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Oluyemisi Amos Akinyemiju

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DIFFERENTIAL EFFECTS OF SIMAZINE AND DIURON ON SURVIVAL, GROWTH AND PHYSIOLOGY OF <u>POPULUS</u> CLONES

By

Oluyemisi Amos Akinyemiju

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ABSTRACT

DIFFERENTIAL EFFECTS OF SIMAZINE AND DIURON ON SURVIVAL, GROWTH AND PHYSIOLOGY OF POPULUS CLONES

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Field and greenhouse studies were performed to evaluate the differential effects of simazine (2-chloro-4, 6-bis (ethylamino) - s - triazine) and diuron (3-(3,4-dichlorophenyl) - 1, 1-dimethyl urea) on survival, growth and physiology of Populus clones. In field studies on three plantation sites in lower Michigan, clonal differences in establishment, growth and biomass yield of several Populus clones was demonstrated in response to application of simazine and diuron. The response of clones to simazine and diuron followed similar pattern. High doses of each herbicide were toxic to all clones except H 47, which appeared to be relatively resistant. Simazine at 2 kg/ha was found adequate for acceptable weed control, survival and biomass yield at the Tree Research Center (TRC) and Dansville. At Manistee, however, simazine was ineffective due to the low pH of the surface soil and the presence of resistant weed species, but diuron at 2 kg/ha was suitable.

A single initial tillage at the beginning of the season benefitted all clones tested at all herbicide rates;

survival, growth and biomass yield were higher on tilled than untilled plots. Supplementing tillage with herbicides further increased survival and growth of the clones above what was obtained with tillage or herbicide alone.

At the TRC plantation, simazine caused an increase in total foliar nitrogen of Populus clones beyond that caused by elimination of competing vegetation. The stimulatory effect of simazine on total foliar nitrogen was dependent on clone, rate of simazine and length of time after application. Clone H 47 and NC 5323 treated with 2 to 3 kg/ha simazine gained nearly 12% in total foliar nitrogen over the untreated weed-free control at the middle of the season, the period of most active growth. Nitrogen contents of clone NE 388 and NE 48 were unaffected, but height growth of NE 388 was significantly inhibited by simazine.

In one greenhouse study 21 Populus clones from section Tacamahaca and Aigeiros were assayed for tolerance to five levels of simazine and diuron. Selected clones included both intra- and intersectional hybrids as well as pure P. deltoides. Each of the clones could be classified as tolerant, intermediate or intolerant to simazine or diuron. Tacamahaca hybrids and intersection crosses between Tacamahaca and Aigeiros were relatively intolerant of both herbicides, whereas Aigeiros clones were relatively tolerant. Examples of intolerant clones include

P. *maximowiczii* x *P*. *trichocarpa* (NE 388) and *P*. *maximowiczii* x *P*. cv. 'berolinensis' (NE 48). The *P*. x *euramericana* clones 'Canada blanc' (NC 5323) and 'I 45/51' (NC 5328) were among the most tolerant tested. A selectivity index with simazine of ca. 11 between NC 5328 and NE 388 was calculated.

In a second greenhouse study, additions of 5 mg/pot of simazine had no deleterious morphological or physiological effects on NC 5328 (Aigeiros) but reduced the rate of CO_2 fixation, increased CO_2 compensation concentrations and lowered the specific leaf weight of NE 388 (Tacamahaca). The deleterious effects of simazine on NE 388 were detected ca. 48 hr after exposure of plants to simazine and generally became more pronounced thereafter. Visual symptoms of injury were evident at ca. 2 week after simazine application.

Toxic responses to simazine in intolerant clone NE 388 were different depending on the position of the crown that was sampled. Inhibition of photosynthesis and increased CO_2 compensation points were more pronounced in the region of recently mature leaves, but somewhat less in the region of expanding leaves. The lower crown region consisting of older mature leaves showed no visual symptoms of injury; rate of photosynthesis and CO_2 compensation concentration were unaffected.

Differential physiological and morphological responses to simazine and diuron among clones in greenhouse studies confirmed the varying clonal responses to the herbicides observed in the field.

This dissertation is dedicated to all
Those in search for knowledge: That in
Patience they may find and
Perseverance overcome.

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	ix
CHAPTER I. INTRODUCTION	1
CHAPTER II. THE EFFECTS OF TILLAGE AND HERBICIDES (SIMAZINE AND DIURON) ON THE ESTABLISHMENT OF POPLAR PLANTATIONS AT DIFFERENT LOCATIONS IN LOWER MICHIGAN	7
Introduction	7
Materials and Methods	9
Results	16
Discussion	29
CHAPTER III. THE INFLUENCE OF SIMAZINE ON TOTAL NITROGEN AND PHOSPHORUS CONCENTRATIONS IN FOLIAGE OF FIELD-GROWN POPLAR CLONES	34
Introduction	34
Materials and Methods	36
Results and Discussion	38
CHAPTER IV. DIFFERENTIAL TOLERANCE OF <u>POPULUS</u> CLONES TO SIMAZINE AND DIURON	45
Introduction	45
Materials and Methods	47
Results	51
Discussion	65
CHAPTER V. EFFECT OF SIMAZINE ON PHOTOSYNTHETIC CO_2 FIXATION, CO_2 COMPENSATION POINT, LEAF STOMATAL CONDUCTANCE AND SPECIFIC LEAF WEIGHT OF SIMAZINE-TOLERANT AND INTOLERANT <u>POPULUS</u> CLONES	70
Introduction	70
Materials and Methods	72
Results	77
Discussion	82
CHAPTER VI. SUMMARY AND CONCLUSIONS	89
BIBLIOGRAPHY	94

LIST OF TABLES

Table		Page
2.1	Soil properties of the experimental plantation sites.	11
2.2	Parentage of Populus clones planted on the three experimental plantation sites	13
2.3	The effect of simazine on survival and height growth of Populus hybrids in their first growing season on tilled and untilled sites in lower Michigan (TRC)	17
2.4	First-year survival and height growth of four Populus hybrids established with varying levels of simazine in lower Michigan, (TRC)	19
2.5	The effect of simazine on survival, growth and biomass yield of Populus hybrids during their second growing season on tilled and untilled sites in lower Michigan, (TRC)	21
2.6	Second-year survival and height growth of four Populus hybrids established with simazine in lower Michigan	22
2.7	The effect of simazine and diuron on survival and height growth of Populus hybrids during their first growing season on tilled and untilled sites in lower Michigan, (Dansville)	24
2.8	First-year survival and growth of three Populus hybrids established with simazine and diuron in lower Michigan, (Dansville)	25
2.9	The effect of simazine and diuron on survival and growth of Populus hybrids during their first growing season on tilled and untilled sites in lower Michigan, (Manistee)	27
2.10	First-year survival and height growth of three Populus hybrids establishment with simazine and diuron in lower Michigan, (Manistee)	28

TABLE		PAGE

3.1 Parentage of Populus clones used in nitrogen experiment. 37

3.2 The effect of simazine on total nitrogen, phosphorus and leader height growth of Populus hybrids, at two dates during the first growing seasons. 38

3.3 The effect of simazine on total kjeldahl nitrogen, leader height growth and survival of four 1-yr-old Populus hybrids . 40

4.1 Parentage of the Populus clones used in herbicide screening. 48

4.2 Criteria used for whole plant and leaf visual scoring. 50

4.3 Analysis of variance for number of leaves per plant, height, dry weight, plant and leaf score four weeks after the application of varying rates of simazine and diuron to Populus clones grown on sand culture in the greenhouse. 52

4.4 Whole plant and leaf visual scores per plant four weeks after the application of varying rates of simazine to 21 Populus clones grown on sand culture in the greenhouse. 53

4.5 Whole plant and leaf visual scores per plant four weeks after the application of varying rates of diuron to 21 Populus clones grown on sand culture in the greenhouse 54

4.6 Relative total dry weight, four weeks after the application of varying rates of simazine to 21 Populus clones grown in sand culture in the greenhouse. . . . 55

4.7 Relative dry weight, four weeks after the application of varying rates of diuron to 21 Populus clones grown in sand culture in the greenhouse 56

TABLE		PAGE

4.8 Toxicity of simazine to 21 Populus clones as shown by ED_{20} and ED_{50} values. 61

4.9 Toxicity of diuron to 21 Populus clones as shown by ED_{20} and ED_{50} values. 62

4.10 Clonal rankings based on ED_{20} values for simazine and diuron four weeks after treatment with varying levels of the herbicides. 63

5.1 Leaf plastochron index (LPI) in relation to leaf serial number and plastochron index (PI) for young Poplars measured at various times for response to simazine . 75

5.2 Physical condition of Populus clones NE 388 and NC 5328 two weeks after treatment with 5 mg/pot simazine. 78

5.3 Abaxial leaf conductance of Populus clones NE 388 and NC 5328 after treatment with 5 mg/pot simazine 80

5.4 CO_2 compensation concentrations of Populus clones NE 388 and NC 5328 after treatment with 5 mg/pot simazine. . . 81

5.5 Leaf photosynthetic rate of Populus clones NE 388 and NC 5328 after treatment with 5 mg/pot simazine 83

5.6 Specific leaf weight of Populus clones NE 388 and NC 5328 after treatment with 5 mg/pot simazine 84

LIST OF FIGURES

FIGURE		PAGE
3.1	Effect of simazine on weed control and survival of Populus clones at the end of their first growing season in lower Michigan (TRC).	42
4.1	Relative total dry weights of Populus clones, four weeks after treatment with varying rates of simazine and diuron	57

CHAPTER I

INTRODUCTION

Among the hardwoods proposed for high-yielding, intensive culture in the north-temperate regions of the United States is the genus *Populus*, especially members of sections Aigeiros (cottonwoods) and Tacamahaca (balsam poplars) and their hybrids (Schreiner, 1970; Dickmann, 1975). Poplars are among the fastest-growing trees in North America, they can easily be propagated vegetatively by hardwood cuttings and coppice regeneration after harvesting is vigorous. However, poplars are "prima donnas" (McKnight, 1970); their high yield potential can only be realized on sites where moisture, aeration, and fertility are adequate throughout the growing season. In addition, a high level of silviculture, especially control of competing vegetation, is required for the attainment of their yield potential.

A number of attempts have been made to identify the most suitable establishment methods for poplar (DeBell and Alford, 1972; Briscoe, 1973; Randall and Kennedy, 1976; Gilmore, 1976; Petersen and Phipps, 1976; Randall and Krinard, 1977). Some of these findings have become routine practices in plantation establishment. However, economic considerations and practicability for large plantations have made a few of them unattractive. For example, Randall and Krinard (1977) found that 1-yr-old rooted cuttings planted in 1 m deep holes provided an excellent alternative to shallow planting. The obvious biological advantages of deep-planting long, rooted cuttings, however, are often outweighed by nursery and planting costs. Similarly, neither survival nor first-year growth of a group of six Stoneville, Mississippi, cottonwood clones was improved by angle planting or cutting the base of cuttings diagonally (Randall and Kennedy, 1976). Survival of cottonwood seedings was higher when seedlings were planted in auger holes as opposed to dibble plantings; however, as Gilmore (1976) pointed out, each manager has to decide between the alternatives of lower survival but lower planting cost, or high survival and higher planting costs.

Site improvement and reduction in competition have also been investigated for poplars. Schreiner (1945a, 1945b) early demonstrated the inhibiting effects of sod on the growth and development of hybrid poplar. A number of approaches to weed control have been investigated. The applicability of black polyelthylene mulch in establishing plantations of hybrid poplar was compared with mechanical cultivation during two dissimilar growing seasons (Bowersox and Ward, 1969). The results indicated that establishment success with polyethylene can equal or exceed that of mechanical cultivation. However, in a season of prolonged drought, polyelthylene film hindered the recharge of soil moisture by light rainfall, nullifying the early growth advantage of the mulched trees. The economics of this technique, though not investigated, do not appear attractive.

Mechanical cultivation has been the most commonly-used method of weed control and site improvement for poplar (Meritt and Bramble, 1966; Bowersox and Ward, 1969; Baker and Blackmon, 1978; Krinard and Johnson, 1975; McKnight and Biesterfeldt, 1969; Minckler and Woerheide, 1965; McKnight, 1970). Kennedy (1975) demonstrated the need for extreme care during cultivation of young cottonwood plantations. Poor first-year cultivation due to improper adjustment of equipment, disking too close to young plants or driving so fast as to cover the cuttings with soil resulted in growth losses. There are also planting spacing and economic limitations to mechanical cultivation.

Biological pest management remains relatively uninvestigated in forestry. The concept of one organism controlling another was recognized by Darwin (1859). Cover crops (Ford and Williamson, 1952) to control unwanted vegetation have proved unsatisfactory in establishing hybrid poplars. Recent advances in allelopathic research may offer future practical utility in the control of unwanted vegetation. A number of plant species have been shown to possess allelochemics which are capable of reducing vegetation (Stachon and Zimdahl, 1980; Toai and Linscolt, 1979; Stewart, 1975; Steenhagen and Zimdahl, 1979; Kelsey and Harrington, 1979; Funk et al., 1979; Lockerman and Putnam, 1979; Buchanan et al., 1978; Fischer et al., 1978). More research still needs to be done before the concept can be of practical utility. Using insects to control weeds in forestry and agriculture has received very little attention (Anderson, 1977). The goal of this approach, based on sound principles of population ecology, is not weed eradication but reduction of a weed's abundance to economically or aesthetically tolerable levels (Goeden, 1977). Limitations to application of biological weed control, however, exist. Breeding insects or biotic agents for species specificity has not met with any appreciable success and the problem of restricting the biotic agent from the preferred plants after the exhaustion of the undesired plants remains a problem (Anderson, 1977).

Reduction of weeds to non-competitive levels continues to be the aim of weed research in agronomy and short-retation intensive forestry where the objective is to maximize the growth potential of the crop on a particular site. Chemicals remain the major economically and efficient method of attaining this objective. A number of attempts have been made to identify suitable pre- and post-emergent herbicides for use in the establishment of poplar (Esau and Morgan, 1977; Shipman, 1974; Woessner, 1972).

Simazine (Dickmann et al., 1978; Martin and Carter, 1966; Shipman, 1974), for example, has been shown to be promising for weed control in poplar. However, field and greenhouse observations of plant injury due to application of simazine or other herbicides such as diuron at levels adequate for acceptable weed control have been reported for some clones of poplar on different soils (Bowersox and Ward, 1969; Dickmann et al., 1978; von Althen, 1979; Esau and Morgan, 1977). The extent and mechanism of differential tolerance in Populus hybrid clones to various herbicides remains relatively uninvestigated (von Althen, 1979).

Soil and environmental factors are among the most important determinants of the efficacy of herbicides. The effect of tillage, pH, clay minerals and other textural fractions, organic matter, nutrient types and levels, on simazine have been investigated (Sheets, 1970; Weber, 1970; Best et al., 1975; Kells et al., 1980; Koren and Shlevin, 1977; Atkinson and Allen, 1976; Hance, 1976; Slack et al., 1978; Nearpass, 1965; Hance et al., 1968, 1977; Walker and Thompson, 1977; Richardson and Banting, 1977). In a few cases diuron was included in the investigations. In a review on ways to influence the activity and persistence of triazine herbicides in soils, LeBaron (1970) stressed the complimentarity of some form of mechanical cultivation and triazine herbicide application. This practice is widespread in agronomy but is little known in intensive forestry (McKnight, 1970).

Differential clonal responses to simazine and diuron, and the interactive effect of soil factors on both the efficacy of the herbicides and performance of poplar clones call for specific site, clonal and chemical evaluations. The potential contribution of tillage in chemical control of weeds in intensive culture of poplar also deserves investigation. Equally important is an understanding of the physiological mechanism of tolerance of poplar to simazine and diuron.

The research reported in this dissertation investigated:

1. The effect of tillage, clone, herbicide (simazine and diuron) and their interaction on the establishment of poplar plantations at different locations in lower Michigan.
2. The influence of simazine on total nitrogen concentrations in foliage of field-grown poplar clones.
3. The dose-response of selected poplar clones treated with varying rates of simazine or diuron in the greenhouse using a sand culture technique.
4. Variation in photosynthesis, leaf conductance and leaf morphology of poplar clones identified as tolerant or intolerant to simazine.

CHAPTER II

THE EFFECT OF TILLAGE, CLONE, HERBICIDE (SIMAZINE AND DIURON) ON THE ESTABLISHMENT OF POPLAR PLANTATIONS AT DIFFERENT LOCATIONS IN LOWER MICHIGAN

INTRODUCTION

Residual activity of herbicides is essential for season long control of weeds. Without residual activity, frequent applications of less persistent herbicides would be necessary, and costs of weed control would be high. Simazine and diuron exhibit varying degrees of persistence in soils. Rates of disappearance of these chemicals are dependent on several environmental and edaphic factors (Hartley, 1976; Muzik, 1976). Soil texture has a greater effect on carry-over of several herbicides than climate (Sheets, 1970), with carry-over greater in course than in fine-textured soils (Harris and Sheets, 1965). The phytotoxicity of diuron was not influenced by soil pH of between 4.3 and 7.5 (Corbin et al., 1971). But persistence and adsorption of simazine by several soils was correlated significantly with percent clay, organic matter and titrable acidity (Nearpass, 1965). Persistence of simazine in the soil was shown to increase with soil pH (Slack et al., 1978), while rate of formation of an unextractable ^{14}C-breakdown product from ^{14}C-atrazine increased with decreasing pH (Kells et al., 1980).

In field studies Best et al., (1975) showed that liming an acid Bladen silt loam which increased pH from 5.5 to 7.5, increased the phytotoxicity of several S-triazines. In forestry, unlike agronomic situations where carry-over could be a problem, the greater the persistence of an herbicide, the greater its effectiveness in weed control.

Soil-type effects on persistence per se are usually difficult to assess from field experiments due to the complicating influence of climate, especially rainfall and temperature. While it is simple to show that persistence varies among soils, determination of the causes for differences is difficult in a crude soil system (LeBarron, 1970). It is essential, therefore, that information is obtained for particular herbicides on specific sites intended for intensive poplar culture.

On some soil types substituting herbicides for cultivation is not only practical but may benefit some crops by avoiding root damage (LeBarron, 1970). However, there has been an increasing realization that the use of herbicides and tillage often complement each other. Tillage in combination with several herbicides has been shown to increase both weed control and yield of soybeans over no-till methods (Kapusta, 1979). Field studies on the persistence of simazine showed less persistence under no-tillage corn than conventionally-tilled corn (Slack et al., 1978), and the rate of formation of an unextractable ^{14}C-compound from ^{14}C-atrazine was greater under no-tillage (Kells et al., 1980). Such information in forestry is lacking, especially in intensive culture of poplar.

The purpose of this study was to investigate the effect of tillage on the phytotoxicity of varying rates of simazine and diuron during establishment of hardwood cuttings of several different clones of hybrid poplar at three sites in lower Michigan.

MATERIALS AND METHODS

The study sites were located in three areas in lower Michigan. The first was at the Tree Research Center (TRC) of Michigan State University (NE¼ X 6 T3N RIW) in Ingham County; the second was also in Ingham County near Dansville (S 33 T2N R1E), and the third was in Mason County South of Manistee (S 25 T20N R17W). The three areas were previously agricultural lands that had been abandoned in the last 15 to 20 years and were occupied by grasses, and broad-leaf weeds. Each of the 3 experiments was a split-split-plot, three-level factorial. The three levels were tillage, herbicide, and clone. Tillage in each experiment was accomplished with an Oliver 550 tractor fitted with a 1.5 m wide tiller, run with the power take-off from the tractor. The tractor made two passes on each tilled area to a depth of 15 cm. The herbicide was sprayed with a tractor mounted, 323.0 liter tank with a rear pump also run from the power take-off. The sprayer consisted of 2 T-jet nozzles (#8006) 46 cm apart, delivering a spray width of 90 cm. Tractor speed was maintained at 3.2 km/hr and tank pressure was maintained at $2x10^6$ dynes $/cm^2$ for all spraying.

Cuttings were ca. 25 cm long and 1.3 cm in diameter on the average and had buds close to the proximal end. Cuttings were planted by hand with ca. 2.5 cm of each cutting left above the soil surface. Triplicate soil samples were taken randomly with a soil auger from each planting site at the beginning of each experiment. Depth to the B horizon was measured for each sample. Textural analysis was done on the samples using the Bouyoucos (1951) hydrometer method. Organic matter, pH and nutrient determinations were done by the Michigan State University Soil Testing Laboratory (Table 2.1).

All data collected were statistically evaluated using the analysis of variance. Means were compared using the least significant difference (LSD) at the 10% significance level (Cochran and Cox, 1957).

TABLE 2.1 SOIL PROPERTIES OF THE EXPERIMENTAL PLANTATION SITES

Character	Horizon	Experimental Sites		
		TRC	Dansville	Manistee
Soil Series	--	Metea Sandy Loam	Belle Fontaine Sandy Loam	Nester Loam
Texture (Class)	AP	Sandy Loam	Loamy Sand	Sandy Loam
	A2	Loamy Sand	Loamy Sand	Loamy Sand
Organic Matter (%)	AP	7.0	3.2	4.0
	A2	2.8	1.4	1.6
Clay (%)	AP	15.0	8.0	11.5
	A2	13.5	6.0	10.0
Nitrate (mg/kg)	AP	4.6	10.5	5.0
Depth (cm)	AP	30	19	27
	A2	17	18	17
pH	AP	6.7	6.1	5.6
	A2	7.4	7.1	6.1

TRC Plantation

This plantation was established in spring of 1978 on a 60 m x 60 m area and followed for 2 years. The area was divided into 5 blocks of 60 m x 12 m each. Each block was partitioned into parallel strips among which simazine levels of 0, 2, 3 and 4 kg/ha active ingredient (a.i.) were randomly partitioned. Tilled and untilled factors were alternatively imposed on each level of simazine. Glyphosate at a rate of 2 kg/ha a.i. was sprayed over the untilled plots to control existing weeds a few days before simazine was sprayed. The control plots were sprayed only with glyphosate and subsequent weed control was done with a mower, repeated twice a month for the first year.

Twelve dormant hardwood cuttings of four Populus clones, P. euramericana (NC 5323), P. "charkowiensis" x P. "caudina" (H 47), P. maximowiczii x P. trichocarpa (NE 388), and P. maximowiczii x P. "berolinesis" (NE 48), were randomized within each tillage factor (Table 2.2). Cuttings were planted on May 27th, 8 days after the area was sprayed with simazine, at a spacing of 1.5 m between rows and 1.2 m between the trees within rows. Two border trees on either end of each row were planted for each treatment combination. There was no rain during the week of planting; the days were sunny and temperatures were in the high 80's. Percent survival was determined 5, 9 and 17 weeks after planting, and leader heights were measured 9

TABLE 2.2 PARENTAGE OF POPULUS CLONES PLANTED ON THE THREE EXPERIMENTAL PLANTATION SITES.

Clone No.	Parentage	Section
NC 5323	Populus x euramericana (Canada Blanc)	Aigeiros
NC 5328	Populus x eruamericana (I 45/51)	Aigeiros
H 47	P. cv. "charkowiensis" x P. cv. "caudina"	Aigeiros
NE 41	P. maximowiczii x P. trichocarpa (Androscoggin)	Tacamahaca
NE 388	P. maximowiczii x P. trichocarpa (Kingston)	Tacamahaca
NE 48	P. maximowiczii x P. cv. "berolinensis"	Tacamahaca x Aigeiros

and 17 weeks after planting. Weed control was assessed visually at the end of the season before the first frost.

Early in the second growing season and twice a month for the rest of the season the entire plantation was mowed. Height and diameter 5 cm above ground level were measured on July 25th and October 25th. The dry weight of wood and bark of both leader and branches were estimated from a regression based on diamter^2 x height (Gottschalk and Dickmann, 1980).

Dansville Plantation

This plantation was established in the spring of 1979 on a 36 m x 72 m area. Unlike the TRC plantation, tillage was the first factor in the split-split plot factorial experiment. Glyphosate at the rate of 2 kg/ha a.i. was sprayed over the untilled plots to control existing weeds. Simazine and diuron each at 2 and 4 kg/ha a.i. plus a control were split over and randomized within each tillage factor in parallel strips. The controls were mowed twice a month for the entire growing period.

Three of the Populus clones used in the TRC plantation (NC 5323, NE 388 and NE 48) were randomized within each herbicide level and control (Table 2.2). Six dormant hardwood cuttings of each clone were planted by hand at a spacing of 2 m between rows and 2 m between each plant within the row. Planting was done on May 26th, a cool cloudy day, 8 days after the area was sprayed with simazine and diuron. There were three replications. Percent survival and leader height growth were determined 9 and 19 weeks after planting. At the last sampling time, 2 randomly selected trees were harvested from each clone for each treatment combination and average weight of both leader and branches was taken after drying at 75 C for 48 hr.

<u>Manistee Plantation</u>

This plantation was established in the spring of 1979 on a 78 m x 60 m area. Tillage was the first factor here as in the Dansville plantation. However, unlike the other plantations, the entire area was tilled once in the fall of 1978. In May, 1979, a second tillage was imposed. A 78 m x 10 m strip was tilled and alternated with an untilled 78 m x 10 m strip in each block. Glyphosate at the rate of 2 kg/ha a.i. was sprayed over the untilled plots to control existing vegetation. Simazine and diuron each at 2 kg/ha a.i. plus a control were sprayed in parallel strips and randomized within each tillage factor.

Nine dormant hardwood cuttings of three <u>Populus</u> clones, NC 5323, <u>P</u>. x <u>euramericana</u> (NC 5328) and <u>P</u>. <u>maximowiczii</u> x <u>p</u>. <u>trichocarpa</u> (NE 41) were planted randomly within each herbicide level and the control at a spacing of 3 m within rows and 2 m between rows (Table 2.2). Simazine and diuron were sprayed separately over the planted cuttings on June 14th, 15 days after planting.

The control was left unweeded for the duration of the experiment. There were 3 replications. Survival and leader height growth determinations were done 11 and 19 weeks after planting. Biomass of wood and bark was determined as for the Dansville plantation.

RESULTS

TRC YEAR ONE

There were generally no significant differences in survival or height growth among the rates of simazine throughout the season (Table 2.3), although highest rates of simazine tended to reduce survival. However, survival in the weed-free controls was significantly better than in plots treated with highest levels of simazine. Height growth in control plots was significantly higher than height growth at 4 kg/ha simazine but not different from that at 2 and 3 kg/ha at the middle and end of season. Weed control at 4 kg/ha simazine was comparable to the weed-free control but higher than at 2 and 3 kg/ha (Table 2.3). Weed control at 2 and 3 kg/ha simazine did not differ.

There were significant differences in survival and growth between tilled and untilled sites (Table 2.3), with survival and height at the end of the season about 20% and 30 cm better on the tilled compared with the untilled sites. Weed control was also slightly better on tilled sites. The effect of different rates of simazine remained the same on

TABLE 2.3 THE EFFECT OF SIMAZINE ON SURVIVAL AND HEIGHT GROWTH OF POPULUS HYBRIDS IN THEIR FIRST GROWING SEASON ON TILLED AND UNTILLED SITES IN LOWER MICHIGAN, (TRC).

		Weeks After Planting					
		5	9		17		
Tillage	Simazine (kg/ha a.i.)	Surv (%)	Surv (%)	Ht (cm)	Surv. (%)	Ht (cm)	Weed Control (%)
Tilled	0	88	81	36	81	97	94
	2	76	69	32	68	91	80
	3	70	65	31	65	89	72
	4	62	52	31	52	78	86
Untilled	0	71	58	21	57	62	91
	2	67	43	17	42	56	74
	3	71	46	18	45	61	71
	4	60	37	17	35	49	88
	LSD .10	13	16	5	16	14	8

either the tilled or untilled sites (Table 2.3). However, simazine and tillage supplemented each other and significantly increased end-of-season survival and growth of plants by over 15% and 20%, respectively, above either tillage or simazine alone.

There were significant clonal differences in survival and growth (Table 2.4), with clones NC 5323 and H 47 surviving better at 5, 9 and 17 weeks after planting compared to clones NE 388 and NE 48. There were generally no differences in survival between each clonal pair. At the middle of the season, 9 weeks after planting, the ranking in height among the four clones was H 47 , NC 5323 , NE 48 ,NE 388. There were no significant differences in height among clones at the end of the season. Clones NC 5323 and H 47 had better survival at all rates of simazine tested than clones NE 48 and NE 388 at the middle and the end of season (Table 2.4). There were also significant differences in height growth within some simazine rates. H 47 grew best at 4 kg/ha whereas NE 388 was the most inhibited at this herbicide rate. At the end of the first season there were no significant differences in height among clones at 2 kg/ha simazine.

TABLE 2.4 FIRST-YEAR SURVIVAL AND HEIGHT GROWTH OF FOUR *POPULUS* HYBRIDS ESTABLISHED WITH VARYING LEVELS OF SIMAZINE IN LOWER MICHIGAN, (TRC).

		Weeks After Planting				
		5	9		17	
Simazine (kg/ha a.i.)	Clone	Surv (%)	Surv (%)	Ht (cm)	Surv (%)	Ht (cm)
0	NC 5323	81	77	29	76	79
	H 47	88	74	32	72	75
	NE 388	80	64	25	66	83
	NE 48	68	63	26	63	78
2	NC 5323	81	61	23	61	70
	H 47	79	68	33	66	77
	NE 388	61	44	21	43	73
	NE 48	65	51	21	51	73
3	NC 5323	73	63	25	63	71
	H 47	75	60	25	58	66
	NE 388	64	48	23	46	82
	NE 48	69	53	24	52	78
4	NC 5323	65	45	22	43	59
	H 47	76	57	28	56	70
	NE 388	51	36	19	33	58
	NE 48	53	40	24	41	65
	$LSD_{.10}$	12	14	3	14	11

TRC YEAR TWO:

The significant differences in growth due to the different rates of simazine carried over into the second year (Table 2.5). Survival at the end of the first year compared to survival the second year within each simazine rate were not significantly different. Trends in survival among different rates of simazine were also the same as for the first year. Height, diameter and biomass at the middle and end of season were significantly lower at 4 kg/ha simazine compared to the control. Differences among the lower rates of simazine and the control were not significant. At the end of the season control plants were taller and greater in diameter than plants in the 2 and 3 kg/ha simazine plots, although biomass was not significantly different. There were no significant differences in growth and biomass production between 2 and 3 kg/ha simazine. Growth and biomass yield were again better on the tilled compared to the untilled sites at the middle and end of the season (Table 2.5).

Survival patterns among clones the second year were not significantly different from the first year (Table 2.6). Growth and biomass yield of clones NC 5323 and H 47 were better than NE 388 and NE 48 at the middle and end of season. There were no significant differences in growth between NC 5323 and H 47, but height at the middle and end of season and diameter at the end of season were lower in NE 48 compared to NE 388.

TABLE 2.5 THE EFFECT OF SIMAZINE ON SURVIVAL, GROWTH AND BIOMASS YIELD OF <u>POPULUS</u> HYBRIDS DURING THEIR SECOND GROWING SEASON ON TILLED AND UNTILLED SITES IN LOWER MICHIGAN, (TRC).

		Period of Measurement							
		Mid-Season				End of Season			
Tillage	Simazine (kg/ha a.i.)	Surv (%)	Ht. (m)	Dia (cm)	Wood & Bark (g)	Surv (%)	Ht (m)	Dia (cm)	Wood & Bark (g)
Tilled	0	78	1.8	3.1	255	78	2.0	3.4	340
	2	65	1.7	3.0	236	65	1.9	3.2	296
	3	66	1.6	3.0	221	66	1.9	3.2	293
	4	51	1.4	2.6	176	51	1.5	2.7	225
Untilled	0	54	1.3	2.7	148	54	1.5	2.9	203
	2	40	1.2	2.5	121	40	1.3	2.6	145
	3	44	1.2	2.5	118	43	1.3	2.7	150
	4	32	1.0	2.2	100	32	1.1	2.3	130
	$LSD_{.10}$	16	0.2	0.30	46	16	0.3	0.3	66

TABLE 2.6 SECOND-YEAR SURVIVAL AND HEIGHT GROWTH OF FOUR *POPULUS* HYBRIDS ESTABLISHED WITH SIMAZINE IN LOWER MICHIGAN, (TRC).

		Period of Measurement							
		Mid-Season				End of Season			
Simazine (kg/ha a.i.)	Clone	Surv (%)	Ht (m)	Dia (cm)	Wood & Bark (g)	Surv (%)	Ht (m)	Dia (cm)	Wood & Bark (g)
0	NC 5323	73	1.6	3.0	222	73	1.9	3.3	305
	H 47	75	1.6	3.0	217	75	1.8	3.2	270
	NE 388	64	1.5	2.8	187	63	1.8	3.0	261
	NE 48	52	1.4	2.8	180	52	1.6	3.0	250
2	NC 5323	62	1.5	2.8	175	61	1.6	3.0	217
	H 47	66	1.6	3.0	226	66	1.8	3.1	276
	NE 388	40	1.3	2.6	148	40	1.5	2.7	186
	NE 48	43	1.4	2.7	164	43	1.5	2.8	201
3	NC 5323	70	1.3	2.8	169	70	1.6	3.0	230
	H 47	68	1.4	2.8	171	67	1.5	2.9	201
	NE 388	37	1.4	2.7	167	37	1.6	2.9	225
	NE 48	45	1.4	2.8	171	45	1.6	3.0	230
4	NC 5323	52	1.3	2.7	156	51	1.5	2.4	195
	H 47	56	1.3	2.9	169	56	1.5	3.0	210
	NE 388	33	1.2	2.5	134	33	1.4	2.6	173
	NE 48	26	0.7	1.6	93	26	0.9	1.7	130
	$LSD_{.10}$	13	0.2	0.3	34	13	0.2	0.3	49

Dansville

In general, survival was very low on this site. Simazine at the two rates investigated caused less mortality than diuron, while the weed-free control tended to have higher survival and height growth at the middle and end of season compared to the herbicide treatments. End-of-season tree biomass was not significantly different among herbicide treatments and the control, although biomass was reduced at highest herbicide levels (Table 2.7).

Survival and growth were more than 50% better on the tilled compared with the untilled sites at both periods of measurement (Table 2.7). Biomass of wood and bark at the end of the season was about 300% better on the tilled sites. Plants in the simazine and diuron treated plots survived and grew better when the site was tilled (Table 2.7). Survival and growth on tilled sites at each herbicide level were significantly better than either herbicide or tillage alone, although the trend in growth and biomass yield with herbicide and herbicide rate was still the same.

There were clonal differences in survival, height growth and biomass yield on this site (Table 2.8). NC 5323 had the highest while NE 388 was poorest. The height and biomass of NC 5323 and NE 48 were not significantly different but higher than NE 388 at the middle and end of the season. The ranking of the clones with respect to survival

TABLE 2.7 THE EFFECT OF SIMAZINE AND DIURON ON SURVIVAL AND HEIGHT GROWTH OF POPULUS HYBRIDS DURING THEIR GROWING SEASON ON TILLED AND UNTILLED SITES IN LOWER MICHIGAN, (DANSVILLE).

			Period of Measurement				
	Herbicide		Mid-Season		End of Season		
Tillage	Type	Rate (kg/ha a.i.)	Surv (%)	Ht (cm)	Surv (%)	Ht (cm)	Wood & Bark (g)
Tilled	Control	0	65	25	61	31	3.5
	Simazine	2	46	21	44	40	3.5
		4	50	22	50	34	4.3
	Diuron	2	43	24	41	37	5.0
		4	22	11	17	17	2.2
Untilled	Control	0	35	14	30	16	1.2
	Simazine	2	30	9	24	19	1.2
		4	11	6	7	12	0.8
	Diuron	2	19	7	15	10	0.5
		4	13	5	6	10	0.6
	$LSD_{.10}$		17	8	13	9	2.0

TABLE 2.8 FIRST-YEAR SURVIVAL AND GROWTH OF THREE *POPULUS* HYBRIDS ESTABLISHED WITH SIMAZINE AND DIURON IN LOWER MICHIGAN, (DANSVILLE).

			Period of Measurement				
			Mid-Season		End of Season		
Type	Rate (kg/ha a.i.)	Clone	Surv (%)	Ht (cm)	Surv (%)	Ht (cm)	Wood & Bark (g)
Control	0	NC 5323	75	17	70	21	1.7
		NE 48	47	22	39	26	2.1
		NE 388	28	20	28	23	3.2
Simazine	2	NC 5323	70	16	58	33	2.6
		NE 48	33	16	33	30	2.6
		NE 388	11	14	11	24	2.0
	4	NC 5323	53	14	53	28	2.0
		NE 48	25	20	25	26	4.6
		NE 388	14	8	8	14	1.0
Diuron	2	NC 5323	47	20	42	29	5.0
		NE 48	25	16	25	24	2.0
		NE 388	20	11	17	18	1.3
	4	NC 5323	36	17	22	29	3.2
		NE 38	17	8	11	12	1.0
		NE 388	0	0	0	0	0.0
		$LSD_{.10}$	17	7	17	13	2

and growth were unaffected by the different levels of both simazine and diuron at both periods of measurement (Table 2.8). NC 5323 showed the best performance at any herbicide rate while NE 388 was the poorest.

Manistee

In general, all herbicide treatments showed better survival and growth than the unweeded controls (Table 2.9). Survival and growth of plants on diuron-treated areas were better than on simazine-treated areas. There were no significant differences in survival and growth of plants among rates within each herbicide. The trend in herbicide efficacy remained the same on tilled versus untilled sites (Table 2.9). Survival and growth were significantly higher on tilled compared with untilled sites at each herbicide rate tested. Survival and growth of plants at different rates of diuron were significantly better than at different rates of simazine on either tilled or untilled sites. Survival and growth in a tillage-herbicide combination were better than either alone.

There were significant clonal differences in survival, height growth and biomass yield (Table 2.10). Clone NC 5323 had better survival compared with NE 41 and NC 5328 at the middle and end of season. Height growth of NE 41 was higher than NC 5328 or NC 5323 at the two periods of measurement. Biomass yield of NE 41 was also higher than NC 5323 and NC 5328. The trends in clonal and herbicidal

TABLE 2.9 THE EFFECT OF SIMAZINE AND DIURON ON SURVIVAL AND GROWTH OF *POPULUS* HYBRIDS DURING THEIR FIRST GROWING SEASON ON TILLED AND UNTILLED SITES IN LOWER MICHIGAN, (MANISTEE).

Treatment			Period of Measurement				
	Herbicide		Mid-Season		End of Season		
Tillage	Type	Rate (kg/ha a.i.)	Surv (%)	Ht (cm)	Surv (%)	Ht (cm)	Wood & Bark (g)
Tilled	Control[a]	0	42	26	39	30	1.6
	Simazine	2	68	31	60	43	5.7
		4	60	33	57	45	5.5
	Diuron	2	77	37	77	56	13.3
		4	68	41	67	72	16.6
Untilled	Control[a]	0	40	26	38	38	5.2
	Simazine	2	41	26	36	40	5.0
		4	43	23	40	33	2.4
	Diuron	2	57	28	54	50	5.1
		4	56	24	49	44	6.0
	$LSD_{.10}$		18	7	17	15	8

[a]Unweeded Control

TABLE 2.10 FIRST-YEAR SURVIVAL AND HEIGHT GROWTH OF THREE *POPULUS* HYBRIDS ESTABLISHED WITH SIMAZINE AND DIURON IN LOWER MICHIGAN, (MANISTEE).

Herbicides			Period of Measurement				
			Mid-Season		End of Season		
Type	Rate (kg/ha a.i.)	Clone	Surv (%)	Ht (cm)	Surv (%)	Ht (cm)	Wood & Bark (g)
Control[a]	0	NC 5323	62	26	62	38	2.2
		NE 41	48	35	42	45	6.7
		NC 5328	13	17	12	19	1.3
Simazine	2	NC 5323	72	28	66	39	5.1
		NE 41	56	34	43	47	5.7
		NC 5328	36	23	34	38	5.2
	4	NC 5323	72	28	68	43	4.0
		NE 41	45	32	44	43	3.0
		NC 5328	38	23	33	31	4.8
Diuron	2	NC 5323	72	33	74	56	7.5
		NE 41	68	34	66	55	10.9
		NC 5328	60	31	57	49	9.4
	4	NC 5323	58	32	56	62	11.0
		NE 41	74	44	70	78	16.2
		NC 5328	54	21	48	34	6.7
		$LSD_{.10}$	24	11	24	21	5

[a]Unweeded Control

behavior were still essentially the same as in the main effects (Table 2.10). NE 41 performed best with regards to biomass production at all herbicide rates except at 4 kg/ha simazine. NC 5328 showed poorest survival and height at all herbicide rates. Survival and growth of the clones were better at various levels of diuron compared to simazine or the unweeded control.

DISCUSSION

In these experiments the successful establishment of unrooted poplar cuttings was influenced significantly by the type and rate of herbicide applied, whether or not the area was tilled and the clone used. The persistence of simazine is influenced by both tillage and pH of the soil. Kapusta (1979) showed that persistence of simazine was increased by tillage, while Slack et al., (1978) demonstrated that simazine persistence was lowest on soils of pH 5.4 or less. Corbin et al, (1971) reported highest activity of triazines at pH 6.5 and that soil pH levels between 4.3 and 7.5 had no effect on phytotoxicity of diuron.

In general the data presented here are in agreement with these reports. The Ap horizon had a pH of 6.7 at the TRC and 6.1 at Dansville but only 5.6 at Manistee (Table 2.1). Simazine at 2 or 3 kg/ha at the TRC and 2 kg/ha at Dansville was adequate for establishment of poplar cuttings on either tilled or untilled sites. At Manistee, however, simazine at both rates was ineffective, probably due to the low pH of the surface soil. The effect of surface soil pH and tillage is similar. Kells *et al*. (1980) found that rate of formation of an unextractable ^{14}C-compound from soil treated with ^{14}C-atrazine was greater under no tillage and increased with decreasing pH. The effect of tillage in increasing persistence of simazine may be through its effects on the pH of the soil, since organic matter incorporation during tillage has the potential of making the soil less acidic. The use of lime to raise soil pH at the Manistee site may be a practical way to make this soil suitable for simazine use (Kells *et al*., 1980).

On each of the three plantation sites, weed control was 70% or better at any herbicide rate on tilled or untilled plots except at Manistee where weed control on simazine treated plots was less than 50% at the end of the first growing season. In general, weed species were effectively controlled at TRC and Dansville; however, at Manistee, witchgrass (*Panicum capillare*) formed a dense mat on simazine-treated plots, irrespective of the rate. Diuron on the other hand, controlled witchgrass. The reduction in survival and growth of *Populus* trees on simazine treated plots at Manistee plantation was probably due to the presence of this resistant weed species in addition to the reduced activity of simazine due to the low pH of the surface soil.

The single initial tillage operation at the beginning of the season benefitted all clones tested at all herbicide rates. Survival, growth and biomass yield were higher on tilled than untilled plots. Supplementing tillage with herbicides further increased survival and growth of the clones above what was obtained with tillage or herbicide alone. The increased performance of the clones with tillage might be due to increased persistence of herbicides on tilled plots (Slack et al., 1978). The enhancement of tree establishment due to increased moisture and soil contact with the planted cuttings probably was also a contributing factor. Grazing on young shoots by insects and animals present under the dead but standing weeds was also a problem for the poplar trees and may contribute to the low survival on the untilled sites.

On the three sites there were clonal differences in survival, height, diameter and biomass. Clones from section Aigeiros generally survived better than clones from section Tacamahaca at all herbicide rates. Tacamahaca clones grew well, however, often better than the Aigeiros clones probably because of their greater leaf area duration (Dickmann, 1979). NC 5323 and H 47, for example, set bud in late August whereas NE 48 and NE 388 set bud in late September. These clonal differences have important implications in selection or breeding of clones for use in intensive plantation establishment. Clones with high survival and growth rate are preferred; however, a trade-off might be made between clones with high survival but less growth and clones with less survival but high growth rates. An understanding of the factors affecting survival of Populus hybrids will enhance the managers' ability to make the best choice of clones intended for plantations.

Differential clonal response to simazine and diuron occurred at the three study areas. Higher doses of each herbicide were more toxic to each of the clones tested. At the TRC 4 kg/ha a.i. simazine caused high mortality and growth inhibition in all clones, but this effect was more severe in Tacamahaca clones NE 388 and NE 48. Simazine at 2 kg/ha a.i. appeared suitable for all clones tested at the TRC, although survival was still higher for NC 5323 and H 47. Productivity of each clone at the high rate of simazine in the second year was still significantly low probably due to the "compound-interest" nature of growth. At Dansville 2 kg/ha a.i. of simazine or diuron would be a suitable application for all clones tested. At Manistee diuron offered the best potential for use with any of the clones tested. Although differential clonal response would still occur, for economic reasons 2 kg/ha a.i. diuron would be preferred. Differential clonal response to herbicides has been reported among Populus hybrids (von Althen, 1979; Dickmann et al., 1978), although the exact reasons are not known. In these experiments, however, it appeared that clones with high establishment qualities, exemplified in percent survival, were little affected by simazine or diuron at rates adequate for effective weed control. This high surviving ability would probably be in addition to any physiological, biochemical or morphological basis for differential tolerance that might exist in the clones.

CHAPTER III

THE EFFECT OF SIMAZINE ON TOTAL FOLIAR NITROGEN AND PHOSPHORUS CONTENT OF FIELD-GROWN HYBRID *POPULUS*

INTRODUCTION

The stimulatory effect of simazine at subtoxic levels on plant growth and nitrogen nutrition has been reported extensively in crop plants (Ries, 1976; Ebert and Dumford, 1976). Bartley (1957) made one of the first observations that simazine may improve growth, noting that corn was greener and taller when simazine was applied at rates up to 18 kg/ha. Later works have shown similar responses for apple and peach trees, certain conifers and oak trees (Ries *et al*., 1963; Conner and White, 1968; Ries, 1976). Ten kg/ha of simazine has been estimated to produce a response in apples equivalent to an application of 200 to 250 kg/ha of nitrogen fertilizer (Karnatz, 1964 as cited in Ries, 1976). However, in several cases no effect on dry matter was observed or additional height growth was offset by a reduction in plant dry weight (Tweedy *et al*., 1971; Ries, 1976; Ebert and Dumford, 1976). Simazine applied preemergence at herbicidal rates to field planted Scotch pine, white spruce and balsam fir, however, did not significantly alter their foliar nitrogen concentration (Conner and White, 1968).

Ries et al. (1963) was one of the first to suggest that simazine may be involved in the alteration of nitrogen nutrition of plants. Since then several investigations on the mechanism of simazine stimulation of protein synthesis have been reported (Ries and Wert, 1972; Doll and Meggitt, 1968; Pulver and Ries, 1973; Tweedy and Ries, 1967; Tweedy et al., 1971; Lund-Hoeie, 1969b). Stimulation of root growth by subherbicidal levels of triazines was reported for conifers (Lund-Hoeie, 1969b). Freney, (1965) suggested that simazine increased plant growth by a direct effect on plant metabolism and not by an interaction with the soil because he could find no simazine effect on mineralization of soil organic nitrogen. It has also been reported that nitrogen content would increase in corn after triazine treatment only when the temperature was low, but when nitrate was replaced by ammonium ion in the nutrient cultures, no extra stimulation of nitrogen uptake occurred (Tweedy and Ries, 1967; Doll and Meggit, 1968). While it has been demonstrated that hill reaction inhibitors like the triazines increase protein synthesis which induces more rapid nitrate uptake in plants, the mechanism of such action is not clear.

In the intensive culture of Poplar, simazine is one herbicide that can be used to suppress weed competition during plantation establishment (Dickmann et al., 1978;

von Althen, 1979). An understanding of the effect of simazine on survival, growth and leaf nutrient content, especially total nitrogen, of hybrid Populus clones will enhance the selection of proper weed control strategies or the breeding of new clones. The study reported here was conducted to evaluate the effect of varying rates of soil-applied simazine on concentrations of total foliar nitrogen and phosphorus in four field-grown Populus hybrids.

MATERIALS AND METHODS

The experimental site was a tilled old-field area on the Michigan State University campus in East Lansing, Michigan. The soil was of sandy-loam texture and contained 4.6 mg/g of NO3 - N and 7% organic matter. Three simazine rates (2,3 and 4 kg/ha a.i.) prepared from an 80% wettable powder formulation, plus a control were randomly applied in 1 m strips on the tilled area. The herbicide was sprayed with a tractor-mounted aggitated tank sprayer on May 19, 1978. The unsprayed control strips were treated with 2 kg/ha glyphosate prior to planting and then mechanically weeded twice a month throughout the growing season.

Twelve dormant hardwood cuttings (25 cm long) of four Populus clones (Table 3.1) were randomly planted within each herbicide level in a split-split-plot factorial design.

TABLE 3.1 PARENTAGE OF *POPULUS* CLONES USED IN THIS EXPERIMENT.

Clone No.	Hybrid Parentage	Section
NC 5323	*Populus* x *euramericana* cv. "*Canada blanc*"	Aigeiros
H 47	*P*. cv. "*Charkowiensis*" x *P*. cv. "*caudina*"	Aigeiros
NE 388	*P*. *maximowiczii* x *P*. *trichocarpa* cv. "*Kingston*"	Tacamahaca
NE 48	*P*. *maximowiczii* x *P*. cv. "*berolinensis*"	Tacamahaca x Aigeires

Spacing was 3.0 m between rows and 1.2 m between trees within a row. Planting was done by hand on May 27, 1978. Each treatment combination was replicated five times.

Percent survival was determined and leader height growth measured at intervals during the growing season. Percent weed control was estimated visually at the end of the season. Two mature leaves from each of ten trees per clone, two trees from each replication, were harvested at the middle and end of the growing season for each treatment combination. The leaves from each treatment were composited, dried at 70 C for 48 hr. and ground with a Wiley mill. Digestion of dried leaf material was done with a Tecator digestion system Model DS-40. Percent total (Kjeldahl) nitrogen and phosphorus were simultaneously determined using the Technicon Auto Analyzer II system. All data were subjected to analysis of variance and means were compared at the 0.1 level of probability using the LSD (Cochran and Cox, 1957).

RESULT AND DISCUSSION

Simazine had a significant effect on total foliar nitrogen and leader height growth of hybrid Populus clones at the middle of the growing season (Table 3.2). Foliage samples at all simazine levels tested had significantly higher total nitrogen than the weed-free control. Differences among the simazine rates were not statistically significant. However, height growth was reduced by simazine treatment except at the 2 kg/ha a.i.

TABLE 3.2 THE EFFECT OF SIMAZINE ON TOTAL NITROGEN, PHOSPHORUS AND LEADER HEIGHT GROWTH OF POPULUS HYBRIDS, AT TWO DATES DURING THE FIRST GROWING SEASON.

	July 30			October 1		
Simazine Kg/ha a.i.	N (%)	P (%)	Ht (cm)	N (%)	P (%)	Ht (cm)
0	3.0	.18	36	3.0	.19	97
2	3.3	.18	32	3.0	.20	91
3	3.3	.18	31	3.0	.20	89
4	3.2	.18	31	3.0	.20	78
LSD.10	.1	.01	5	.2	.02	8

The end-of-season total nitrogen data showed no significant differences between control and simazine treatments, or among the different simazine rates (Table 3.2). Heights at the same period were again statistically higher for control plants than for those treated with the heaviest levels of simazine. The slightly higher seasonal height growth of control plants versus those treated with 2 kg/ha a.i. simazine was probably due to an early-season growth advantage of the control plants. The non-significant difference in foliar nitrogen at the end of the season indicates the transient nature of the simazine effect. Apple leaves also showed increased in nitrogen early in the growing season after simazine applications but the effect was diminished by June (Solecka _et al_., 1969).

Simazine had no significant effect on total phosphorus of any clone at the two periods of sampling. Phosphorus levels were similar to those reported for other field-grown _Populus_ plants. (Blackmon and White, 1972; Einspahr, 1971).

There were significant differences among clones in the extent to which foliar nitrogen accumulation was stimulated by simazine (Table 3.3). Compared with the weed-free control, clone NC 5323 treated with 3 kg/ha simazine had significantly higher total foliar nitrogen at the middle of the season, while clone H 47 treated with 2 kg/ha simazine showed significantly higher total foliar nitrogen at the middle and end of season. Total foliar

TABLE 3.3 THE EFFECT OF SIMAZINE ON TOTAL KJELDAHL NITROGEN, LEADER HEIGHT, GROWTH AND SURVIVAL OF FOUR 1-YR-OLD *POPULUS* HYBRIDS

		Date of Sampling					
		July 30			October 1		
Clone	Simazine (Kg/ha a.i.)	N (%)	Ht (cm)	Surv (%)	N (%)	Ht (cm)	Surv (%)
NC 5323	0	3.2	36	90	3.2	95	88
	2	3.2	29	85	3.3	87	85
	3	3.6	33	67	3.1	91	67
	4	3.3	30	48	3.2	77	48
H 47	0	3.5	42	80	3.1	94	78
	2	3.9	46	93	3.5	97	90
	3	3.8	33	71	3.3	84	72
	4	3.7	33	70	3.5	83	70
NE 388	0	2.8	34	83	2.9	104	83
	2	3.1	26	40	2.9	92	40
	3	3.0	27	57	2.8	88	52
	4	2.8	25	43	2.7	70	42
NE 48	0	2.7	33	72	3.0	95	71
	2	2.9	29	56	2.5	89	57
	3	2.9	31	67	2.9	93	67
	4	3.0	34	45	2.7	81	45
	LSD .10	.4	8	20	.4	20	19

nitrogen of H 47 was also higher than controls at the highest simazine rates, but height growth was suppressed.

At the other extreme, the nitrogen content of NE 388 appeared to be unaffected by simazine treatment at all rates tested at the middle and end of the growing season. Height growth of NE 388 at all simazine rates was lower than the control, but there were no significant differences in height among the different herbicide rates. The height growth response of NE 388 is probably an inhibitory effect of simazine since this clone has been shown to be very sensitive to this herbicide (von Althen, 1979; Chapter 2). Total foliar nitrogen and height growth of clone NE 48 was unaffected at both sampling periods by simazine (Table 3.3).

Survival among the four clones was generally lower in simazine-treated plots than in control plots (Table 3.3). Clone H 47 exhibited highest survival, whereas clone NE 388 showed the least survival, at all rates of simazine tested. Overall survival and weed control at the end of the season for the 2 and 3 kg/ha rates of simazine represented an acceptable performance on this sandy-loam soil. (Figure 3.1).

These data suggest that untreated plants in the weed-free control might actually be more efficient in nitrogen utilization; i.e., a simazine-induced increase in nitrogen content does not necessarily produce correspondingly greater growth. Simazine appeared to reduce nitrogen efficiency in clones NC 5323, NE 388 and NE 48 but not in

FIGURE 3.1 EFFECT OF SIMAZINE ON WEED CONTROL AND SURVIVAL OF *POPULUS* CLONES AT THE END OF THEIR FIRST GROWING SEASON IN LOWER MICHIGAN (TRC).

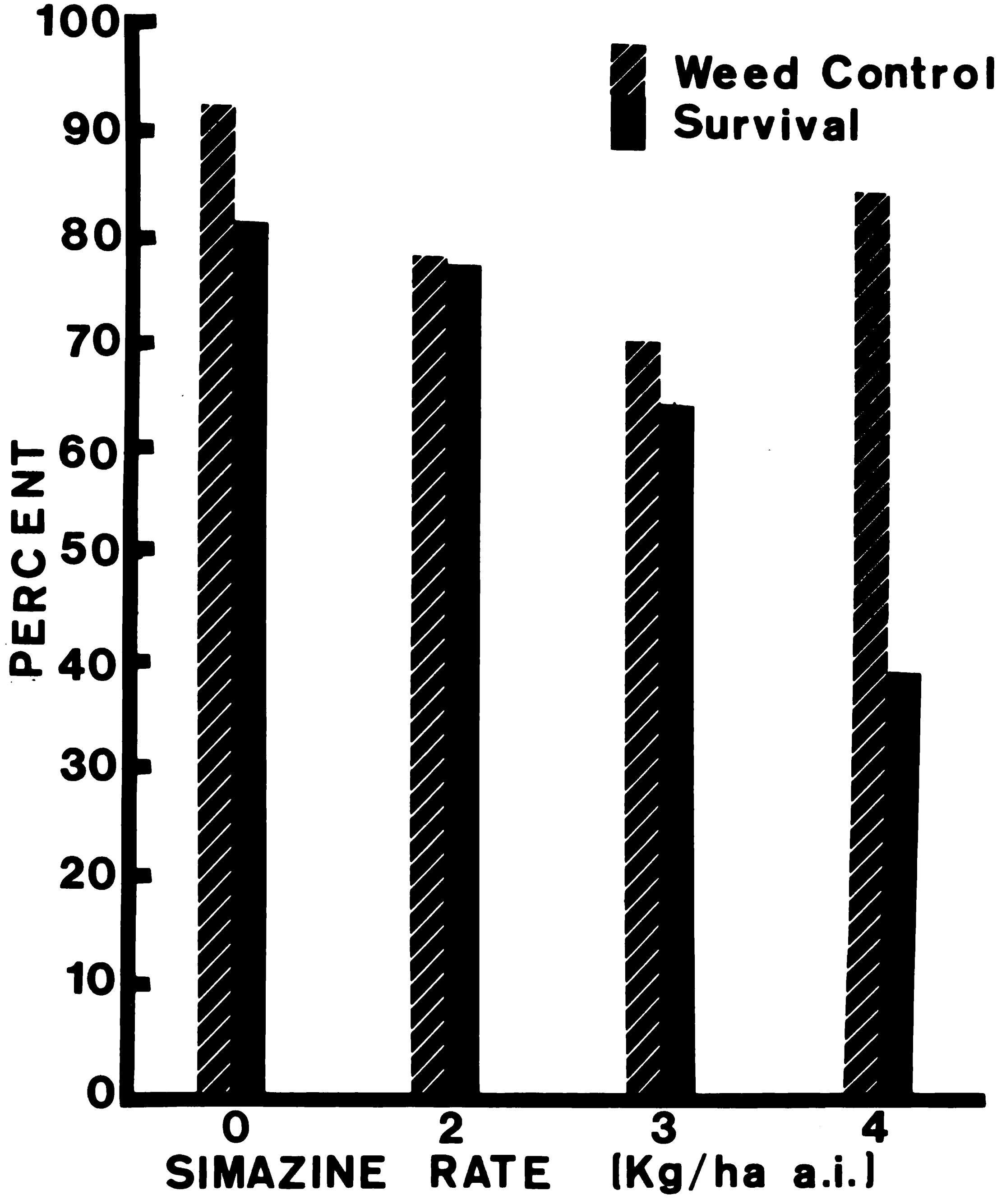

100
90
80
70
60
50
40
30
20
10
0
PERCENT
Weed Control
Survival
0
2
3
4
SIMAZINE RATE (Kg/ha a.i.)

Figure 3.1

H 47. Apparently the potential stimulatory effect of higher nitrogen levels is offset by the phytotoxic effects of simazine on sensitive poplar clones.

The practical utility of nitrogen stimulation by the triazines in agronomic or tree crops is elusive (Ries, 1976); however, any additional growth in a tree crop eventually shortens the length of the rotation, a definite economic advantage. The direct exploitation of this phenomenon in tree breeding may be still far in the future, however, owing to the problem of elucidating the mechanism of such simazine stimulation of nitrogen uptake (Ebert and Dumford, 1976). Screening, with a view to selecting clones of Populus exhibiting total nitrogen stimulation by simazine coupled with undiminished height growth and acceptable survival, as in H 47 and NC 5323, will be of immediate practical utility in selection of plant material for high-yielding plantations.

CHAPTER IV

DIFFERENTIAL TOLERANCE OF *POPULUS* CLONES TO SIMAZINE AND DIURON

INTRODUCTION

Simazine (2-chloro-4, 6-bis (ethylamino) - s - triazine) and diuron (3-(3,4-dichlorophenyl) - 1, 1-dimethyl urea) have the potential to be economical forms of weed control in poplar plantations due to their proven ability to control weed competition and their ease of application (von Althen, 1979; Dickmann *et al*., 1978; Chapter 2). The greatest disadvantage of these chemicals is the susceptibility of many poplar species and clones to dosage necessary for effective weed control (von Althen, 1979; Chapter 2). Little quantitative data is available, however, on the susceptibility of various poplar species and clones to simazine and diuron. In a field experiment von Althen (1979) showed that the Euramerican poplar clone I-45/51 was very tolerant to simazine up to 4.5 kg/ha in two consecutive years of application. He also demonstrated that other poplar clones exhibit a wide variance in tolerance to simazine in the field. In Chapter 2, it was reported that NC 5323 was relatively tolerant while NE 388 was intolerant to simazine and diuron at several different plantation sites in lower Michigan.

It has been demonstrated that the phytotoxicity of simazine and diuron are affected by percent clay, organic matter and soil pH (Slack et al., 1978; Kells et al., 1980). Corbin et al. (1971) showed that phytotoxicity of the s-triazine prometone increased as soil pH increased, reaching a maximum at pH 6.5, while soil pH levels between 4.3 and 7.5 had no effect on the phytotoxicity of diuron. The differential phytotoxicity of simazine and diuron due to soil pH was also reported in Chapter 2; on the Manistee site where pH was 5.6 simazine was ineffective while diuron was still effective in controlling competiting vegetation. These herbicide-soil interactions impose a severe limitation on the use of field soils for the evaluations of relative tolerance of poplar to simazine and diuron.

The purpose of this experiment was to investigate the relative tolerance of 21 different Populus clones to varying rates of simazine and diuron in the greenhouse using a sand-culture technique modified from Clay and Davison (1978). It was reasoned that this approach would obviate some of the drawbacks inherent in field tolerance trials.

MATERIALS AND METHODS

The screening experiment was conducted in a greenhouse between June, 1979 and June 1980. Washed sand of pH 7.5-8.0 was used to fill specially prepared 0.03 m^3 plastic pots. The four holes at the base of each plastic pot were sealed with screen mesh and fibre wool to prevent the leaching of sand but still allow for free drainage and aeration. The top 2.5 cm of each pot was left unfilled.

Dormant hardwood cuttings 30 cm in length and about 2.0 cm in diameter were made from one-year-old stool shoots. Twenty-one clones of Populus species and hybrids were used in the screening (Table 4.1). Selection of clones reflected the genetic diversity of the genus and included representatives of sections Aigeiros, Tacamahaca and intersectional crosses between them.

Simazine (Princep 80W) and diuron (Karmex 80WP) were used in the screening. Each herbicide was applied at five dose levels (1, 5, 10, 20 and 50 mg per pot) in 1 litre of water. Selection of the dose levels for both herbicides was based on a preliminary experiment. There was an untreated control for each herbicide. Replication was at least two times, giving a minimum total of twelve trees per clone for each herbicide. Evaluation of clones NE 388 and NC 5328 were repeated at two separate occasions. The experimental design was a split-split-plot factorial.

TABLE 4.1 PARENTAGE OF THE *POPULUS* CLONES USED IN HERBICIDE SCREENING

Clone Number	Section Tacamahaca
NE 41	*P. maximowiczii* x *P. trichocarpa*
NE 388	*P. maximowiczii* x *P. trichocarpa*
NC 5260	*P. tristis* x *P. balsamifera*
	Section Tacamahaca x Aigeiros
NE 48	*P. maximowiczii* x *P.* "berolinensis"
NE 298	*P.* "betulifolia" x *P. trichocarpa*
NE 302	*P.* "betulifolia" x *P. trichocarpa*
NE 207	*P. deltoides* x *P. trichocarpa*
Jac 4	*P. balsamifera* x *P. deltcides*
Jac 7	*P. balsamifera* x *P. deltoides*
NE 218	*P. deltoides* x *P. trichocarpa*
	Section Aigeiros
NC 5323	*P.* x *euramericana* "Canada blanc"
NC 5326	*P.* x *euramericana* "Eugenei"
NC 5328	*P.* x *euramericana* "I 45/51"
NC 5377	*P.* x *euramericana* "Wisconsin #5"
H 47	*P.* x "charkowiensis" x *P.* "caudina"
NE 353	*P. deltoides* x *P.* "caudina"
NE 308	*P.* "charkowiensis" x *P.* "incrassata"
NE 17	*P.* "charkowiensis" x *P.* "caudina"
NE 58	*P.* "rasumowskyana" x *P.* "incrassata"
PD 184	*P. deltoides*
PD 222	*P. deltoides*

Trees were about four weeks old before a single application of herbicide directly to the surface of the sand was made. The four weeks allowed the trees to establish in the pots and hence eliminated the comfounding effect of inherent differences in clonal rooting and survival. When watering was necessary 1 litre of nutrient solution was added rapidly to the sand surface; this was sufficient to flood the pot and aerate the sand (Clay and Davison, 1978). The nutrient solution was prepared from a commercial water-soluble fertilizer (Stern's Miracle-Gro) containing 15% total nitrogen (N), 30% available phosphoric acid (P_2O_5), 15% soluble potash (K_2O), and the micro-nutrients Cu, Fe, Mn and Zn. The fertilizer was applied as 15 g to 5 litres of water.

Response of the trees to varying doses of simazine and diuron was monitored for four weeks. During this time the degree of injury to the plants and leaves were quantitatively recorded at weekly intervals by visual assessment (Table 4.2). In addition, measurements of height and number of leaves were taken weekly. At the end of the experiment, dry weights of leaves, stem and roots were determined after drying 24 hr. at 75 C. The various measurements were statistically assessed using the analysis of variance, and a planned comparison of treatment means at the 1% level of probability was done.

TABLE 4.2 CRITERIA USED FOR WHOLE PLANT AND LEAF VISUAL SCORING[a]

A. Whole Plant Visual Score

0 = Dead

1 = Moribund

2 = Alive with some green tissue; no growth

3 = Very stunted but still making growth

4 = Considerable (50%) growth inhibition

5 = Readily distinguishable growth inhibition

6 = Some detectable adverse effect

7 = Indistinguishable from control

B. Leaf Visual Score

0 = Dead

1 = Ca. 85% leaf area dead

2 = Ca. 50% leaf area dead

3 = Ca. 10% leaf area dead

4 = Normal leaf

[a]Modified from Clay and Davison (1978).

RESULTS

Herbicides and rate: There were no statistically significant differences detected in the phytotoxicity of simazine and diuron to poplar clones in all parameters evaluated throughout the duration of the experiment (Table 4.3). Significant differences in phytotoxicity were observed, however, among the rates of each herbicide (Table 4.3). The trends in rate within each herbicide were similar (Tables 4.4-4.7, Figure 4.1). The lower rates of each herbicide were less phytotoxic, but at the same concentration diuron appeared to be less toxic than simazine. The whole plant and leaf visual scores showed lower phytotoxicity at 10, 20, and 50 mg/pot of diuron compared to the same rates of simazine (Tables 4.4 and 4.5). Total dry weights at 10, 20 and 50 mg/pot of diuron were significantly higher than the corresponding values at the same rates of simazine (Table 4.6 and 4.7). The difference in phytotoxicity between simazine and diuron at the same rates began to be apparent ca. 3 weeks after the application of the herbicides.

Clone: Highly significant treatment differences occurred due to the clonal material used in this experiment (Table 4.3). All parameters evaluated showed significant clonal differences at the 1% level of probability at the fourth week of treatment. There were also significant

TABLE 4.3 ANALYSIS OF VARIANCE FOR NUMBER OF LEAVES PER PLANT, HEIGHT, DRY WEIGHT, PLANT AND LEAF SCORE FOUR WEEKS AFTER THE APPLICATION OF VARYING RATES OF SIMAZINE AND DIURON TO *POPULUS* CLONES GROWN ON SAND CULTURE IN THE GREENHOUSE

Source of Variation	Degrees of freedom	Probability Level of F-Statistic: Number of leaves per plant	Height	Dry Weight	Plant Score	Leaf Score
Herbicide	1	.97	.188	.26	.361	.335
Rate	5	.002	.0005	.0005	.0005	.0005
Herbicide x Rate	5	.308	.095	.023	.053	.054
Clone	22	.0005	.0005	.0005	.0005	.0005
Herbicide x Clone	22	.0005	.0005	.0005	.0005	.0005
Rate x Clone	110	.083	.0005	.0005	.0005	.0005
Herbicide x Rate x Clone	110	.995	.926	.023	.001	.0005

TABLE 4.4 WHOLE PLANT AND LEAF VISUAL SCORES PER PLANT FOUR WEEKS AFTER APPLICATION OF VARYING RATES OF SIMAZINE TO 21 POPULUS CLONES GROWN ON SAND CULTURE IN THE GREENHOUSE

Clones	Rates of Simazine (mg/pot)					
Section Tacamahaca	0	1	5	10	20	50
NE 41	11	11	8	4	0	0
NE 388	11	10	6	0	0	0
NC 5260	11	11	10	9	2	0
Section Tacamahaca x Aigeiros						
NE 218	11	11	11	11	8	3
Jac 7	11	11	10	9	6	1
NE 302	11	11	8	4	3	0
NE 207	11	11	8	6	3	0
NE 48	11	4	1	0	0	0
Jac 4	11	11	11	9	2	0
NE 298	11	11	10	7	0	0
Section Aigeiros						
NE 17	11	11	10	6	5	0
NE 5377	11	11	11	6	5	0
NE 5328	11	11	11	11	9	2
NC 5323	11	11	11	10	5	0
PD 222	11	11	11	8	7	3
NE 308	11	11	11	10	6	1
H 47	11	11	11	8	6	6
NE 58	11	11	11	6	5	0
NC 5326	11	10	9	7	4	0
NE 353	11	11	11	10	3	0
PD 184	11	11	8	4	3	0

TABLE 4.5 WHOLE PLANT AND LEAF VISUAL SCORES PER PLANT FOUR WEEKS AFTER THE APPLICATION OF VARYING RATES OF DIURON TO 21 *POPULUS* CLONES GROWN ON SAND CULTURE IN THE GREENHOUSE

Clones	Rates of Diuron (mg/pot)					
Section Tacamahaca	0	1	5	10	20	50
NC 5260	11	11	11	11	6	0
NE 388	11	11	9	6	4	0
NE 41	11	11	10	10	8	0
Section Tacamahaca x Aigeiros						
Jac. 7	11	11	11	11	11	4
NE 302	11	11	9	9	6	0
NE 298	11	11	11	8	6	5
NE 207	11	11	11	10	8	3
Jac 4	11	11	11	11	4	0
NE 218	11	11	10	4	3	3
NE 48	11	10	6	3	1	0
Section Aigeiros						
NE 353	11	11	11	11	8	5
NC 5323	11	11	11	11	8	6
NC 5326	11	11	11	11	8	2
NE 17	11	11	9	9	7	0
NC 5328	11	11	11	11	10	5
NC 5377	11	11	11	11	6	3
H 47	11	11	8	8	3	0
NE 58	11	11	11	11	6	3
PD 184	11	11	11	11	8	0
PD 222	11	11	11	11	8	4

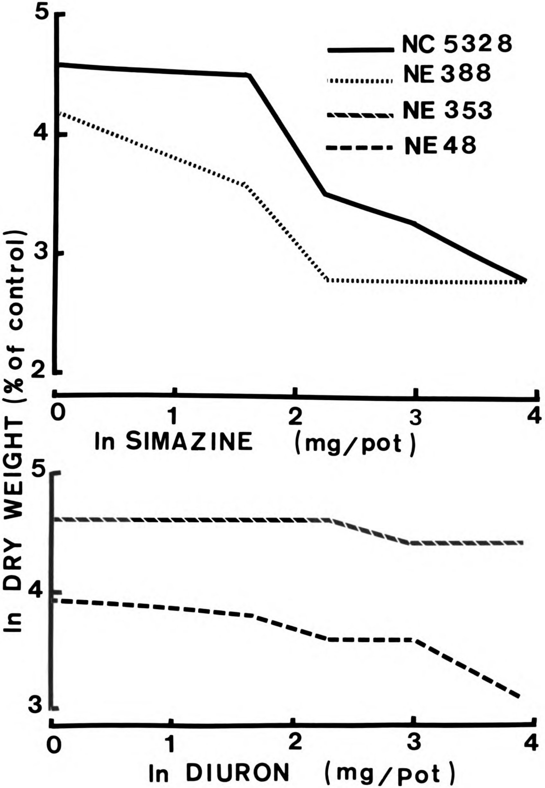
TABLE 4.6 RELATIVE TOTAL DRY WEIGHT, FOUR WEEKS AFTER THE APPLICATION OF VARYING RATES OF SIMAZINE TO 21 POPULUS CLONES GROWN IN SAND CULTURE IN THE GREENHOUSE

Clones	Rates of Simazine (mg/pot)				
Section Tacamahaca	1	5	10	20	50
	. . . . . .% of control.				
NE 41	72	50	25	16	16
NE 388	68	39	18	13	16
NC 5260	59	35	21	12	7
Section Tacamahaca x Aigeiros					
NE 218	81	81	49	44	33
Jac 7	100	65	44	19	19
NE 302	100	38	20	11	7
NE 207	85	45	35	16	15
NE 48	82	32	18	18	23
Jac 4	100	65	44	19	19
NE 298	55	27	28	23	18
Section Aigeiros					
NE 17	100	100	48	34	26
NC 5377	100	97	32	18	13
NC 5328	100	87	34	26	15
NC 5323	85	82	58	54	51
PD 222	100	80	48	35	31
NE 308	100	69	42	42	27
H 47	94	75	37	25	31
NE 58	100	72	38	18	14
NC 5326	100	39	38	13	10
NE 353	74	30	23	23	18
PD 184	70	45	24	17	9

TABLE 4.7 RELATIVE TOTAL DRY WEIGHT, FOUR WEEKS AFTER THE APPLICATION OF VARYING RATES OF DIURON TO 21 POPULUS CLONES GROWN IN SAND CULTURE IN THE GREENHOUSE

Clones	Rates of Diuron (mg/pot)				
Section Tacamahaca	1	5	10	20	50
	. . . . . .% of control.				
NC 5260	100	100	100	47	31
NE 388	100	82	46	12	18
NE 41	91	64	60	27	20
Section Tacamahaca x Aigeiros					
Jac 7	100	100	100	96	96
NE 302	100	100	78	41	20
NE 298	100	100	37	25	23
NE 207	100	100	27	28	17
Jac 4	100	77	77	22	22
NE 218	94	56	38	38	36
NE 48	50	46	36	36	23
Section Aigeiros					
NE 353	100	100	100	83	83
NC 5323	100	100	100	80	58
NC 5326	100	100	100	91	21
NE 17	100	100	100	73	73
NC 5328	100	100	96	55	48
NC 5377	100	100	100	46	14
H 47	100	100	80	70	23
NE 58	100	100	100	30	27
PD 184	100	95	34	45	12
PD 222	100	94	24	34	22

FIGURE 4.1 RELATIVE TOTAL DRY WEIGHTS OF <u>POPULUS</u> CLONES, FOUR WEEKS AFTER TREATMENT WITH VARYING RATES OF SIMAZINE AND DIURON



NC 5328
NE 388
NE 353
NE 48
5
4
3
2
0
1
2
3
4
ln SIMAZINE (mg/pot)
ln DRY WEIGHT (% of control)
5
4
3
0
1
2
3
4
ln DIURON (mg/pot)

Figure 4.1

herbicide x clone and rate x clone interactions for tree heights and number of leaves at the end of the experiment. Total dry weights, whole plant and leaf visual scores showed significant herbicide x clone, rate x clone and herbicide x rate x clone interactions (Table 4.3). Injury symptoms, as measured by the whole plant and leaf visual scores showed significant clonal differences beginning from the second week after treatment at all levels of simazine and diuron. For example, clone NE 48 exhibits injury symptoms at 1 mg/pot simazine or diuron (Table 4.4 and 4.5) whereas clone NC 5328 showed only minor damage at 20 mg/pot of simazine or diuron. At the end of the experiment a few clones were still alive but most were completely killed at 50 mg/pot simazine or diuron (Table 4.4 and 4.5). Total dry weights at the end of the experiment were significantly different among the clones at each herbicide rate (Tables 4.6 and 4.7). At the higher concentrations most clones were reduced in dry weights to below 50% of the controls (Tables 4.6 and 4.7). Tree heights and number of leaves showed similar clonal differences at the 4th week of treatment.

A few clones were observed that exhibited differential tolerance to simazine or diuron. Clone NE 353 showed only minor injuries with diuron but was heavily reduced in dry weights by simazine. *P. deltoides* clone 222 was more inhibited by diuron than simazine (Tables 4.6 and 4.7).

Dose-response curves: To rank the clones used in this experiment on a relative scale convenient for comparing their tolerance to simazine and diuron, dose-response curves were prepared for each clone (Figure 4.1). The doses of simazine or diuron causing 20% (ED_{20}) and 50% (ED_{50}) growth inhibition were obtained from the dose-response curves of each clone using total dry weights expressed as a percent of the control (Tables 4.8 and 4.9). The dose response curves for different clones indicated that the degree of response to increasing doses varied among clone (Figure 4.1). Owing to differences in the degree of response to increasing doses of simazine or diuron, trends in ED_{20} and ED_{50} values obtained for the clones were different (Tables 4.8 and 4.9). For example, based on ED_{20} values, NC 5328 was ca eleven times more tolerant than NE 388 to simazine (Table 4.8). However, when this selectivity index was calculated from ED_{50}, (Fryer and Makepeace, 1977) clone NC 5328 was only two times more tolerant than NE 388 to simazine. A similar trend was observed in the tolerance of clones of diuron. Because of the larger clonal differentials, ED_{20} values were employed in ranking the tolerance of poplar clones to simazine or diuron.

Clonal rankings: Table 4.10 is a ranking of clone in order of decreasing ED_{20} values. Based on this ranking, clones NE 17, NC 5377, NC 5323, NE 218 and PD 222 were considered relatively tolerant to simazine within the range

TABLE 4.8 TOXICITY OF SIMAZINE TO 21 *POPULUS* CLONES AS SHOWN BY ED_{20} AND ED_{50}[a] VALUES.

Clones	ED_{20}	ED_{50}
Section Tacamahaca		
NE 41	1.0	4.7
NE 388	1.0	2.1
NC 5260	1.0	1.7
Section Tacamahaca		
NE 218	5.2	9.5
Jac 7	2.2	8.2
NE 302	1.5	3.2
NE 207	1.2	3.8
NE 48	1.0	2.3
Jac 4	1.0	3.3
NE 298	1.0	1.2
Section Aigeiros		
NE 17	6.2	9.8
NC 5377	5.9	8.6
NC 5328	5.5	7.4
NC 5323	5.2	50.0
PD 222	5.0	7.0
NE 308	4.3	8.2
H 47	3.5	7.4
NE 58	2.9	9.8
NC 5326	1.3	2.8
NE 353	1.0	2.1
PH 184	1.0	6.6

[a]Doses(mg/pot) causing 20 and 50% reduction in total dry weights, respectively.

TABLE 4.9 TOXICITY OF DIURON TO 21 *POPULUS* CLONES AS SHOWN BY ED_{20} AND ED_{50}[a] VALUES.

Clones	ED_{20}	ED_{50}
Section Tacamahaca		
NC 5260	12.2	20.1
NE 388	5.0	8.6
NE 41	2.0	11.6
Section Tacamahaca x Aigeiros		
Jac 7	50.0	50.0
NE 302	10.2	20.1
NE 298	6.0	7.5
NE 207	5.6	7.0
Jac 4	3.2	12.2
NE 218	1.5	6.1
NE 48	1.0	1.0
Section Aigeiros		
NE 353	50.0	50.0
NC 5323	22.2	50.0
NC 5326	22.2	28.5
NE 17	20.0	50.0
NC 5328	13.5	40.5
NC 5377	12.2	16.4
H 47	11.0	25.8
NE 58	11.0	14.9
NE 308	10.0	50.0
PD 184	5.8	7.8
PD 222	5.2	6.0

[a]Doses (mg/pot) causing 20 and 50% reduction in total dry weights, respectively.

TABLE 4.10 CLONAL RANKINGS BASED ON ED_{20} VALUES FOR SIMAZINE AND DIURON FOUR WEEKS AFTER TREATMENT WITH VARYING LEVELS OF THE HERBICIDES.

Clonal Ranking (Simazine)[a]	Clonal Ranking (Diuron)[b]
Tolerant	**Tolerant**
NE 17 (A)[c]	NE 353 (A)
NC 5377 (A)	Jac 7 (T X A)
NC 5328 (A)	NC 5323 (A)
NC 5323 (A)	NC 5326 (A)
NE 218 (T X A)	NE 17 (A)
PD 222 (A)	NC 5328 (A)
Intermediate	**Intermediate**
NE 308 (A)	NC 5377 (A)
H 47 (A)	NC 5260 (T)
NE 58 (A)	H 47 (A)
Jac 7 (T X A)	NE 58 (A)
	NE 302 (T X A)
	NE 308 (A)
Intolerant	**Intolerant**
NE 302 (T X A)	NE 298 (T X A)
NC 5326 (A)	PD 184 (A)
NE 207 (T X A)	NE 207 (T X A)
NE 48 (T X A)	PD 222 (A)
NE 353 (A)	NE 388 (T)
Jac 4 (T X A)	Jac 4 (T X A)
PD 184 (A)	NE 41 (T)
NE 41 (T)	NE 218 (T X A)
NC 5260 (T)	NE 48 (T X A)
NE 298 (T X A)	
NE 388 (T)	

[a]ED_{20} ranges for simazine tolerant clones = $>$5 mg/pot; intermediate = 2 to 5 mg/pot; Intolerant = $<$ 2 mg/pot.

[b]ED_{20} ranges for diuron tolerant clones = $>$13 mg/pot; intermediate = 10 to 13 mg/pot; Intolerant = $<$10 mg/pot.

[c] A = Section Aigeiros; T = Section Tacamahaca; T X A = Tacamahaca X Aigeiros

of doses tested. These clones tolerated more than 5 mg/pot of simazine before a 20% reduction in total dry weight occurred. These tolerant clones belong to the section Aigeiros except NE 218 which is an intersectional cross of Aigeiros x Tacamahaca. Those clones considered intermediate in tolerance to simazine had ED_{20} values between 2 and 5 mg/pot. These intermediate clones were NE 308, H 47, NE 58 and Jac-7, all Aigeiros clones except the inter-sectional cross Jac-7. Clones that had ED_{20} values less than 2 mg/pot were considered intolerant to simazine. The group of intolerant clones consisted of three Aigeiros clones NC 5326, NE 353 and PD 184; three Tacamahaca clones NE 41, NE 388 and NC 5260; and the five intersectional crosses NE 302, 207, 48 298 and Jac-4 (Table 4.10).

Clones that tolerated more than 13 mg/pot before a 20% reduction in total dry-weight occurred were considered relatively tolerant to diuron. Tolerant clones included NE 353, Jac 7, NC 5323, NC 5326, NE 17 and NC 5328, again, all from section Aigeiros except Jac-7. Clones considered intermediate in tolerance to diuron had ED_{20} values between 10 and 13 mg/pot. Clones in this intermediate category were Aigeiros clones NC 5377, H 47, NE 58 and NE 308; Tacamahaca clone NC 5260 and Aigeiros x Tacamahaca clone NE 302. Clones that had ED_{20} values less than 10 mg/pot were considered relatively intolerant to diuron and included Tacamahaca clones NE 388 and NE 41; Aigeiros clones PD 184 and PD 222; and Aigeiros x Tacamahaca clones NE 298, 207, 218, 48 and Jac-4 (Table 4.10).

DISCUSSION

Simazine and diuron appeared to have similar phytotoxic effects on poplar clones used in this experiment. The toxicity of both herbicides increased with increasing concentration. This suggested that their mode of action (the sequence of events that leads to death following the primary response) are similar, although the mechanism of action (the primary biochemical or biophysical interference impose by a herbicide that leads to lethality) may not be the same (Moreland, 1980). Climatic factors, soil textural fractions and pH affect simazine and diuron to different degrees in the field. Simazine, an s-triazine, is believed to be protonated in acid soil systems and adsorbed by negatively-charged soil colloids resulting in reduced concentrations in the solution available for plant uptake (Weber, 1970). Diuron, a substituted urea, on the other hand is unaffected by pH in the range of 4.3 to 7.5 (Corbin _et al_., 1971). A differential pH-dependent phytotoxicity of simazine and diuron was reported (Chapter 2) from a field soil of pH 5.4. It seemed reasonable, then, that on a sand culture of pH 7.5 to 8.0 in a greenhouse, simazine and diuron should express comparable phytotoxicity.

Simazine was, however, more toxic than diruon at higher concentrations and cuased more reduction in heights and dry weights. This difference in phytotoxicity at the high rates was manifested in visual symptoms about the third week of treatment. Simazine is less soluble than diuron (3.5 versus 42 ppmW in H_2O at 25 C; Mullison, 1979). This differential solubility might have resulted in greater leaching of diuron making it less available to the plants. This difference in solubility is probably responsible for about four months greater persistence of simazine than diuron when applied at the same rate in the field (Brown, 1978). Diuron has also been reported to be more toxic to fruit crops than simazine (Clay and Davison, 1978).

The *Populus* clones used in this study were found to vary substantially in their susceptibility to simazine and diuron. Differences in susceptibility were visually observed by the second week of treatment and by the end of the experiment, leaf, stem and root dry weights showed substantial clonal differences in phytotoxicity. Clonal differences in total number of leaves and height at harvest was probably due to the lethal effect of the herbicides at high rates rather than inhibition of leaf production or height growth during the treatment period. Von Althen (1979) similarly found that height growth of clones used in his experiment was unaffected by simazine treatment, while Clay and Davison (1978) found that leaf number in strawberries showed the least response to increasing doses of simazine. Rate of leaf production and height growth are also believed to be more under genetic influence and less controlled by environment or cultural practices. For this reason rate of leaf production or height growth may be less reliable for monitoring the response of tree crops to soil-acting herbicides. In spite of the obvious drawback in the use of number of leaves and height growth in ranking the relative tolerance of crops to herbicides, however, Clay and Davison (1978) found significant correlation between these parameters and other measures of herbicide effects.

In general, dry weights, ED_{20} or ED_{50} values or plant and leaf visual scores are more reliable and frequently used in evaluating the tolerance of crops to soil-acting herbicides (Clay and Davison, 1978; Clay, 1980; Talbert and Fletchall, 1964; Fryer and Makepeace, 1977). Clay (1980) observed that ED_{20} values gave more useful estimates of relative tolerance in different plant species than ED_{50} values.

Several parameters were employed in ranking the clones tested in this experiment, but only the ranking based on ED_{20} values are shown here. Trends in all the rankings are similar and indicated that Aigeiros poplars are relatively more tolerant to simazine and diuron than Tacamahaca poplars. Tacamahaca x Aigeiros clones appeared to be intermediate or intolerant to simazine and diuron. However, at least one Aigeiros clone was found to be consistently intolerant, while one Tacamahaca x Aigeiros clone was tolerant among the clones tested. However, no Tacamahaca clone was found to be tolerant. These results are similar to the field observation of von Althen, (1979) who observed that cuttings of clones NC 5328 (I-45/51) and NC 5323 (DN 21), both in the section Aigeiros, were tolerant to dosages of up to 4.5 kg/ha simazine but DJac 14, a Tacamahaca x Aigeiros clone, was injured by a dose of 2.2 kg/ha. The present results also confirmed the results from field experiments reported in Chapter 2 that showed Aigeiros clones NC 5323, NC 5328 and H 47 to be tolerant to doses of up to 4 kg/ha simazine or diuron while Tacamahaca clones NE 388 and NE 48 were injured by a dose of 2 kg/ha simazine or diuron on various plantation sites in lower Michigan.

The basis for this differential selectivity to simazine and diuron among Populus clones is not known. Due to its sparingly soluble nature, the major basis of selectivity of simazine in the field, especially in plants lacking any active metabolic pathway of degrading simazine molecules (e.g. as in maize) is placement (Ebert and Dumford, 1976). The same is true for diuron. In a sand-culture such as used in this experiment all clones have equal exposure to simazine or diuron within experimental limits. Any differential tolerance, e.g., a difference in selectivity index of about eleven between NC 5328 and NE 388, cannot be simply due to a physical barrier. Simazine was reported to be actively metabolized to non herbicidal hydroxysimazine in Norway spruce (Picea abies) by Lind-Hoeie (1969a), whereas Dhillon et al., (1968) did not observe any degradation of simazine in red pine (<u>Pinus resinosa</u>) seedlings. Similar data for <u>Populus</u> is not available.

The differential tolerance to simazine and diuron in this experiment may be due to some form of inactivation of the herbicide molecules, e.g., by active metabolism to hydroxysimazine (Shimabukuro, 1968) or compatmentalization on active binding sites (Arntzen <u>et al</u>., 1979; Pfister <u>et al</u>., 1979). If this inactivation occurs in <u>Populus</u> it is apparently under relatively strong genetic control and could be exploited by tree geneticists in selecting or breeding new clones insensitive to soil-acting herbicides.

CHAPTER V

EFFECT OF SIMAZINE ON PHOTOSYNTHETIC CO_2 FIXATION, CO_2 COMPENSATION POINT, LEAF CONDUCTANCE AND SPECIFIC LEAF WEIGHT OF SIMAZINE-TOLERANT AND INTOLERANT *POPULUS* CLONES

INTRODUCTION

The effects of triazine herbicides on photosynthesis and water balance of agronomic crops and weeds have been studied extensively, but very few reports exist on forest trees (Ebert and Dumford, 1976; Moreland, 1980). In nearly all studies, the extent of transpiration reduction was related directly to herbicidal susceptibility of the particular plant species. In all cases of transpiration reduction by triazines, partial or complete stomatal closure occurred (Ebert and Dumford, 1976). Imbamba and Moss (1971) showed that CO_2 uptake is reduced more than transpiration in the light. The inhibitory effect of triazines on photosynthesis is very likely the initial cause which leads to reduced transpiration (van Oorschot, 1976). Besides depriving the cell of energy, inhibiting photosynthesis would also raise the CO_2 concentration of the cells. The effect of high concentrations of CO_2 causing stomatal closure in the light has been well documented (Ebert and Dumford, 1979). If photosynthesis is partially or completely inhibited by the triazines, then, stomata would be expected to remain closed or to only partially open.

More than one reaction in the photosynthetic process is probably affected by simazine. In addition to inhibition of the Hill reaction, simazine (triazines) also inhibit photosynthetic CO_2 fixation (Moreland and Hilton, 1976; Moreland, 1980). Any condition that alters the process by which CO_2 is fixed or released will alter the CO_2 compensation equilibrum that exists between photosyntheses and respiration (McClelland _et al_., 1978). CO_2 compensation concentration, then, can be used to compare effects of environmental conditions or chemical treatments on photosynthesis and respiration. A highly significant negative correlation has been demonstrated to exist between photoshythetic rate and CO_2 compensation point (Heichel and Musgrave, 1969; Dickmann, 1971; Larcher, 1980).

Differences in phytotoxicity of simazine in _Populus_ clones have been reported in the field (Dickmann, _et al_., 1978; von Althen, 1979; Chapter 2) and confirmed in greenhouse studies (Chapter 4). The selectivity among _Populus_ clones to simazine appears to be under genetic control. Most of the clones tested in the Aigeiros section were relatively tolerant of simazine whereas clones in the Tacamahaca x Aigeiros intersectional hybrids were relatively intolerant (Chapter 4). For example, clone NC 5328 (section Aigeiros) tolerated up to 4.5 kg/ha simazine, whereas clone

NE 388 (section Tacamahaca) was injured by less than 2 kg/ha simazine in field trials (von Althen, 1979; Chapter 2).

A selectivity index to simazine of eleven between NC 5328 and NE 388 was observed using a sand-culture technique in the greenhouse (Chapter 4). It was concluded that tolerance to simazine in poplar could not be due to positional selectivity (Chapter 4), but must depend on certain genetically-controlled physiological responses.

The objective of this study was to further characterize the differential tolerance to simazine that exist among Populus clones. The effect of simazine on CO_2 fixation, CO_2 compensation point, leaf conductance and specific leaf weight in simazine-tolerant NC 5328 and intolerant NE 388 clones of Populus were evaluated.

MATERIALS AND METHOD

Cultural: Unrooted hardwood cuttings of Populus x euramericana cv. "I-45/51" (NC 5328) and P. maximowiczii x P. trichocarpa cv. "Kingston" (NE 388) were selected for uniformity in length, diameter and bud size. They were raised in a greenhouse in washed sand of pH 7.5-8.0. Temperature in the greenhouse was ca. 25 C during an 18 hr day and 15 C at night. Relative humidity was between 60 and 80%. Watering and aeration of the pots was accomplished by pouring 1 litre of nutrient solution directly on the sand when the surface was dry. The nutrient solution was prepared from a 15-30-15 (N-P-K) commercial water-soluble plant food supplemented with the micronutrients Cu, Fe, Mn and Zn.

The plastochron index (PI) and leaf plastochron index (LPI) concepts as developed for cottonwood by Larson and Isebrands (1971), were used to select the sampled leaves. At the 4th week of growth, height, diameter and number of leaves 20 mm or greater in length were recorded. Length of the index leaf (the first leaf below the apex at least 20 mm in length) and the leaf immediately above it were measured for calculation of PI and LPI. At a PI of 12, the index leaf (LPI 0), the 12th leaf from the base, would be exactly 20 mm long. The next older leaf below the index leaf has a LPI of 1 and so on down the plant.

Simazine treatment: As plants approached a PI of 10, pairs were selected within each clonal pool. Pairing was based on uniformity in height, diameter and number of leaves (PI). At a PI of 11 one plant from each pair was randomly selected; 5 mg of simazine (80% wettable powder) was added in 1 litre of water to the sand surface in the pot. The other plant in the pair was left untreated. Based on previous studies, 5 mg/pot simazine was high enough to cause injury but not enough to kill the intolerant clone NE 388 during the experimental period. Treated plants were scored at each sampling time for visual injury symptoms on the leaves and whole plant (see Chapter 4 for criteria of visual assessment). Heights and number of leaves were also recorded.

Photosynthesis, leaf conductance and CO_2 compensation point: Measurements were made on plants 24 hr, 48 hr, 1 week and 2 weeks after treatment with simazine (Table 5.1). The measurements corresponded with PI's of 11, 13, 18 and 25 for NE 388, and 11, 12, 16 and 21 for NC 5328. For both clones determinations were made on leaves of LPI 4 and 9 at 24 hr, 4 and 10 at 48 hr, and 5 and 11 at 1 week after treatment. Two weeks after treatment determinations were made on leaves of LPI 5, 12 and 21 for NE 388 and LPI 5, 11 and 18 for NC 5328. The purpose of this scheme was to monitor physiological activity within the developing, newly mature and older leaf zones. The sampling scheme consisted of both a vertical series and a horizontal or aging series (Table 5.1). The vertical series allows comparison of leaves in similar states of development while the horizontal series allows comparisons of the same leaf position with time (Dickmann, 1971).

Photosynthetic measurements were made in the greenhouse where the plants were grown. Prior to determinations, conductance of the abaxial leaf surface was measured with a Li Cor LI-65 Autoporometer equipped with a horizontal Kanemasu-type sensor (Kanemasu et al., 1969). Leaf temperature was measured with the bead thermister built into the

TABLE 5.1 LEAF PLASTOCHRON INDEX (LPI) IN RELATION TO LEAF SERIAL NUMBER AND PLASTOCHRON INDEX (PI) FOR YOUNG POPLARS MEASURED AT VARIOUS TIMES FOR RESPONSE TO SIMAZINE.

	Period After Treatment With 5 mg/pot Simazine[a]							
	24 hr.		48 hr.		1 week		2 weeks	
Leaf No. (From Base)	NC 5328 (PI 11)	NE 388 (PI 11)	NC 5328 (PI 12)	NE 388 (PI 13)	NC 5328 (PI 16)	NE 388 (PI 18)	NC 5328 (PI 21)	NE388 (PI 25)
	. . . . .LPI.							
1	10	10	11	12	15	17	20	24
2	9*	9*	10*	11*	14	16	19	23
3	8	8	9	10	13	15	18*	22
4	7	7	8	9	12	14	17	21*
5	6	6	7	8	11*	13	16	20
6	5	5	6	7	10	12	15	19
7	4*	4*	5*	6	9	11*	14	18
8	3	3	4	5*	8	10	13	17
9	2	2	3	4	7	9	12	16
10	1	1	2	3	6	8	11*	15
11	0	0	1	2	5*	7	10	14
12			0	1	4	6	9	13
13				1	3	5*	8	12*
14					2	4	7	11
15					1	3	6	10
16					0	2	5*	9
17						1	4	8
18						0	3	7
19							2	6
20							1	5*
21							0	4
22								3
23								2
24								1
25								0

[a]Leaves on which physiological measurements were made are followed by an asterisk.

porometer by appressing it to the lower leaf surface. Photosynthetically active photon flux density (PAPFD) was determined with a Li Cor LI-85 light meter equipped with a quantum sensor. Measurement of PAPFD was made parallel to the leaf surface, assuring in situ radiation conditions by maintaining continuity of canopy architecture (Kriedemann et al., 1964). Air containing 332 ppm CO_2 (5.0 uCi l^{-1} $^{14}CO_2$) was passed over both surfaces of a leaf by clamping the treatment chambers of a pulse labeling devise (McWilliam et al., 1973) over the leaf for 20 seconds. Flow rate was 80 ml min^{-1}, sufficient to minimize the boundary layer resistance and prevent depletion of CO_2 in the chambers. The exposed area (0.5 cm^2)immediately cut from the leaf with a 1 cm cork borer and placed into a scintillation vial containing 1.5 ml NCS tissue solubilizer (Amersham Corp.)

Scintillation vials containing leaf discs and NCS were placed in an oven for 24 hr at 50C. Samples were allowed to cool for 30 min and then bleached by the addition of 0.5 ml H_2O_2. Twenty-four hours later, 17 ml of a scintillation fluid containing 1000 ml tolune, 400 ml methyl cellosolve and 60 ml Spectrafluor (Amersham Corp) was added. After an additional 24 hr. dark equilibration period, samples were counted in an Isocap 300 liquid scintillation system (Nuclear Chicago) at room temperature. Rate of photosynthesis, expressed as weight of CO_2 taken up by the leaf per unit time and leaf area, was calculated using a formula similar to that of McWilliam et al. (1973).

Leaves used for photosynthetic measurements were excised and CO_2 compensation points determined using a Mylar-bag technique (Dickmann and Gjerstad, 1973). The area of each individual leaf then measured with a Li Cor LI-3000 leaf area meter.

Leaves were dried in an oven at 75 C for 48 hr. and weighed for calculation of specific leaf weight (SLW), an index of the relative density of a leaf and a sensitive measure of environmental influences (Ledig, 1974). SLW was determined to monitor morphological changes occurring in leaves following simazine treatment.

The experimental design was a split-split-plot factorial. Two replications of each paired measurement were made, representing eight trees and 16 leaves per measurement time. Means were compared at the 0.05% level of probability by Duncan's New Multiple Range Test.

RESULTS

The condition of leaves and plants, heights and number of leaves two weeks after treatment with 5 mg/pot simazine is shown in Table 5.2. There were no injury symptoms visible in both NC 5328 and NE 388 from the time of treatment through the first week. In the second week after treatment clone NE 388 began to show significant injury symptoms, whereas NC 5328 did not (Table 5.2). The

TABLE 5.2 PHYSICAL CONDITION OF *POPULUS* CLONES NE 388 AND NC 5328 TWO WEEKS AFTER TREATMENT WITH 5 MG/POT SIMAZINE.

Clone	Treatment	Leaf Score[a]	Plant Score[b]	Height (cm)	Number of Leaves
NE 388	Control	4	7	62	25
	Simazine	2	5	58	25
NC 5328	Control	4	7	53	21
	Simazine	4	7	55	22

[a]Leaf score: 0 = dead leaf; 4 = normal leaf.

[b]Plant score: 0 = dead plant; 7 = normal plant.

injury symptoms in NE 388, which consisted of acute chlorosis and "burning" of leaves, was confined to the zone between LPI 11 and 18 (PI 25). This chlorotic region corresponded to the zone of recently mature leaves and consisted of about one-third of the entire leaf area of the plant.

The leaf conductance of NE 5328 in this experiment ranged between 0.08 and 0.13 cm s^{-1} (Table 5.3). There were no significant differences detected between treated and control plants nor among the different leaf positions within each treated plant for both NC 5328 and NE 388 throughout the duration of the experiment.

There were no significant differences detected in CO_2 compensation points between treated and control plants or among the different positions within treated plants of NC 5328 at 24 hr, 48 hr, 1 week and 2 weeks after treatment with 5 mg/pot simazine (Table 5.4). Compensation points of NE 388 plants treated with simazine, however, showed significant increases over control plants at 48 hr, 1 week and 2 weeks after treatment. There was also more variability in compensation points within NE 388 plants than within NC 5328 plants. Leaves in the recently mature zone, where symptoms of simazine injury were most apparent, showed higher compensation points than leaves in the zones below and above.

Significant differences were found between NC 5328 and NE 388 in the rate of CO_2 fixation. No significant

TABLE 5.3 ABAXIAL LEAF CONDUCTANCE OF *POPULUS* CLONES NE 388 AND NC 5328 AFTER TREATMENT WITH 5 MG/POT SIMAZINE.

Clone	Treatment	Leaf[b] Position	Period After Treatment[a]			
			24 hr	48 hr	1 week	2 weeks
			. . . . cm s^{-1}			
NE 388	Control	E	.12	.08	.09	.11
		RM	.09	.12	.13	.09
		OM	---	---	---	.09
	Simazine	E	.12	.10	.11	.11
		RM	.11	.11	.11	.10
		OM	---	---	---	.09
NC 5328	Control	E	.10	.11	.09	.10
		RM	.10	.11	.11	.10
		OM	---	---	---	.12
	Simazine	E	.11	.10	.10	.11
		RM	.10	.10	.11	.10
		OM	---	---	---	.10

[a]No means within columns differ significantly at the 5% level.

[b]E = expanding leaves; RM = recently mature leaves; OM = older mature leaves.

TABLE 5.4 CO_2 COMPENSATION CONCENTRATIONS OF *POPULUS* CLONES NE 388 AND NC 5328 AFTER TREATMENT WITH 5 MG/POT SIMAZINE.

Clone	Treatment	Leaf Position[b]	Period After Treatment[a] 24 hr.	48 hr.	1 week	2 weeks
			. . . .	. . .ppm.	. . . . . .	. .
NE 388	Control	E	69 bc	74 b	70 a	55 a
		RM	65 abc	71 b	58 a	47 a
		OM	--	--	--	49 a
	Simazine	E	67 abc	87 c	123 b	80 b
		RM	66 abc	71 b	182 c	115 c
		OM	--	--	--	55 a
NC 5328	Control	E	66 abc	69 b	59 a	47 a
		RM	64 abc	61 a	56 a	40 a
		OM	--	--	--	40 a
	Simazine	E	70 c	68 b	61 a	44 a
		RM	65 ab	61 a	57 a	44 a
		OM	--	--	--	40 a

[a]Means within columns followed by the same letter do not differ significantly at the 5% level.

[b]E= expanding leaves; RM = recently mature leaves; OM = older mature leaves.

differences in rate of photosynthesis were detected between treated and control plants of NC 5328, although treated plants showed slightly higher rates of CO_2 fixation at 24 hrs, 48 hrs, and 2 weeks after treatment. The rate of CO_2 fixation was significantly reduced in simazine treated NE 388 plants 1 week and 2 weeks after treatment (Table 5.5). There were also significant differences in the degree of inhibition among different leaf positions on NE 388 plants; expanding leaves were less inhibited than recently mature leaves at 1 week and 2 weeks after treatment. Older mature leaves at 2 weeks after treatment did not show any inhibition at all (Table 5.5).

A significant difference in the response of NE 388 and NC 5328 to simazine treatment was also reflected in specific leaf weights (Table 5.6). Clone NC 5328 was again not affected but a significant reduction in specific leaf weight occurred 1 week and 2 weeks after treatment of clone NE 388. All leaf positions sampled were uniformly reduced.

DISCUSSION

Differences in photosynthetic response to simazine treatment have been demonstrated in *Populus* clones NC 5328 and NE 388. Additions of simazine at 5 mg/pot had no deleterious morphological or physiological effects on NC 5328 but reduced the rate of CO_2 fixation, increased CO_2 compensation concentrations and lowered the specific leaf weight of NE 388. These deleterious physiological effects of

TABLE 5.5 LEAF PHOTOSYNTHETIC RATE OF *POPULUS* CLONES NE 388 AND NC 5328 AFTER TREATMENT WITH 5 MG/POT SIMAZINE

Clone	Treatment	Leaf[b] Position	Period After Treatment[a] 24 hr	48 hr	1 week	2 weeks
			. . . . . . .mg CO_2 dm^{-2} hr^{-1}. .			
NE 388	Control	E	1.7 a	11.6ab	5.9 b	8.2 b
		RM	5.8 ab	14.6b	5.3 b	10.6 bcd
		OM	--	--	--	9.7 bc
	Simazine	E	4.3 a	8.7a	2.3 a	3.3 a
		RM	10.0 bc	11.3ab	1.7 a	2.8 a
		OM	--	--	--	8.6 b
NC 5328	Control	E	11.0 c	11.5ab	5.5 b	13.3 cd
		RM	9.4 bc	10.7ab	5.3 b	11.0 bcd
		OM	--	--	--	7.9 b
	Simazine	E	12.1 c	14.5b	5.4 b	13.9 d
		RM	9.0 bc	12.1ab	5.0 b	12.9 cd
		OM	--	--	--	7.3 b

[a]Means within columns followed by the same letters do not differ significantly at the 5% level.

[b]E = expanding leaves; RM = recently mature leaves; OM = older mature leaves.

TABLE 5.6 SPECIFIC LEAF WEIGHT OF *POPULUS* CLONES NE 388 AND NC 5328 AFTER TREATMENT WITH 5 MG/POT SIMAZINE

			Period After Treatment[a]			
Clone	Treatment	Leaf[b] Position	24 hr	48 hr	1 week	2 weeks
			. . . . mg cm^{-2}.			
NE 388	Control	E	3.0 a	4.1 a	4.3 b	4.0 b
		RM	3.7 a	4.2 a	4.4 b	4.1 b
		OM	--	--	--	4.4 b
	Simazine	E	3.8 a	4.3 a	2.6 a	2.4 a
		RM	3.5 a	4.5 a	3.0 a	2.7 a
		OM	--	--	--	2.7 a
NC 5328	Control	E	4.0 a	4.2 a	4.4 b	4.0 b
		RM	3.7 a	4.5 a	4.1 b	4.6 b
		OM	--	--	--	4.4 b
	Simazine	E	4.0 a	4.4 a	4.5 b	4.3 b
		RM	4.0 a	4.0 a	4.2 b	4.3 b
		OM	--	--	--	4.4 b

[a]Means within columns followed by the same letters do not differ significantly at the 5% level.

[b]E= expanding leaves; RM = recently mature leaves; OM = older mature leaves.

simazine on NE 388 were detected 48 hr. after exposure of plants to simazine and generally became more pronounced thereafter. Visual symptoms of injury did not become evident until two weeks after treatment, however. The length of this period before onset of visual injury symptoms was similar to that reported for simazine and diuron on intolerant clones of Populus in Chapter 4.

The differential effect of simazine on CO_2 fixation in two clones of Populus reported here is similar to observations of Radosevich and Appleby (1973) that photosynthesis was completely inhibited by simazine in susceptible biotypes of common groundsel, whereas resistant biotypes were unaffected. Similar differential effects of triazine herbicides on photosynthesis in different biotypes of plant species have also been reported by Souza Machado et al.(1977) and Radosevich et al. (1979). The effect of triazines on CO_2 compensation concentration in susceptible and tolerant biotypes has received less attention. In one report bentazone application to entire morning glory increased CO_2 compensation by ca. 300% over untreated control plants 4 days after application (McLelland et al., 1978). The increased CO_2 compensation points observed after the intolerant NE 388 was treated with simazine in this experiment correlates with the reduced rates of CO_2 fixation also shown by this clone.

The basis for this differential response of NE 388 and NC 5328 might be due to differences in translocation, accumulation, active metabolism, or binding of the simazine compound in different plant parts. These factors have been reported as responsible for differential tolerance between susceptible and resistant biotypes of some crops and weeds (Werner and Putnam, 1980; Radosevich et al., 1979). The basis for observed differential response of NE 388 and NC 5328 to simazine is being investigated further.

Leaf stomatal conductance appeared not to be affected by simazine in both tolerant and intolerant clones of *Populus* within the experimental period. Stomatal conductance, a reciprocal of stomatal diffusion resistance, is directly proportional to the stomatal pore width (Larcher, 1980). Thus, by showing no effect on conductance, simazine probably did not directly affect the rate of gas flow through the stomata in this experiment. It is now generally agreed that the effect of photosynthetic inhibitors like the triazines on transpiration is secondary (van Oorshot, 1974, 1976; Ebert and Dumford, 1976). Sharkey (1980), for example was able to inhibit photosynthesis for a period of 4 days without any apparent effect on stomatal pore size in excised leaves of *Xanthium strumarium* using DCMU (diuron) or atrazine. The results reported here further confirm that within a short period (2 weeks in this experiment) photosynthesis could be reduced substantially without affecting stomatal conductance. However, the necrosis induced by herbicides would ultimately lead to decreased transpiration rate and conductance. The onset of this necrotic processes is indicated in clone NE 388 by the reductions in SLW that occurred after 1 week.

The response to simazine toxicity in the tolerant clone NE 388 varied depending on the position of the crown sampled. Inhibition of photosynthesis and increased CO_2 compensation points were more pronounced in the region of recently mature leaves, but was also apparent in the region of expanding leaves. The differential response between leaf positions may be due to the degree of vascularization and its causal effects on xylem translocation. For example, Larson and Isebrands (1971) observed that internodes in the recently mature zone (LPI 9, 10, 11 or 12 in this experiment) have complete secondary vascular tissues with well developed secondary vessels and rays. They also observed that the expanding leaf zone (LPI 4 or 5 in this experiment) consisted of immature primary vascular tissues. Field observations at periods of drought stress in Populus plantations correlates well with the described trend in vascularization of the crown.

For example, wilting is first observed in the apex and uppermost leaves while drought-induced leaf scenescence occurs first in the lower-most leaves (Dickmann, 1980), indicating that the resistance to water movement into leaves is highest in these regions. Because simazine is transported in the xylem, the crown region into which water most freely moves would be expected to accumulate greater levels of simazine. It is also known that the lower leaves of *Populus* plants are declining in physiological activity (Dickmann, 1971; Dickmann and Gordon, 1975), reducing their sensitivity to simazine.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Fast-growing forest trees like the *Populus* are prime candidates for intensive production of wood. However, growth can be substantially limited if competition for water, space and nutrient is not eliminated or reduced by controlling competiting vegetation. Chemicals, when applied properly, offer the most effective and cheap method of weed control. Only a few chemicals among which are simazine and diuron, have potential for use in poplar plantations. But at rates effective for adequate weed control injury to some *Populus* clones often occurs. The studies reported here examined the differential effect of simazine (2-chloro-4, 6-bis (ethylamino) - s - triazine), and diuron (3-(3,4 -dichlorophenyl) - 1, 1-dimethyl urea) on survival, growth and physiology of selected hybrid *Populus* clones.

In field studies, clonal differences in establishment, growth and biomass yield of simazine and diuron treated *Populus* was demonstrated. Clones NC 5323 and H 47 performed well at the Tree Research Center (TRC); NC 5323 and NE 41 were the best at Dansville and Manistee. The response of the clones to simazine and diuron follow similar pattern. High doses of each herbicide were toxic to all clones except H 47, which appeared to be relatively tolerant. Simazine at 2 kg/ha was found adequate for acceptable weed control, survival and biomass yield at TRC and Dansville. However, at Manistee simazine was ineffective due to the low pH of the surface soil but diuron at 2 kg/ha was suitable.

A single initial tillage operation at the beginning of the season benefitted all clones tested at all herbicide rates on all sites. Survival growth and yield were higher on tilled plots when supplemented with herbicides compared to either tillage or herbicide alone. This was probably due to the increased persistence of the herbicides on the tilled sites. However, the enhancement of tree establishment due to increased moisture and soil contact was probably also significant.

The effect of simazine in causing an increase in total foliar nitrogen of Populus clones beyond that due to elimination of competiting vegetation was demonstrated. The stimulatory effect of simazine on total foliar nitrogen was dependent on the clone, rate of simazine and length of time after application. Clone H 47 and NC 5323 treated with 2 to 3 kg/ha simazine gained nearly 12% in total foliar nitrogen over the untreated weed-free control at the middle of the season, the period of most active growth. There was also a corresponding increase in height growth. Nitrogen content of clone NE 388 and NE 48 were unaffected but height growth of NE 388 was significantly inhibited by simazine.

In greenhouse studies, *Populus* clones from sections Tacamahaca and Aigeiros were assayed for tolerance to five levels of simazine and diuron. Selected clones included both intra and intersectional hybrids as well as pure P. *deltoides*. Washed sand of pH 7.5-8.0 was used to eliminate or reduce the influence of edaphic factors on herbicidal behavior. Although no morphological basis for selectivity was observed, each of the clones could be classified as either tolerant, intermediate or intolerant to simazine or diuron in the range of concentrations tested. Tacamahaca hybrids and intersection crosses between Tacamahaca and Aigeiros were relatively intolerant to both herbicides, whereas Aigeiros clones were relatively tolerant. Examples of intolerant clones include *P. maximowiczii* x *P. trichocarpa* (NE 388) and *P. maximowiczii* x *P.* cv. "berolinensis" (NE 48). The *P.* x *euramericana* clones "Canada blanc" (NC 5323) and "I 45/51" (NC 5328) were among the most tolerant tested. A selectivity index of eleven between NC 5328 and NE 388 was observed with simazine.

Based on the field and greenhouse studies, it is evident that clonal differences in the response of *Populus* clones to simazine could not be due to positional selectivity, but must depend on certain genetically-controlled physiological responses. Differential photosynthetic responses to simazine were observed in *Populus* clones NC 5328 and NE 388. Additions of 5 mg/pot simazine had no deleterious morphological or physiological effects on NC 5328 but reduced the rate of CO_2 fixation, increased CO_2 compensation points and lowered specific leaf weight of NE 388. The deleterious effects of simazine on NE 388 were detected 48 hr. after exposure of plants to simazine and generally became more pronounced thereafter. Visual symptoms of injury in intolerant clone NE 388 lagged ca. 12 days behind the onset of inhibitory effects on physiological processes and changes in specific leaf weights.

The response to simazine toxicity in the intolerant clone NE 388 was different depending on the position of the crown that was sampled. Inhibition of photosynthesis and increased CO_2 compensation points were more pronounced in the region of recently mature leaves, followed by the region of expanding leaves. The lower crown region with older mature leaves showed no visual symptoms of injury; rate of photosynthesis and CO_2 compensation concentrations were not affected. The differential response due to leaf position was probably due to morphological patterns of vascularization and their causal effect on translocation of herbicide into the different regions. Since simazine transport is apoplastic, the degree of development of the xylem system in each crown region will differentially affect its distribution and subsequent toxicity.

The differential clonal responses to simazine and diuron demonstrated in the field and greenhouse studies could be due to a number of factors, such as differential uptake and translocation, metabolism of simazine to non-toxic hydroxysimazine or chloroplast binding characteristics among _Populus_ clones. A study to evaluate differential simazine uptake, translocation or metabolism in intolerant and tolerant clones is already underway. Further studies will be necessary to examine differential herbicide-binding to the chloroplast as a possible major factor in differential simazine or diuron tolerance among _Populus_ clones. Stuides to confirm heritability of the mechanism of tolerance among the _Populus_ clones is essential to know if such mechanism could be transferred to clones with other desirable characteristics but susceptible to herbicide toxicity.

Breeding for herbicide tolerance among _Populus_ may be further down the road; immediate selection of herbicide tolerant _Populus_ clones based on the parentages can now be achieved with a high degree of safety.

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