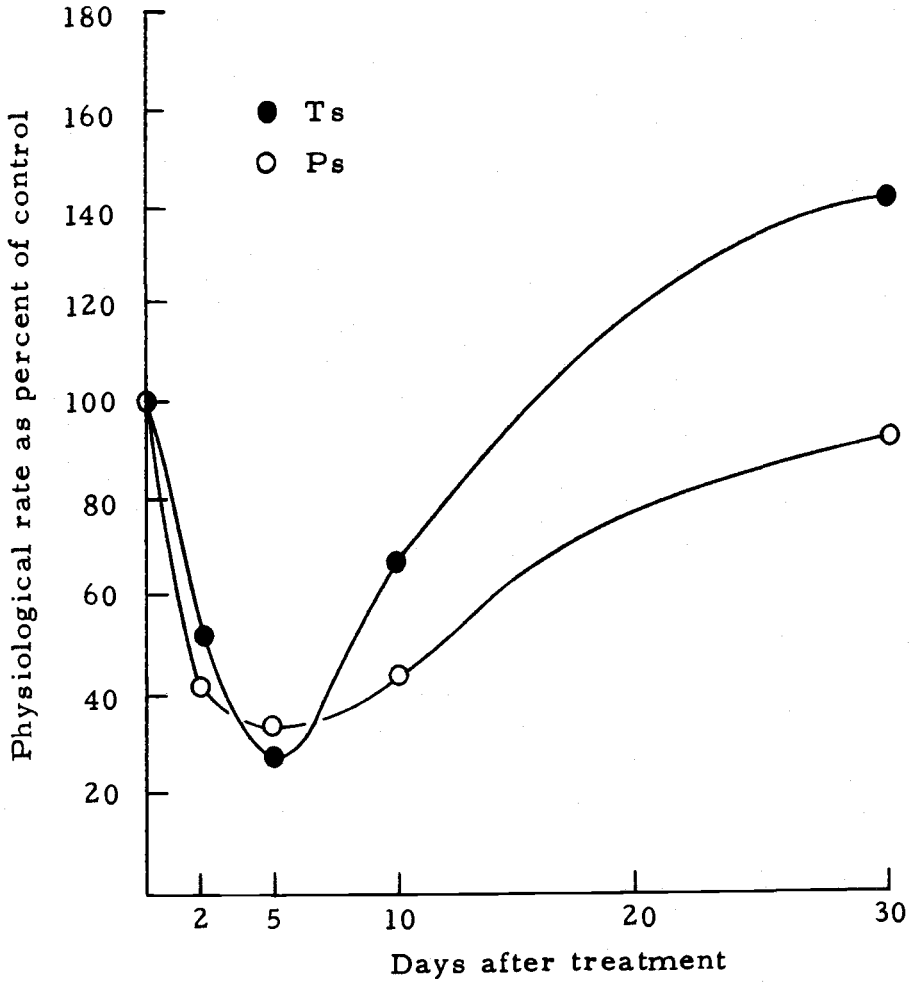


Figure 5. Photosynthesis and transpiration of Douglas-fir seedlings treated with 1/4 ppm atrazine.

Uptake of Atrazine

The rate of atrazine uptake from the nutrient solution was monitored to determine if the process was active or passive. Two experiments were conducted to determine which of these processes was taking place in Douglas-fir seedlings. The first experiment was to withdraw 0.9 ml aliquots sequentially from the nutrient solution into which the seedlings had been placed, and to count the radioactivity, using a liquid scintillation counter. This experiment showed that there was an initial drop in the concentration of atrazine, with an equilibrium being reached in a few minutes (see Table 6). The small decrease observed at first could be accounted for by adsorption of the chemical by organic matter surrounding the roots or by diffusion into cellular free space. Moody et al. (41) studied the release of atrazine from roots which had been placed in nutrient solution to which atrazine had been added. They found that 90 percent of the herbicide was released from the roots within 30 minutes after placing them in pure water. The rapid release, they said, indicated low energy physical or chemical attachment of the molecules to the cells. In the study reported in this paper, an equilibrium was maintained after an initial drop in concentration, indicating that some of the herbicide was adsorbed within five minutes. The nearly constant

Table 6. Changes in ^{14}C activity in nutrient solution during uptake by Douglas-fir seedlings.

Time elapsed (minutes)	Counts per minute*
0	3362 a
5	3228 b
15	3230 b
30	3211 b
60	3238 b
120	3258 b
240	3182 b
390	3187 b
750	3195 b

Letters indicate means which are significantly different at the 0.05 level.

*Radioactivity measured by liquid scintillation counting of 0.9 ml aliquots withdrawn from the nutrient solution containing atrazine.

Table 7. Calculated cumulative uptake of atrazine from nutrient solution based on measurements of transpiration (ng atrazine per g dry weight needles).

Atrazine level (molar)	Time after treatment (days)			
	2	5	10	30
Control	0	0	0	0
1.16×10^{-6}	6.15	9.39	48.68	115.06
5.80×10^{-7}	5.73	12.09	21.02	54.13
2.90×10^{-7}	2.12	3.86	7.75	20.58
1.45×10^{-7}	1.07	2.40	4.83	13.43

concentration which existed following the first five minutes indicates that the uptake of the herbicide into the plant is a passive process.

The second way of measuring herbicide uptake was to correlate the rate of transpiration with the accumulation of radioactive material in the leaves of the plants. This correlation was found to be significant at the 0.001 level ($r = 0.93$). This again, would indicate a passive process. If, indeed, atrazine uptake is a function of transpiration, a temperature sensitive process, this would explain the reports in the literature of toxicity being related to temperature (36, 51, 81). All of these reports indicate that with increasing temperature there is increased susceptibility to the herbicide. See Table 7 for tabulations of atrazine uptake.

Metabolism of Atrazine

The study of metabolism of atrazine by Douglas-fir seedlings was undertaken to determine if the partial resistance to the herbicide is related to the ability of the seedlings to detoxify the compound. Also, the investigation of the metabolism of atrazine made it possible to determine the concentration of the herbicide in the leaves of the plant and relate these levels to the physiological responses noted.

Three methods were used to determine the amount of herbicide taken up by the plants. The first was to measure the rate of

herbicide uptake from the growing medium. This was discussed in the previous section under herbicide uptake. The other two processes involved measuring the levels of radioactivity in the various portions of the plant. The first of these, a dry combustion process, made possible the quantification of all ^{14}C labeled material in the plant, without showing specific compounds. The second method, extraction of labeled material from the plant and chromatographic separation, made it possible to calculate the concentration of both the undegraded atrazine and the metabolites. A detailed description of the dry combustion and the thin layer chromatographic processes is found in the Appendix.

No attempt was made to do a detailed kinetic study of metabolism, nor was the order of appearance of the various metabolites investigated, because the seedlings had been exposed to the herbicide for two days before the first measurements of metabolism were made. The amounts of the various compounds and the total radioactivity found in the needles after each treatment period were determined.

Results of Metabolism Study

It is apparent from the data collected in this study that Douglas-fir seedlings are able to metabolize atrazine (Table 8). After one month's exposure to the highest concentration of the herbicide

Table 8. Concentration of the various ^{14}C-labeled compounds found after treating Douglas-fir seedlings with atrazine. (counts per minute/mg dry weight needles)

Treatment (molar)	Days after treatment	R_f values*			
		0.00	0.42 (atrazine)	0.65	total
1.16×10^{-6}	3	0.095	0.225	0.180	0.500
	6	0.215	0.205	0.746	1.166
	12	1.128	0.228	2.284	3.640
	30	1.049	0.131	2.963	4.143
5.80×10^{-7}	3	0.091	0.038	0.266	0.395
	6	0.134	0.037	0.384	0.555
	12	0.278	0.159	0.648	1.085
	30	0.669	0.164	1.282	2.115
2.90×10^{-7}	3	0.030	0.128	0.078	0.236
	6	0.055	0.183	0.136	0.374
	12	0.208	0.039	0.336	0.583
	30	0.315	0.079	0.954	1.348
1.45×10^{-7}	3	0.049	0.021	0.149	0.219
	6	0.122	0.044	0.328	0.494
	12	0.121	0.023	0.334	0.478
	30	0.329	0.030	0.677	1.036

*Baker-flex silica gel 1-B sheets developed in ethanol-water-acetic acid (26:57:7.5) to a 15 cm front.

(1.16×10^{-6} molar), only three percent of the compound taken up was in the undegraded form.

The concentration of undegraded atrazine in the leaves of the seedlings varied considerably, with concentration of the herbicide in the growing medium and period of time after initiation of treatment both affecting the level of atrazine found. A ten-fold variation in concentration was observed, the highest level being 68.7 nmoles per gram dry weight in those seedlings treated with 1.16×10^{-6} molar atrazine and the lowest level, 6.4 nmoles per gram, found in seedlings exposed to the 1.45×10^{-7} molar atrazine solution.

There were two dissimilar metabolites isolated from the needles of the treated seedlings. One, which accounted for about one-third of the radioactivity, remained at the origin in the solvent solution used. The other metabolite, accounting for approximately two-thirds of the activity, had an Rf of 0.6 to 0.7. Atrazine in this same solvent had an Rf of 0.4 and accounted for only a small percentage of the activity except during the first two measurement periods.

Identification of the Metabolites

Without labeled reference compounds available to compare with those isolated in this experiment it was impossible to make positive identification of the two metabolites found in the treated

seedlings. Chromatograms were developed using three different solvent solutions reported in the literature (39, 68, 79) and comparisons made between the metabolites the authors had identified and those found in this experiment. Using these comparisons it appears the metabolite which remained at the origin is most likely hydroxyatrazine and the substances with an Rf of 0.6 to 0.7 are probably peptide conjugates.

Discussion of Metabolism

The inability of the plant to metabolize the herbicide, at least initially, results in a build-up of atrazine to a level which affects the physiological processes of the seedlings. One of the manifestations of these toxic levels was seen in the reduced photosynthetic rate of the seedlings treated with the highest concentration of the herbicide (Table 2). Low concentrations of atrazine, on the other hand, had the opposite effect on the photosynthetic rate. When the level of atrazine in the leaf was below 20 nmoles per gram, there was a marked increase in the rate of CO_2 uptake.

The amounts of atrazine metabolized during the study period indicate the partial resistance of Douglas-fir seedlings to the herbicide noted in an earlier study (50) is the result of the plant's ability to degrade the herbicide. Several investigators have suggested that resistance to herbicide damage is correlated with the

ability of the plant to detoxify the parent compound (53, 66, 67, 79). Shimabukuro and Swanson (69) reported that metabolism of atrazine by cotton resulted in the intermediate tolerance noted for that plant. Similar results were reported with corn and peas by Negi et al. (45).

The levels of undegraded atrazine found in the leaves of these seedlings bracket those reported by Thompson (79) for wild cane, a resistant species. He found an atrazine concentration of 29 nmoles per gram fresh weight in the leaves of plants exposed to the chemical in a nutrient solution.

The metabolites of atrazine found in the seedlings treated in this study were compared to those reported in the literature for other plants. Montgomery and Freed (42) reported chlortriazines were detoxified by replacing the chlorine atom with a hydroxyl group. Corn was identified as one plant which was able to metabolize large quantities of atrazine by hydroxylation (26). Later studies by Shimabukuro and coworkers (68) showed other products of metabolism, namely the two dealkylated analogs of the herbicide. Recent reports by Lamoureux et al. (39) and Thompson (79) indicate a third pathway of metabolism of atrazine by some plants. The products they isolated were peptide conjugates which accounted for a majority of the radioactivity found in the plants which had been exposed to either simazine or atrazine.

The two metabolites (presumably hydroxyatrazine and the peptide conjugates) found in the Douglas-fir seedlings used in this experiment were apparently the same two products Thompson (78) observed in wild cane, Panicum and Setaria. The levels of hydroxyatrazine and peptide conjugates assayed from the coniferous seedlings were similar to those Thompson detected in his investigations of various monocots treated with atrazine.

IMPLICATIONS OF FINDINGS

When interpreting the results of these experiments it must be borne in mind that these tests were conducted under controlled conditions with greenhouse grown seedlings. The environmental conditions under which the plants were grown were carefully controlled and the measurements of the physiological processes were conducted under a single set of conditions, i.e., light intensity, temperature and humidity. An attempt was even made to insure genetic homogeneity through the use of half sibs. Using the data obtained from seedlings in this study, which were tested under one set of environmental conditions, it may be possible to extrapolate to other regimes through the use of information other investigators have published. Krueger and Ferrell (38), Salo (62) and Pope[1] have conducted experiments with several ecotypes of Douglas-fir seedlings under varying regimes of temperature, humidity, light and season. For the study of photosynthesis, Webb's (84) and Pope's development of response surface graphs of photosynthesis under varying environmental conditions may make possible extrapolations from these tests, which would give only a few points on the surface, to a wider range of temperature and light conditions.

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[1] Personal conversation, W.W. Pope, California State University, Humboldt, Department of Forestry.

The implications of these findings can be understood by observing the effects of atrazine on: a) the physiological processes of the plants, b) the growth of the resistant species, and c) the communities into which it has been introduced.

The finest level of resolution in this investigation was obtained by monitoring the rates of photosynthesis, respiration and transpiration. These responses, in turn, were correlated with the level of atrazine present in the leaves of the seedlings. From these observations of the physiological processes, particularly photosynthesis, two critical concentration thresholds for Douglas-fir seedlings, and possibly other plants, were quantified. It was shown that a balance point between growth inhibition and growth promotion occurs around 30 nmoles per gram needles. A second threshold is found when foliar concentration reaches approximately 70 nmoles per gram. Internal levels exceeding this point result in death of the plant. Exposure of the seedlings to levels of atrazine between the first and second threshold results in an initial inhibition, with a slow recovery, of photosynthetic efficiency.

This study suggests the selectivity noted for this herbicide could be the result of either the plants having different damage thresholds or the plants having different abilities to metabolize atrazine.

Looking at the results from an organism level, the whole plant, the dual properties of the herbicide become apparent. When internal concentrations of the herbicide exceed the threshold level, we find growth is inhibited, while at lower concentrations, the growth promotion properties of the compound become visible.

In field application of atrazine the effects of the substance on the crop species is seen in two ways. The reduction of competition on the site quite often means the difference between complete decimation of the crop plants, and survival. Once the plants become established the beneficial physiological effects of the herbicide become important and the increased growth rates noted in this study are realized. It is serendipitous that a compound which can insure survival also promotes growth once the competing vegetation has been suppressed. Preest's (54) study of the growth of Douglas-fir seedlings for three years after establishment showed that volume increment was increased 82 percent by repeated applications of herbicides.

Using low concentrations of atrazine, it may be possible to achieve growth enhancement when plants are grown in a controlled environment, i.e., where the concentration of atrazine in the growing medium can be regulated so damage to the plants does not occur. The levels of the chemical in the growing medium could most easily monitored if the plants were grown in a hydroponic solution. Careful measurements of soil characteristics, coupled with the use of a

predictive equation for herbicide withdrawal, may make possible regulation of atrazine levels in field applications. These possibilities will be discussed in greater detail below.

The ecological impact of the use of atrazine can be seen when plants with different susceptibilities to the compound are present. This means that plants which have the ability to metabolize the compound at a more rapid rate than the competing vegetation, or plants with a higher threshold, will be favored by the use of this chemical and would eventually dominate the treated area. The use of such a chemical is desirable because it is much less disruptive to the ecosystem than the alternative means presently available for controlling competing vegetation. The use of fire, mechanical or hand methods of vegetation control are expensive and often less effective means of establishing desirable plants on a site. This is especially true in forestry when irregular terrain and steep slopes make the use of aerially applied herbicides particularly attractive.

Implications for the Future

The herbicidal properties of atrazine have been widely studied and agriculturists and foresters are already putting them to good use in the field. This study has shown that atrazine possesses the dual properties of growth inhibition at higher levels and growth promotion

at lower concentrations. This latter property presents an attractive alternative use of the compound which has seen only limited exploration.

If it were possible to prescribe treatment in a manner that would insure the exposure of the plants to low concentrations of the compound these beneficial effects could be derived operationally. As mentioned above this would be relatively easy if the plants were grown in a hydroponic solution, but the calculation of the atrazine which would be present when plants are grown in soil is more difficult. One possible way to predict atrazine concentration in the soil would be to develop a process whereby it would be possible to determine the rate at which atrazine is withdrawn from the soil solution, which renders it unavailable to plants growing on the site. In order to utilize this concept, a withdrawal factor would have to be estimated for each soil so that concentrations could be maintained in a desirable range.

There are reports in the literature (66, 87) of correlations between certain soil characteristics and the percent of atrazine withdrawn from the soil solution. Through the use of such relationships, atrazine withdrawal factors could be estimated from soil analyses. This information could then be coupled with concentration-time curves which would enable a resource manager to predict the concentration of the substance in the soil solution at any time.

Some of the following studies indicate how atrazine levels can be predicted. The formula derived by Day et al. (12) could be used for predicting triazine levels in the soil solution from measurements of percent organic matter and cation exchange capacity. The equation offered by Harris and Sheets (28) would necessitate calculations of percent adsorption and organic matter content to predict atrazine levels in the growing medium. The use of either of these equations would enable one to predict the concentration of the herbicide to which the plants would be exposed. To determine the total herbicide level in the soil at any given time after treatment, a means of estimating its disappearance from the soil would be necessary. The curves (Figure 6) presented by Sikka and Davis (72) would be useful in this regard because they show the decrease in atrazine concentration with time, from the soil.

A resource manager may eventually be able to use a soil withdrawal factor, a time-concentration curve and the calculated tolerance of a crop species to prescribe an application rate which would insure adequate control of competing vegetation without damage to the crop. These calculations would also enable him to prescribe lower dosages so that the enhancement effects of low levels of atrazine could be obtained once vegetation control had been effected.

The rationing of atrazine to continue the growth promotion which is apparent when plants are exposed to low concentrations of the

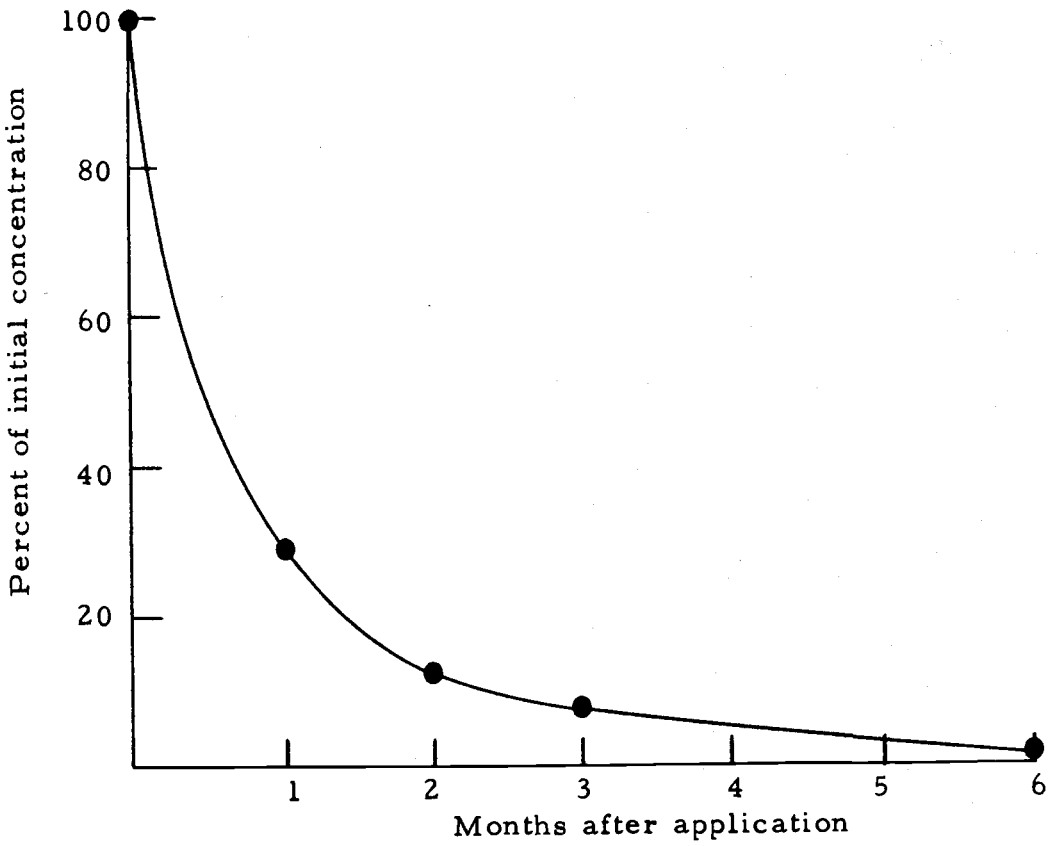


Figure 6. Atrazine concentration in field soil through a 6-month period (from Sikka and Davis, 1966).

herbicide could be accomplished in a couple of ways. First, with repeated light applications of the compound to the growing medium using the various factors discussed above to predict active levels, or secondly, by encapsulating the herbicide in a substance which would slowly decompose, releasing the atrazine into the soil solution over a period of time.

PROPOSED MODE OF ACTION

There is evidence from the data gathered in this study, that the effects of atrazine on these plants, and probably others, is concentration dependent. The reaction of the plant to differing levels of the herbicide indicate that the chemical is affecting two separate metabolic processes, resulting in strikingly different responses; growth promotion at lower levels and photosynthetic inhibition, even death, at higher concentrations. A discussion of the evidence for the effect of atrazine on each system follows.

Process A - Growth Promotion

The evidence for growth enhancement which occurs when plants are exposed to low concentrations of triazines has been accumulating for some time. Exposing plants to low levels of triazines has brought reports of increased growth of corn (14), citrus trees (22), and Douglas-fir seedlings (50). In addition, Ries (59) has reported increased yields from cereal crops when they were treated with simazine at low application rates.

Photosynthetic stimulation has been found in some laboratory experiments (40, 50) where the plants were exposed to low concentrations of the herbicide. In the study reported here the photosynthetic rate of seedlings treated with the lowest levels of atrazine exceeded the control plants by over 30 percent.

A number of studies have shown that low levels of triazines markedly increase the percent nitrogen content in plants and the rate of nitrogen uptake. Ries (59) reported levels of nitrate reductase up to eight times greater in plants growing in simazine. This led him to propose that simazine brought about an increase in enzymatic activity by: a) increasing the uptake of nitrate, b) increasing the rate of nitrate reductase synthesis, c) stimulating protein synthesis with a consequential rise in enzyme activity, or d) some combination of the three.

Another metabolic process that appears to be altered by the level of atrazine present in the plant is the metabolism of the herbicide itself. There is evidence in this study that the rate of degradation of atrazine increases after a few days.

Several recent studies (8, 17, 18) have been undertaken to elucidate the mechanisms by which atrazine is able to alter the growth rate of resistant plants. It appears that atrazine stimulates nucleic acid metabolism and increases protein synthesis when the plants are exposed to low levels of atrazine treatment (9, 52, 73). Foy and Hiranpradit (21) reported that low levels of atrazine markedly retarded senescence and that the chemical exhibited cytokinin-like activity. Further evidence that low levels of atrazine produce a reaction similar to a cytokinin is seen in the elevated rates of transpiration observed in this experiment.

A study by Bush and Ries (8) showed that atrazine in 10^{-8} molar concentration caused a significant increase in fresh weight of non-photosynthetic tissue of red kidney beans. They reported that triazines stimulated protein synthesis or RNA synthesis, or both. They found that the effect of the herbicide on protein synthesis was shown within one or two hours after treatment.

From a study by Penner and Early (52) it could be hypothesized that the initial effect of the triazines on the plants is an increase in nucleic acid synthesis, which results in an increase in protein synthesis. The increased protein synthesis could, in turn, stimulate nitrate uptake.

Process B - Photosynthetic Inhibition

The second process that is affected by atrazine is photosynthesis. It has been found in this and many other studies (11, 50, 64, 83) that the photosynthetic rate is drastically reduced by high concentrations of the herbicide. Studies with isolated chloroplasts have shown that the photosynthetic rate was inversely correlated with the concentration of the triazine present in the solution (43). Another researcher (21) has shown that adding sucrose to the growing medium ameliorates the problem by serving as an alternative source of carbohydrate for the plant. The ultimate effect of exposure to atrazine in high

concentration is disruption of chloroplasts and finally, death of the plant (2).

The concentration of the herbicide in the leaf of the plant determines whether process A or B is predominating. Herbicide levels in the leaf are, in turn, determined by several factors, including concentration of the herbicide in the soil solution, rate of transpiration and concommitant herbicide uptake, and the rate of metabolism of the compound by the plant.

In this study it was found that internal levels of the compound greater than 30 nmoles per gram of dry weight needles brought out the herbicidal properties of atrazine in Douglas-fir seedlings. The minimum concentration needed to trigger the growth enhancing properties of the compound was not determined, but it was found that levels less than ten nmoles per gram of dry weight needles gave the greatest stimulation to photosynthesis. Lower levels were not tested in this study.

The ability of the plants to metabolize the herbicide taken up appears to be the key to the selectivity noted for this herbicide. Those plants which are able to metabolize the compound into non-active forms will be favored over those which are unable to detoxify the compound, especially in an environment in which detoxification maintains foliar concentrations within the stimulatory range while competitors are inhibited.

SUMMARY

The use of herbicides to manipulate vegetation can be easy and economical if the chemical used exhibits selective herbicidal properties. Atrazine is being used extensively in such a manner to control competing vegetation in numerous crops. Recently, atrazine has been used by foresters as an aid in the establishment of coniferous seedlings in nurseries and in forest plantations. Here, the selectivity of the chemical enables the trees to become established by ameliorating the competition for water, sunlight and nutrients posed by the grasses and herbaceous vegetation which formerly occupied the site.

This study was undertaken to investigate the physiological effects of atrazine on Douglas-fir seedlings and to follow the metabolism of the herbicide under controlled environmental conditions. The tree seedlings were exposed to varying concentrations of atrazine for a 30 day period while the uptake of the chemical, the physiological responses of the plants and the metabolism of the herbicide were monitored. Measuring the rates of uptake and metabolism made it possible to correlate the physiological responses noted with the foliar concentration of the undegraded chemical, thereby helping to elucidate the species specificity attributed to atrazine.

Monitoring the physiological responses and the leaf concentrations of the herbicide simultaneously made it possible to correlate the rates of photosynthesis, respiration and transpiration with actual levels of atrazine in the plant.

The responses noted in this study indicate that photosynthesis is altered most drastically by atrazine. The photosynthetic rate was reduced as much as 50 percent when the concentration of atrazine in the leaves exceeded 60 nmoles per gram dry weight. This was irrespective of the external levels of atrazine to which the plants were exposed. Conversely, low levels of the herbicide stimulated photosynthesis and the rate of CO_2 uptake was elevated as much as 30 percent when foliar levels were less than 10 nmoles per gram.

Respiration was not markedly affected by atrazine, except when photosynthetic rates were greatly altered. Only then was a concomitant change in respiration noted. It was found that elevated levels of atrazine slowed the rate of carbon dioxide evolution, while low concentrations caused an acceleration in the rate.

Transpiration was affected only by high concentrations of atrazine. When foliar levels of the herbicide exceeded 60 nmoles per gram the transpiration rate was inhibited greatly. This reduction was attributed to the closing of the stomata caused by a reduction in photosynthesis.

The uptake of atrazine by the tree seedlings was found to be a passive process, that is, the rate of herbicide uptake paralleled the rate of water uptake from the growing medium.

The investigation of metabolism was most important because it made possible the determination of the foliar concentration of atrazine. It was found that the partial resistance to atrazine attributed to Douglas-fir is most likely a result of the plant's ability to detoxify a portion of the herbicide present in the leaves. The rate of metabolism was such that at the end of the 30 day experiment only three percent of the herbicide taken up by the plant was in the undegraded form.

A proposed model for the mode of action of atrazine suggests that in the plant the chemical is affecting two distinct processes. When the foliar level of atrazine is less than 30 nmoles per gram dry weight the compound stimulates the growth processes of the plant. The manifestations of this growth enhancement are seen in elevated levels of photosynthesis, respiration, transpiration and metabolism. Higher concentrations of atrazine, however, make apparent the herbicidal property of the compound, namely, photosynthetic inhibition. The action of atrazine in this process is noted when the levels of the herbicide exceed 30 nmoles per gram. It is theoretically possible to regulate atrazine concentration in the soil so

as to provide for control of sensitive species while maintaining levels in a range actively beneficial for Douglas-fir or other resistant species.

BIBLIOGRAPHY

1. Ashton, F.D., E.M. Gifford and T. Bisalputra. Structural changes in Phaseolus vulgaris induced by atrazine. I. Histological changes. Botanical Gazette 124:329-335. 1963.

2. _____. Structural changes in Phaseolus vulgaris induced by atrazine. II. Effects on fine structure of chloroplasts. Botanical Gazette 124:336-343. 1963.

3. Audus, L.J. The physiology and biochemistry of herbicides. London and New York, Academic Press. 1964.

4. Avron, M. and N. Shavit. Inhibitors and uncouplers of photophorphorylation. Biochimica et Biophysica Acta 109:317-331. 1965.

5. Bentley, J.R., S.B. Carpenter and D.A. Blakeman. Early brush control promotes growth of ponderosa pine planted on bulldozed site. U.S. Forest Service Pacific Southwest Forest and Range Experiment Station Research Note No. P.S.W.-238. 1971.

6. Best, J.A. and J.B. Weber. Disappearance of S-triazines as affected by soil pH using a balance-sheet approach. Weed Science 22:364-373. 1974.

7. Burnside, O.C., C.R. Fenster and G.A. Wicks. Dissipation and leaching of monuron, simazine, and atrazine in Nebraska soils. Weeds 11:209-213. 1963.

8. Bush, P.B. and S.K. Ries. Effect of atrazine on elongation at the embryonic axis of red kidney beans. Weed Science 22:227-229. 1974.

9. Chen, L.G., C.M. Switzer and R.A. Flectcher. Nucleic acid and protein changes induced by auxin-like herbicides. Weed Science 20:51-55. 1972.

10. Clausen, J. and T.T. Kozlowski. Water relations in nursery seedlings and transplants treated with herbicides. University of Wisconsin. Forestry Research Notes No. 111. 1964.

11. Cough, R.W. and D.E. Davis. Effect of atrazine, bromocil, and diquat on $C^{14}O_2$-fixation in corn, cotton and soybeans. Weeds 14:251-255. 1966.

12. Day, B.E., L.S. Jordan and V.A. Jolliffe. The influence of soil characteristics on the adsorption and phytotoxicity of simazine. Weeds 16:209-213. 1968.

13. Dhillon, P.S., W.R. Brynes and C. Merritt. Simazine distribution and degradation in red pine seedlings. Weed Science 16:374-376. 1968.

14. Fink, R. and O.H. Fletchall. The influence of atrazine or simazine on forage yield and nitrogen components of corn. Weeds 15:272-274. 1967.

15. Freeman, J.A., A.J. Renney and H. Driediger. Influence of atrazine and simazine on leaf chlorophylls and fruit yield of raspberries. Canadian Journal of Plant Science 46:454-455. 1966.

16. Freeman, F.W., D.P. White and M.J. Bukovac. Uptake and differential distribution of C^{14}-labeled simazine in red and white pine seedlings. Forest Science 10:330-334. 1964.

17. Freney, J.R. Increased growth and uptake of nutrients by corn plants treated with low levels of simazine. Australian Journal of Agricultural Research 16:257-263. 1965.

18. Foy, C.L. and H. Hiranpradit. Cytokinin-like activity of atrazine, bromacil and fluometuron. (Abstracts) Weed Science Society of America. 1970. p. 38.

19. Funderbuck, H.H., Jr. and D.E. Davis. The metabolism of C^{14} chain- and ring-labeled simazine by corn and the effect of atrazine on plant respiratory systems. Weeds 11:101-104. 1963.

20. Gast, A. and J. Grab. Triazines in top fruit and viticulture. In: Proceedings of the Seventh British Weed Control Conference, Brighton, England, 1964. Vol. 1. London, British Weed Control Council. p. 217-226.

21. Good, Norman. Inhibitors of the Hill reaction. Plant Physiology 36:788-803. 1961.

22. Goren, R. and S.P. Monselise. Some physiological effects of triazines on citrus trees. Weeds 14:141-144. 1966.

23. Graham, J.C. and K.P. Buchholtz. Alteration of transpiration and dry matter with atrazine. Weed Science 16:389-392. 1968.

24. Gysin, H. and E. Knusli. Chemistry and herbicidal properties of triazine and derivatives. Advances in Pest Control Research 3:289-358. 1960.

25. Gupta, G.N. A simple in-vial combustion method for assay of hydrogen-3, carbon-14, and sulfur-35 in biological, biochemical, and organic matter. Analytical Chemistry 38:1356-1359. 1966.

26. Hamilton, R.H. Tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. Journal of Agricultural and Food Chemistry 12:14-17. 1964.

27. Hance, R.J. The speed of attainment of sorption equilibria in some systems involving herbicides. Weed Research 7:29-36. 1967.

28. Harris, C.I. and T.J. Sheets. Influence of soil properties on adsorption and phytotoxicity of CIPC, diuron, and simazine. Weeds 13:215-219. 1965.

29. Hill, E.R., E.C. Putala and J. Vengris. Atrazine-induced ultrastructural changed of barnyardgrass chloroplasts. Weed Science 16:377-380. 1968.

30. Hilton, H.W., O.H. Yuen and N.S. Nomura. Distribution of residues from atrazine, ametryne, and pentachlorophenol in sugarcane. Journal of Agricultural and Food Chemistry 18:217-220. 1970.

31. Humburg, N.E. and C.A. Kust. Transpiration of peas as influenced by chloropropham, trifluralin, or atrazine. (Abstracts) Weed Science Society of America, 1970. p. 53.

32. Jordan, L.S. Simazine translocation and degradation in citrus seedlings. (Abstracts) Weed Science Society of America, 1970. p. 62.

33. Kaufman, D.D. and P.C. Kearney. Microbial degradation of triazine herbicides. Residue Reviews 32:234-266. 1970.

34. Kittams, J.A. and R.A. Ryker. Habitat type and site preparation affect survival of planted Douglas-fir in central Idaho brushfields. USDA Forest Service Research Note INT-198. 1975.

35. Kozlowski, T.T. and J.E. Kuntz. Effects of simazine, atrazine, propazine and eptam on growth and development of pine seedlings. Soil Science 95:164-174. 1963.

36. Kozlowski, T.T., S. Sasaki and J.H. Torrie. Influence of temperature on phytotoxicity of triazine herbicides to pine seedlings. American Journal of Botany 54:790-796. 1967.

37. Krueger, K.W. Comparative photosynthesis and respirational rates of Douglas-fir seedlings from Vancouver Island and Montana under various conditions of light and temperature. Ph.D. thesis. Corvallis, Oregon State University, 1963. 80 numb. leaves.

38. Krueger, K.W. and W.K. Ferrell. Comparative photosynthetic and respiratory responses to temperature and light by Pseudotsuga menziesii var. menziesii and var. glauca seedlings. Ecology 46:794-801. 1965.

39. Lamoureux, G.L., R.H. Shimabukuro, H.R. Swanson and D.S. Frear. Metabolism of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) in excised sorghum leaf sections. Journal of Agricultural and Food Chemistry 18:81-86. 1970.

40. Lund-Hoie, K. The effects of simazine on the photofixation of CO_2 and on translocation of assimilates in Norway spruce (Picea abies). Weed Research 9:185-191. 1969.

41. Moody, K., C.A. Kust and K.P. Buchholtz. Release of herbicides by soybean roots in culture solutions. Weed Science 18:214-218. 1970.

42. Montgomery, M.L. and V.H. Freed. Metabolism of triazine herbicides by plants. Journal of Agricultural and Food Chemistry 12:11-14. 1964.

43. Moreland, D.E., W.A. Gentner, J.L. Hilton, and K.L. Hill. Studies on the mechanism of herbicidal action of 2-chloro-4,6 bis (ethylamino)-s-triazine. Plant Physiology 34:432-435. 1959.

44. Nearpass, D.C. Effects of soil acidity on the adsorption, penetration, and persistence of simazine. Weeds 13:341-346. 1965.

45. Negi, N.S., H.H. Funderbuck, Jr. and D.E. Davis. Metabolism of atrazine by susceptible and resistant species. Weeds 12:53-57. 1964.

46. Newton, M. Constructive use of herbicides in forest resource management. Journal of Forestry 73:329-336. 1975.

47. Newton, M. Environmental management for seedling establishment. Oregon State University Forest Research Laboratory Research Paper 16. 1973.

48. Newton, M. Progress report on FRL project F834 - environmental and biochemical effects of herbicides used in reforestation. School of Forestry, Oregon State University, Corvallis, Oregon. 1970.

49. Pallas, J.E. and J.E. Box. Explanation of the stomatal response of excised leaves to kinetin. Nature 227:87-88. 1970.

50. Peterson, C.A. Some physiological effects of atrazine on Douglas-fir seedlings. M.S. thesis. Corvallis, Oregon State University, 1969. 44 numb. leaves.

51. Penner, D. Effects of temperature on phytotoxicity and root uptake of several herbicides. Weed Science 19:571-576. 1971.

52. Penner, D. and R.W. Early. Effects of atrazine on cromatin activity in corn and soybean. Weed Science 20:367-370. 1972.

53. Plimmer, J.R., P.C. Kearney and U.I. Klingebiel. Atrazine dealkylation: A study of the mechanism of detoxication of some S-triazines. (Abstracts) Weed Science Society of America No. 202. 1969.

54. Preest, D.S. Effects of herbaceous weed control on young Douglas-fir moisture stress and growth. Ph.D. thesis. Corvallis, Oregon State University, 1975. 55 numb. leaves.

55. Raschke, K. Stomatal action. Annual Review of Plant Physiology 26:309-340. 1975.

56. Raschke, K. Zur Steuerung der Transpiration durch die Photosynthese. Bot. Ges. 80:138-144. 1967.

57. Ries, S.K., H. Chmiel, D.R. Dilley and P. Filner. The increase in nitrate reductase activity and protein content of plants treated with simazine. Proceedings of the National Academy of Sciences 58:526-532. 1967.

58. Ries, S.K. and A. Gast. The effects of simazine on nitrogenous components of corn. Weeds 13:273-274. 1965.

59. Ries, S.K. and V. Went. Simazine-induced nitrate absorption related to plant protein content. Weed Science 20:569-572. 1972.

60. Roeth, F.W. and T.L. Lavy. Atrazine translocation and metabolism in sudangrass, sorghum and corn. Weed Science 19:98-106. 1971.

61. Roeth, F.W. and T.L. Lavy. Atrazine uptake by sudangrass, sorghum and corn. Weed Science 19:93-97. 1971.

62. Salo, D.J. Factors affecting photosynthesis in Douglas-fir. Ph.D. thesis. Seattle, University of Washington, 1974.

63. Santelmann, P.W., J.B. Weber and A.F. Wiese. A study of soil bioassay technique using prometryne. Weed Science 19: 170-174. 1971.

64. Sasaki, S. and T.T. Kozlowski. Effects of herbicides on photosynthesis of red pine seedlings. University of Wisconsin Forest Research Notes No. 118. 1965.

65. Sheets, T.J. Persistence of triazine herbicides in soils. Residue Review 32:287-310. 1970.

66. Shimabukuro, R.H. Atrazine metabolism and herbicidal selectivity. Plant Physiology 42:1269-1276. 1967.

67. Shimabukuro, R. H. Atrazine metabolism in resistant corn and sorghum. Plant Physiology 43:1925-1930. 1968.

68. Shimabukuro, R. H., R. E. Kadunce and D. S. Frear. Dealkylation of atrazine in mature pea plants. Journal of Agricultural and Food Chemistry 14:392-395. 1966.

69. Shimabukuro, R. H. and H. R. Swanson. Atrazine metabolism in cotton as a basis for intermediate tolerance. Weed Science 18:231-234. 1970.

70. Shimabukuro, R. H. and H. R. Swanson. Atrazine metabolism, selectivity, and mode of action. Journal of Agricultural and Food Chemistry 17:199-205. 1969.

71. Shimabukuro, R. H. and H. R. Swanson. Metabolism of root-applied versus foliarly-applied atrazine in corn. (Abstract) Weed Science Society of America No. 197. 1969.

72. Sikka, H. C. and D. E. Davis. Dissipation of atrazine from soil by corn, sorghum and johnsongrass. Weeds 14:289-293. 1966.

73. Singh, R. P. and S. H. West. Influence of simazine on chloroplast ribonucleic acid and protein metabolism. Weeds 15:31-34. 1967.

74. Stranger, C. A., Jr. and A. P. Appleby. A proposed mechanism for diuron induced phyto-toxicity. Weed Science 20:357-363. 1972.

75. Smith, C. N., Jr. and J. D. Nalewaja. Uptake and translocation of foliar applied atrazine. Weed Science 20:36-40. 1972.

76. Sun, C-N. and R. S. Adams, Jr. Effects of the phosphorus-manganese-atrazine interaction in soybean plants. Journal of Agricultural and Food Chemistry 19:325-330. 1971.

77. Sutton, D. L., D. A. Durham, S. W. Bingham and C. L. Foy. Influence of simazine on apparent photosynthesis of aquatic plants and herbicide residue removal from water. Weed Science 17:56-59. 1969.

78. Thompson, L., Jr. Metabolism of chloro-triazine herbicides by Panicum and Setaria. Weed Science 20:584-587. 1972.

79. Thompson, L., Jr. Metabolism of simazine and atrazine by wild cane. Weed Science 20:153-155. 1972.

80. Thompson, L., Jr., J.M. Houghton, S.W. Slife and H.S. Butler. Atrazine, Panicum and large crabgrass. Weed Science 19:409-412. 1971.

81. Thompson, L., Jr., S.W. Slife, and H.S. Butler. Environmental influence on the tolerance of corn to atrazine. Weed Science 18:509-514. 1970.

82. Vostral, H.J., K.P. Buchholtz and C.A. Kurst. Effect of root temperature on absorption and translocation of atrazine in soybean. Weed Science 18:115-117. 1970.

83. van Oorschot, J.L.P. Effect of transpiration rate of bean plants on inhibition of photosynthesis by some root-applied herbicides. Weed Research 10:230-242. 1970.

84. Webb, W.L., M. Newton and D. Starr. Carbon dioxide exchange of Alnus rubra, a mathematical model. Oecologia 17:281-291. 1974.

85. Weber, J.B. Mechanisms of adsorption of s-triazines by clay colloids and factors affecting plant availability. Residue Review 32:93-130. 1970.

86. Wheeler, H. and R.H. Hamilton. The leaf concentrations of atrazine in cereal crops as related to tolerance. Weed Science 16:7-10. 1968.

87. Wills, G.D., D.E. Davis and H.H. Funderbuck, Jr. The effect of atrazine on transpiration in corn, cotton, and soybeans. Weeds 11:253-255. 1963.

APPENDIX

Preliminary Study of Photosynthesis

Before launching the study reported in the main body of this report, innumerable measurements of photosynthesis were made to determine the range of atrazine concentrations to be tested. Photosynthetic measurements were made on Douglas-fir seedlings exposed to atrazine concentrations ranging from one part per million (4.64×10^{-6} molar) to 1/32 ppm (1.45×10^{-7} molar).

From the data obtained in this preliminary study it appeared that 1/4 ppm (1.16×10^{-6} molar) atrazine in nutrient solution was the highest concentration from which the plants were able to recover and it was chosen as the upper limit for this investigation. Seedlings exposed to a herbicide concentration of 1/32 ppm exhibited photosynthetic enhancement and this level was selected as the lower limit for this study. These data are presented in Appendix Tables 1 and 2. A response surface graph of the photosynthetic rates obtained in this early study is shown in Appendix Figure 1.

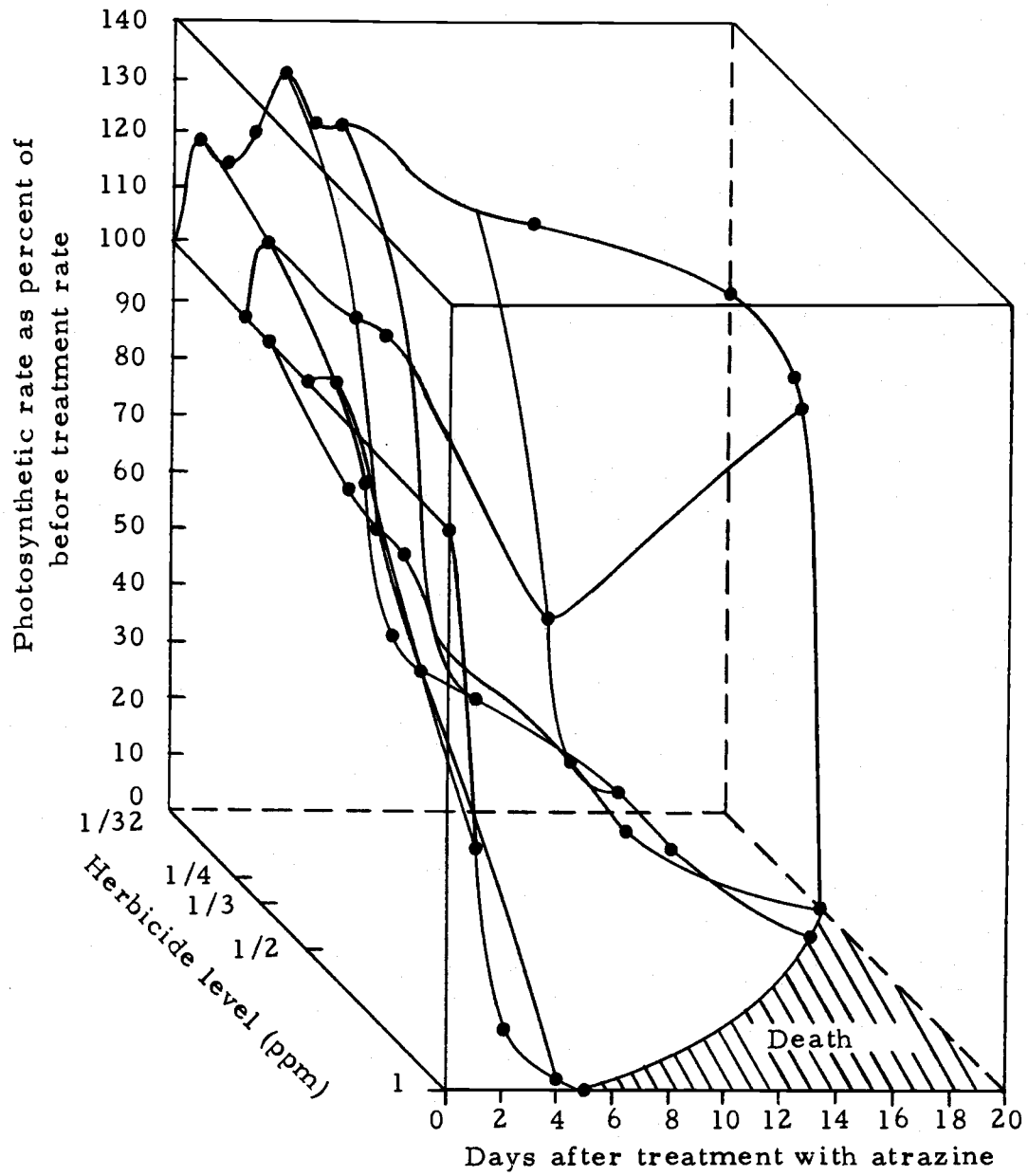
Appendix Table 1. Photosynthetic rate of Douglas-fir seedlings treated with 1 ppm atrazine.

Time after treatment (hours)	Photosynthetic rate (% of before treatment)
1	88
3	62
4	68
5	71
9	41
10	38
24	43
28	16
48	11
96	2

Appendix Table 2. Photosynthetic rate of Douglas-fir seedlings treated with atrazine (rate expressed as a percentage of before treatment rate).

Time after treatment (days)	Atrazine levels (ppm)			
	1/2	1/3	1/4	1/32
1	102	- *	112	119
2	83	-	-	114
3	56	74	-	120
4	49	67	99	131
5	51	64	97	122
6	45	48	90	122
11	29	26	47	106
13	18	13	-	104
20	3	dead	84	92

* - indicates there were no measurements made that day.



Appendix Figure 1. Response surface graph of preliminary photosynthesis data.

Detailed Procedure for Extraction and Isolation of Atrazine and Its Metabolites

The extraction of the labeled atrazine and its metabolites from the seedlings follows the procedure outlined below:

1. Homogenize the fresh material with 80 ml of 95 percent methanol per 25 grams fresh weight.
2. Filter and resuspend twice in 95 percent methanol.
3. Evaporate methanol.
4. Wash the residue twice with water and concentrate to 5 ml.
5. Chromatograph the solution on silica gel 1-B thin layer sheets and develop in ethanol-water-acetic acid (26:57:7.5), using a 15 cm front.
6. Scan the chromatogram sheets.

Assay for ^{14}C Using Dry Combustion

1. Air dry the material and grind to pass a 20 mesh screen in a Wiley mill.
2. Burn 3-7 mg of the material in a sealed vial filled with pure oxygen.
3. Trap the $^{14}CO_2$ in the vial in 0.2 ml of ethanolamine.
4. Assay using the liquid scintillation counter by adding 15 ml of the scintillation solution, composed of 5:2 mixture of toluene

and 2-methoxyethanol, 5 g per liter of PPO and 300 mg per liter of POPOP, one hour after combustion.

5. Count for 20 minutes or 10,000 counts in liquid scintillation counter.

Nutrient Solution Used in the Experiment

	milliliter per liter water
$Ca(NO_3)_2 \cdot 4\ H_2O$	12.5
KNO_3	17.5
NH_4Cl	0.5
$NaH_2PO_4 \cdot H_2O$	2.5
$MgSO_4 \cdot 7\ H_2O$	5.0
Fe - DTPA with sequestene	1.0
A - 5 (minor elements)	0.25