DISSERTATION

GROWTH, NUTRIENT, AND WATER STATUS OF MONTEZUMA PINE AS AFFECTED BY ALDER IN THE STATE OF HIDALGO, MEXICO

Submitted by

Miguel Angel Lopez-Lopez

Forest, Rangeland, and Watershed Stewardship

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MIGUEL ANGEL LOPEZ-LOPEZ ENTITLED GROWTH, NUTRIENT, AND WATER STATUS OF MONTEZUMA PINE AS AFFECTED BY ALDER IN THE STATE OF HIDALGO, MEXICO BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee Graduate Work

Celedonio Aguirre Bravo

CELEDONIO AGUIRRE-BRAVO

RAJIV KHOSLA

Mohammed A. Kalkhan

Adviser

Robin M. Reich

Department Head

ABSTRACT OF DISSERTATION

GROWTH, NUTRIENT, AND WATER STATUS OF MONTEZUMA PINE AS AFFECTED BY ALDER IN THE STATE OF HIDALGO, MEXICO

In order to study the relationships between alders and pine trees in *Pinus montezumae-Alnus arguta* stands in terms of pine growth, nutrient status, needle water potential, and soil water status, a series of experiments were developed in two ejidos and one private forest in the state of Hidalgo, Mexico.

Results indicated that pine growth may be either positively or negatively affected by the presence of alder. When alders were well exposed to sunlight, pine growth generally increased with increasing alder density. Contrarily, when alders were in the understory of a closed pine canopy, even the lowest alder densities exerted negative effects on pine growth. Pine nutrient status also varied depending on alder condition in terms of light exposure. Pine N requirements were fully met when alders were exposed to sunlight. When alders were in the understory, however, N status became worse as alder density increased. Competition for N between pine trees and alders occurred under this condition. Pine P showed a similar behavior as with N when alders were in the understory. Pine K status, on the other hand, was improved with increasing alder density, regardless of the alder condition. However, it seems that K status improvement when alder was in the understory came from a concentration process.

Needle mass generally increased with the application of N fertilizer regardless of the area of regeneration but it decreased with the presence of understory alders in the area of regeneration 1987.

Results indicate that alder clumps significantly decreased soil moisture both, during the rainy and during the dry season of the year. Nonetheless, depletion of soil water did not contribute to significant changes in pine predawn water potentials.

Miguel Angel Lopez-Lopez
Department of Forest, Rangeland, and Watershed Stewardship
Colorado State University
Fort Collins, CO. 80523
Spring 2004

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Dedication

To the Mexican Forest Farmers

Our planet's life largely depends on how you will do your tomorrow's work!

To Elizabeth, Miguel Angel, Lucia Elizabeth, and Alma Gloria

Life always rewards you for the hard times you have had while trying noble goals.
We did it!

To the memory of my parents

I never heard it from you,
cause you were so quiet and sober,
but your endless love showed to me
you sowed into your mind
the highest goals for me
I am a bit of you and you have achieved one more of them!

To the memory of Ignacio and Ester

To my brothers and sisters

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INTRODUCTION

Nitrogen is one of the nutrients that most commonly limit plant growth in many terrestrial ecosystems. Paradoxically, this element is also the most abundant one in the earth's atmosphere. Nonetheless, the molecular form (N_2) in which this element is found in the atmosphere is very little reactive and consequently, it is not available for plant absorption. Therefore, the molecular form of this element must undergo a fixation process before nitrogen may be used by plants.

There are several processes by which molecular nitrogen may be fixed to plant available forms: lightning, industrial, and biological processes. Biological fixation of nitrogen is by far responsible for most of the fixed nitrogen in earth. This route for fixation of nitrogen may be realized by free-living organisms or by symbiotic systems formed by superior plants and organisms such as bacteria or actinomycetes. The most important process for biological nitrogen fixation as judged by the amount of fixed nitrogen is the symbiotic one.

The symbiotic system formed by the association of Alder species (*Alnus* sp) and actinomycetes of the genus *Frankia* is able to fix important amounts of nitrogen in most cold-temperate ecosystems. The nitrogen fixed by this association is primarily incorporated into the metabolism of the plant, which, usually presents higher tissue nitrogen concentrations than non-nitrogen fixing plants. After leaf abscission or organ death, these materials are decomposed in soil and nitrogen released, remaining available not only for nitrogen-fixing plants, but also for associated ones in the ecosystem.

In nitrogen-limited ecosystems, positive responses of plants to this phenomenon are expected to occur. However, at the same time as nitrogen fixation takes place, competition effects between nitrogen-fixing plants and non-nitrogen fixing plants, which frequently are the economically valuable crops, occur and may even mask the positive effects of the improvement in nitrogen availability. The final balance in this trade-off in forest ecosystems depend to a large extent on the silvicultural system adopted. Therefore, all stages in the silvicultural system should be studied previously if reasonable predictions of crop reactions to treatments are to be pursued.

The present research work was intended to elucidate several reactions of young natural-regenerated *Pinus montezumae* to varying proportions of alder (*Alnus arguta*) in three areas of regeneration in the state of Hidalgo, Mexico. The responses of crop trees were evaluated not only in terms of growth and nutrient status, but nutrient- and water-competition effects between the species were studied too.

One additional intention of this work was to motivate scientists and technicians to engage in studies that may go deeply into the details of the management of mixed-species forests in Mexico, alder species have traditionally been viewed as weeds and their soil-improving potential invariably ignored in practice.

The thesis has been divided into four chapters. Chapter 1 has to do with the effects of alder on pine dasometric variables. In chapter 2, the nutrient status (nitrogen, phosphorus, and potassium) of pine trees as affected by the presence of alder was studied. The competition effects for nutrients between pines and alders were also discussed in this chapter. As a means to reinforce the results dealing with the effects of alder on pine growth and nutrient status, we ran an experiment using nitrogen fertilizer to evaluate the effects of varying alder densities on pine growth under the assumption that the response of pines to the presence of alders would be stronger when no nitrogen fertilizers were applied than when they were. This study is presented in Chapter 3. Finally, chapter 4 has to do with the effects of competition for water between pines and alders on pine and soil water status.

CHAPTER 1

RELATIONSHIP BETWEEN PINE GROWTH AND PINE/ALDER RATIO

ABSTRACT

A field study was carried out in Central Mexico in order to investigate how alder (*Alnus arguta*) affects pine (*Pinus montezumae*) growth in three pine-alder young mixed stands. Our results indicate that low-to-intermediate alder densities positively affected pine growth in all three areas of regeneration studied. The intensity of the effects seems to be modified by the overstory pine canopy since a very low alder density (20 trees per ha^{-1}) was optimum for pine growth in the stand with the highest pine density, indicating that alders are acting as weed shrubs. Moreover, our data shows that the role of alder in a system may change from beneficial to detrimental in terms of its effects on pine growth, apparently depending on pine leaf area in the overstory.

INTRODUCTION

Alder species' N-fixation capacity is supposed to affect growth rates of associated plant species by improving nitrogen availability in soils. This is especially true for nitrogen-poor ecosystems.

Alder (*Alnus* spp.) roots are known to associate with actinomycetes of the genus *Frankia*. This association gives rise to certain structures in alder roots, which are called nodules. Nitrogenase activity takes place within the root nodules resulting in the chemical reduction of the molecular nitrogen (N_2) to ammonia (Binkley 1993, Fisher and Binkley 2000).

Most of the nitrogen fixed by the root nodules is primarily translocated to the alder leaves where it is incorporated to the various functions it has within plants. While nitrogen stays in the leaves, it may reach concentrations as high as 2.8 % (Gutierrez 1998). This value is rather high if compared with nitrogen concentrations in wild non-nitrogen-fixing plants, in which case values are often lower than 2 %.

Part of the nitrogen content of alder leaves is supposed to come from the nitrogen fixation process. Therefore, when alder leaves fall during the autumn every year and decompose, they potentially represent a nitrogen input for the ecosystem.

The input of nitrogen into the ecosystem is expected to eventually improve the nitrogen nutrition not only of alders but also of co-occurring species, thus improving growth of such species, especially in nitrogen-limited soils.

The objectives of the present work are to:

1) determine how different pine/alder ratios affect the growth of pine trees, and
2) determine the pine/alder ratio at which maximum pine growth may occur.

REVIEW OF LITERATURE

Biological nitrogen fixation is a process through which N_2 is reduced to NH_3 with the participation of living microorganisms in soil.

$$N_2 + 8e^- + 8H^+ \longrightarrow 2NH_3 + H_2$$

In the symbiotic N-fixation systems, some kinds of soil microorganisms associate with superior plants to give rise to nodules in the plant roots. One of the most common of this kind of symbiosis is that formed by the association of bacteria of the genus *Rhizobium* with legume species. In temperate forests, maybe the most important microorganisms that associate with plants to fix nitrogen are those actinomycetes of genus *Frankia*, which form nodules in the roots of plants from several genuses, among them, the genus *Alnus* (alder). For the fixation process to be carried out, energy must be provided by the tree, which takes advantage of the nitrogen fixed by the organisms (actinomycetes or bacteria) living in the nodules (Finck 1988). Then the nitrogen is transported to the foliage of the host plant, where it is used mainly for the formation of different aminoacids.

The use by the tree of the nitrogen fixed in the nodules, results in an increase of the nitrogen concentration in its foliage. Indeed, nitrogen-fixing plant foliage reaches nitrogen concentrations as high as 2.4-3.7 % (Beer, 1980), although Silvester (1983) reports 2.73, 2.57 and 2.1 % N in foliage of *Alnus incana, A. glutinosa* and *A. rubra,* respectivelly. Gutierrez (1998) reported concentrations of nitrogen in *Alnus firmifolia* leaves as high as 2.8 %. Other wild plant species (non-N-fixing species) frequently show N concentrations lower than 2 %. As a consequence of the high N concentrations, foliage or other parts of the N-fixing plants falling on the forest soil, represent potentially important amounts of nitrogen. This nitrogen, however, is not readily available for other plants. Instead, it must undergo some other processes in order to be ready for absorption by other plants.

Through the process of mineralization, organic nitrogen is converted into ammonium (NH_4). Moreover, some ammonium is further converted into nitrate (NO_3) during other microbial process called nitrification. Both forms of nitrogen, ammonium and nitrate are suitable for absorption by plants, although they may also be lost to the atmosphere (ammonium) or leached to deeper horizons in soil (nitrate), especially in rainy regions (Pritchett 1986). NO_3 may also be returned to the atmosphere as N_2, through the microbiological process called denitrification. During this process, some other nitrogen oxides such as NO, NO_2 and, N_2O (Finck 1988), which are collectively called NOx may also be released to the atmosphere.

The factors affecting the rates of nitrogen fixation are not well known but, in general, the factors improving the growth and productivity of the host trees affect their rate of nitrogen fixation (Binkley 1993, Binkley *et al.* 1994). A high photosynthetic rate of host plants is especially necessary to provide the energy needed for the process of fixation to take place (Schlesinger 1997). This means that alder trees in a mixed stand, as long as possible, should be free of competition for resources other than nitrogen, including water and light. This implies that in such mixed stands, as the proportion of alder increases, nitrogen fixation also increases since more resources in the site are used by this species. This in turn may result in an increased availability of N for pine trees, however, an increased competition for other resources is likely to appear (Binkley and Giardina 1997), which may become limiting for pine growth.

According to this rationale, the more alder trees in an area, the more nitrogen is fixed. If the appropriate conditions for mineralization are present as would be expected in young stands (Smith *et al.* 1997), the nitrogen fixed may be available for pine trees which, according to Liebig's limiting factor law (Pritchett 1986), should respond in growth if N was the former limiting factor. However, if so many alder trees are established per unit ground area, it is expected that these trees to compete with the pine trees for resources other than N, possibly resulting in slow growth of pine trees (Binkley and Giardina 1997, García-Montiel and Binkley 1998) and accumulation of nitrogen in pine tissues.

MATERIALS AND METHODS

Location of the study area

This research was carried out in the municipality of Santiago Tulantepec, Hidalgo, Mexico, in areas regenerated during 1987, 1989, and 1994. The area regenerated in 1994 is in a private forest while the areas regenerated in 1987 and 1989 belong to the ejido Los Romeros, Santiago Tulantepec (Figure 1.1). The region is located between 20° 00′ and 20° 04′ latitude north and between 98° 25′ and 98° 28′ longitude west.

Physiography and climate

According to Aldrete (1990), the average elevation in the zone is around 2650 m above sea level with flat topography and a mean slope of 10 %. The annual precipitation in the zone varies from 600 to 1000 mm (Aldrete 1990). This precipitation predominantly occurs during the period from May through September, but some precipitation may take place even in October and November. The mean annual temperature is around 15 °C (Aldrete 1990).

Vegetation

The dominant tree species in both the ejido and the private forest is *Pinus montezumae* Lamb., but some individuals of *P. patula* Schl. *et* Cham., *P. leiophylla,* and

P. teocote may be found in some sites. Among the main shrub species and broad-leaved trees are *Alnus arguta* (Schl.) Spach. (alder) and some *Quercus* species such as *Q. rugosa* and *Q. laurina* (UCODEFO No. 1, 1996).

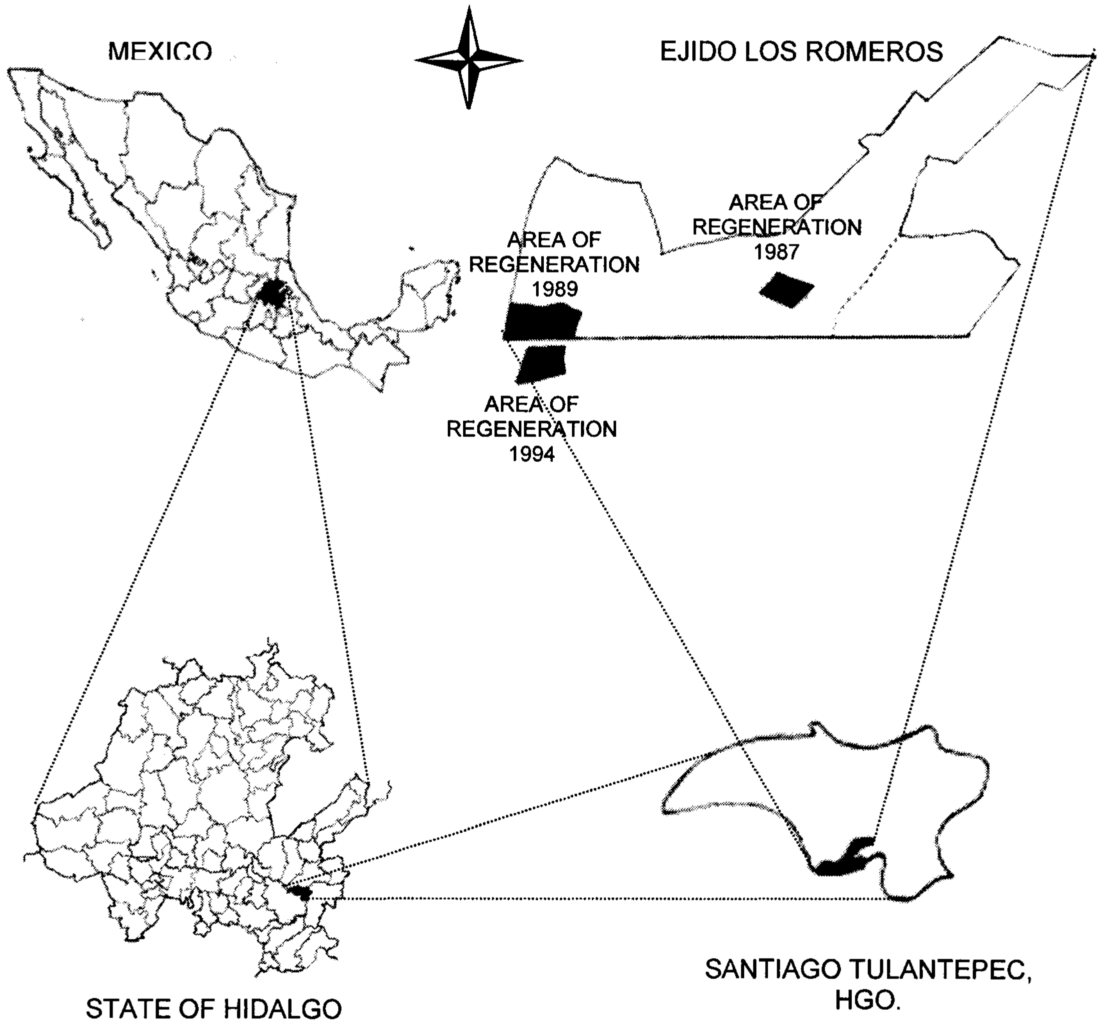


Figure 1.1. Location of the study sites.

Study area management history

In all regeneration areas, alders were eliminated at the time of regeneration. In the areas 1987 and 1989, after the regeneration treatments (seed trees), mechanical control of alders was carried out annually until 1991 to allow pine seedlings to become established. From 1992 to 2000 no control of alders was done, except in 1998. A forest fire in the area 1989 eliminated most alders and many pine trees in March 2001.

In the 1994 (private forest) regeneration area, after the clearcutting was carried out, the alders were mechanically controlled and in 1995 the plantation activities were initiated. After 1995, no control of alders was carried out until March 2001, when a fire spread along the area and bured most alders.

Experimental design

Three separate experiments were carried out corresponding to each of the regeneration areas. The experimental design in all the regeneration areas was a randomized complete block design. Five treatments corresponding to pine/alder ratios were tested and the blocks or replicates corresponded to six (four in the area 1994) different levels of pine tree density. The inclusion of pine density (i.e. number of pine trees per plot) was due to the difficulty in precisely replicating the treatments. The pine/alder ratios and pine densities corresponding to the treatments and blocks are shown in Appendix 1.

The experimental unit was a 15 X 15-m plot. The plots were selected based primarily on the visual appreciation that they had around 65 trees per plot for the regeneration areas in the Los Romeros ejido. In the private forest 1994, the regeneration plots were selected on a systematic basis since regeneration in this area was artificially established and consequently, the number of seedlings per plot is nearly constant.

In the ejido plots, once we determined that the site met the condition concerning pine density, the plot was established by using a compass and a 50-m tape. Wood stakes were put in each corner of the plots. To determine the precise pane/alder ratio (PAR) in the plot, the plot was divided into three sections by using ropes and the amount of pine trees and alders in each section was determined.

By using this procedure, a total of 60 plots were established in each of the ejido regeneration areas. The area in the private forest (area of regeneration 1994) was sufficient to establish just 45 plots.

After the plots were established and the PARs determined, the required PAR series were selected. For doing this, all of the plots in a regeneration area were divided into two sets according to the amount of pine trees per plot. This was done to reduce the variance of pine density among plots. From the whole number of plots, just 30 plots were selected in the ejido areas and 25 plots in the private forest area. For each of the regeneration areas, the selected plots were sorted in descending order according to the pine/alder ratio and the set was divided into five PAR levels (treatments).

In the ejido regeneration areas, each treatment consisted of six replicates or blocks (pine densities). In the other regeneration area each treatment had just four replications per treatment.

Variables measured

Tree biomass and leaf area

Regression models were developed to estimate pine and alder leaf areas since this variable would be used in several stages of the various experiments. At the time of collecting the data for construction of the leaf area models, data was also collected to develop stem (ejido areas) or wood (including branch wood, area 1994) biomass equations. Nine (ejido areas of regeneration) or eight (area of regeneration 1994) pine trees of varying size were cut. Diameter was measured at breast height (DBH, ejido areas) or at the root collar level (area of regeneration 1994). Foliage was manually removed and weighed using a scale (10 g in precision). Eight needle-and-wood samples of varying size were taken from each of the trees and returned to the laboratory where fresh weight was determined (scale 1/10000 in precision), and then oven dried (70 °C until constant weight) and their dry weight determined using the same scale.

Pine Growth

Pine growth was determined in each of the plots. Data used in this process included total tree height, periodic tree height, breast height diameter (DBH), age, and bole biomass. Tree height was measured using a telescopic ruler. When trees were taller than 6.5 m, a suunto clinometer was used to measure tree height.

Since most measurements were carried out during the growing season, tree height was measured from tree base to the last whorl, ignoring the current growth. This was done to eliminate the influence of date of measurement.

Pine tree age was estimated based on the number of whorls. *P. montezumae* is a species with preformed growth (Aldana and Aureoles 1991); that is, it produces just one growth flush per year , so that one whorl corresponds to just one year of age. Tree age consisted of the total number of whorls. A few times the lowest whorls were undetectable (they were covered by bark), but fortunately *P. montezumae* frequently produces some fascicles exactly in the places where old whorls are, making it easier to find the whorls. When the lowest whorl was higher than 20 cm from the soil surface, one additional whorl was supposed to be below that one. This was done because it was observed that most small trees had one whorl before reaching 20 cm in height.

Bole biomass was estimated from DBH or root collar diameter using the equations discussed previously.

Data analysis

Just dominant trees were considered in this analysis since they are the ones that may show responsiveness to any site quality change (Daniel *et al.* 1982, Arteaga 1983) exerted by alder.

In the case of the ejido plots, dominant trees were selected as the 25 % of the tallest trees that had regenerated following the regeneration treatments. In the 1994 (private forest) regeneration area, competition among pine trees is very likely to be absent since they are very young. Small trees (height lower than percentile 25) were filtered out from our database since these trees are likely to have been suppressed during some stage of their life by alder trees, grasses or any other plant species.

Pine trees of species other than *P. montezumae* were also filtered out from our database since they may have different growth rates than *P. montezumae*. Nonetheless, it should be mentioned that the presence of pine species other than *P. montezumae* is rather uncommon in our areas of regeneration.

The effects of site (area of regeneration), PAR level, and the interaction of these factors on growth variables, were analyzed using an analysis of variance. Orthogonal contrast analysis was used to compare pine/alder-level groups within an area of regeneration. That is, the lowest + highest PARs were grouped and contrasted against the other group formed by the three intermediate PARs. This was done to verify the quadratic behavior of the growth variables.

Regression equations were generated for each of the variables studied, with PAR as the independent variable. When quadratic curves were obtained, maximum values for the variables were calculated from the equations of the curves by getting the first derivates of each of the variables with respect to PAR, equaling them to zero and solving for PAR:

$$\frac{d\,(\text{Growth variable})}{d\,(\text{Pine/alder ratio})} = 0$$

Statistical analyses included in all experiments were performed using SAS (Statistical Analysis System, V 6.12). Graphs were built in Excel 2000.

RESULTS

Tree biomass and leaf area models

Based on fresh and dry weight of needles and wood, total tree leaf and stem or wood biomass were estimated and related to diameter at breast height according to the model recommended by Garcidueñas *et al.* (1988) to estimate montezuma pine leaf and stem biomass. The following equations resulted from this process:

$NDW = 0.0088*DBH^{2.0809}$, $R^2 = 0.8172$, RMSE = 0.2775, N = 9

where:

NDW = Needle dry weight (Kg)

DBH = Diameter at breast height (cm)

and

$SB = 1.7759\, e^{0.1497DBH}$, $R^2 = 0.8696$, RMSE = 0.1569, N = 9

where:

SB = Stem biomass (Kg)

Since trees in the area of regeneration 1994 were so small that they did not have a DBH, another equation was developed to estimate pine leaf area and wood biomass from diameter at the root collar (RCD). The methodological procedures and equipment used in this case were the same as those used for big trees. The models that resulted from this process are shown below:

$NDW = 0.1882\ RCD^{3.9449}$, $R^2 = 0.9275$, RMSE = 0.2130, N = 8

where:

NDW = Needle dry weight (g)

RCD = Root Collar Diameter (cm)

and

$WB = 0.0076444\ RCD^{2.562}$, $R^2 = 0.9446$, RMSE = 0.1601, N = 6

where:

WB = Wood biomass (Kg)

Needle area of 20 needle groups of varying size was estimated using the formula developed by Johnson (1984). Dry weight of the needle groups was also determined by using a scale (1/10000 g in precision), and a regression model was developed to estimate needle area as a function of needle dry weight.

The Johnson (1984) equation is:

$$A=2L(1+(\pi/n))\ (V_n/\pi L)^{1/2}$$

where:

A = Total surface area (cm^2)

V_n = Displaced volume of the total needle sample using Archimedes principle (cm^3)

L = Cumulative needle length in the sample (cm)

n = Number of needles per fascicle

The model for pine needle area estimation from needle dry weight was:

$$PLA = (0.0073963 * NDW) - 0.00014055, R^2 = 0.9685, RMSE = 3.1562, N = 10$$

where:

PLA = Pine needle area (m^2)

NDW = Needle dry weight (g)

Two models were also developed to estimate alder leaf area. The first model was used to estimate alder leaf area from shoot basal diameter. The other model was needed to estimate alder leaf area in the areas of regeneration that were burnt. In this model, number of alders per plot was used as the independent variable for estimating alder leaf area (ALA).

Such models are:

$$ALA = 0.19113 * SD^{1.5392536}, R^2 = 0.9168, RMSE = 0.2748, N = 33$$

where:

ALA = Alder leaf area (m^2)

SD = Sprout collar diameter (cm)

and,

$ALA = 3.9435 + 1.7486\ NA$, $R^2 = 0.7138$, $RMSE = 0.1723$, $N = 12$

where:

ALA = Alder leaf area (m^2)

NA = Number of alders per plot

Pine growth

In the areas of regeneration 1987 and 1994, most variables show increasing values as the proportion of alders in the plots goes up [decreasing pine/alder ratios (PARs), Figure 1.2a-e and k-o, respectively]. In the area of regeneration 1989 (Figure 1.2f-j), though a higher variation was found in comparison to the other areas of regeneration, it appears that there exists a trend showing increased values for all growth variables with increasing density of alders.

On the other hand, the trends of the curves in the areas 1987 and 1994 show a quadratic shape, which seems to be well defined in the area 1987. That is, pine growth increases with increasing the density of alders within the plot and, after reaching a peak, a reduction in growth takes place when the proportion of alders is too high. The values for the PAR that maximize pine growth vary among the areas of regeneration. As an average, in the area 1987, such PAR (LA/LA) is around 11.16 (Table 1.1) for all growth variables (considering 60 pine trees per plot with 20 cm DBH, this ratio equals 2,666 pine stems ha^{-1} / 20 alder trees ha^{-1}, see appendix 5 for calculations), while in the area 1994

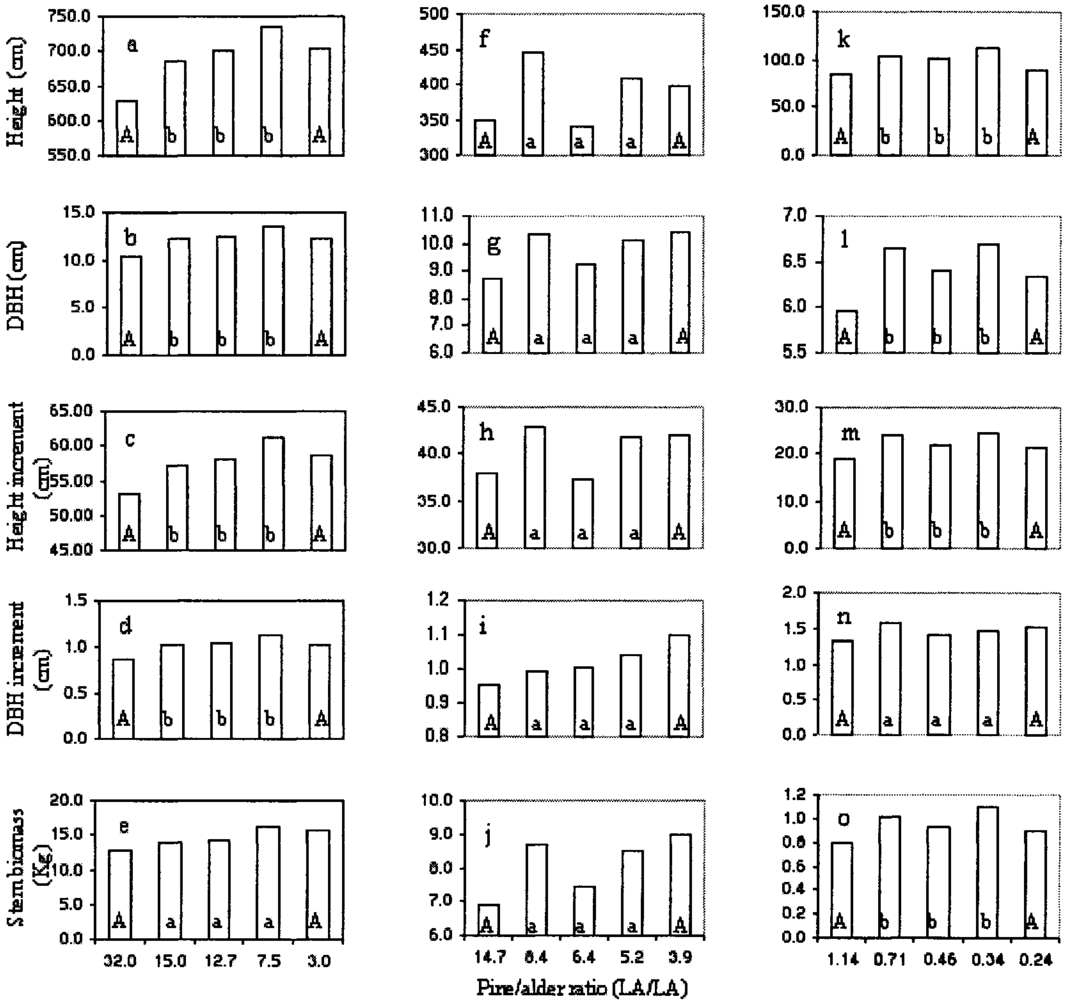


Figure 1.2. Behavior of pine growth variables with varying pine/alder ratios (LA/LA), from the areas of regeneration 1987 (left column), 1989 (central column), and 1994 (right column). A bar represents the average of six (regeneration area 1987), five (regeneration area 1989), and four plots (regeneration area 1994). Different-case letters were statistically compared for a variable within a regeneration area. Different letters between the case-groups compared indicate groups are statistically different at the 0.05 level of significance.

it is around 0.57 (considering an average of 16 pine trees per plot with a root collar diameter of 9 cm, this ratio equals 711 pine trees ha^{-1} / 267 alders ha^{-1}, see appendix 5 for calculations).

Table 1.1. Pine/alder ratios (PAR, LA/LA) that maximize the growth variables in the areas of regeneration 1987 and 1994. N = 5.

AREA	**MODEL**	**R^2**	**RMSE**	**Optimum PAR**
1987	$H = -0.192PAR^2 + 4.3285\ PAR + 687.39$	0.9700	8.6732	11.27
1994	$H = -71.373PAR^2 + 86.005PAR + 79.442$	0.5670	10.0083	0.60
1987	$DBH = 0-.0054\ PAR^2 + 0.1177\ PAR + 12.103$	0.8895	0.5076	10.90
1994	$RCD = -1.9799PAR^2 + 2.2579PAR + 5.968$	0.7606	0.2028	0.57
1987	$HI = -0.0138\ PAR^2 + 0.2994\ PAR + 57.616$	0.9839	0.4746	10.85
1994	$HI = -15.778PAR^2 + 18.351\ PAR + 18.457$	0.7055	1.7775	0.58
1987	$DBHI = -0.0004\ PAR^2 + 0.0093\ PAR + 1.0108$	0.8287	0.0524	11.63
1994	$RCDI = -0.46449\ PAR^2 + .4816\ PAR + 1.3831$	0.4663	0.1113	0.52
1987	*			
1994	$SB = -0.6466\ PAR^2 + .7236\ PAR + 0.8082$	0.5883	0.1030	0.56
1987	*AVERAGE*			*11.16*
1994	*AVERAGE*			*0.57*

H=Pine height (cm), DBH=Diameter at breast height (cm), RCD=Root Collar Diameter (cm), HI=Height increment (cm), DBHI=Diameter at breast height increment (cm), RCDI=Root Collar Diameter Increment (cm), SB=Stem biomass (Kg), *No quadratic curve.

The analysis of variance for all three areas of regeneration indicated that the effect of the site (area of regeneration) is significant (p-value=0.0001) for the three growth variables considered in this analysis (total height, height increment, and stem biomass; diameters were not analyzed since two areas used DBH and the other area of regeneration used root collar diameter). The effects of PAR level (LA/LA) for pine height and height increment were also significant (p-value=0.0078 and p-value=0.0012, respectively), but this independent variable did not significantly affect stem biomass (p-value=0.4456).

Table 1.2. Growth variable mean values for the PAR groups contrasted in all regeneration areas.

Variable	PAR group	Area 1987	Area 1989	Area 1994
Height (cm)	L1+L5	666.300	373.338	87.355
	L2+L3+L4	707.800	398.556	105.152
Diameter (cm)	L1+L5	11.300	9.579	6.152
	L2+L3+L4	12.700	9.875	6.679
Height Increment (cm yr^{-1})	L1+L5	55.850	39.924	20.167
	L2+L3+L4	58.970	40.675	23.508
Diameter increment(cm yr^{-1})	L1+L5	0.942	1.027	1.422
	L2+L3+L4	1.061	1.014	1.483
Stem biomass (Kg)	L1+L5	14.104	7.935	0.845
	L2+L3+L4	14.741	8.195	1.010

L1-L5 = Pine/alder ratio levels 1 through 5

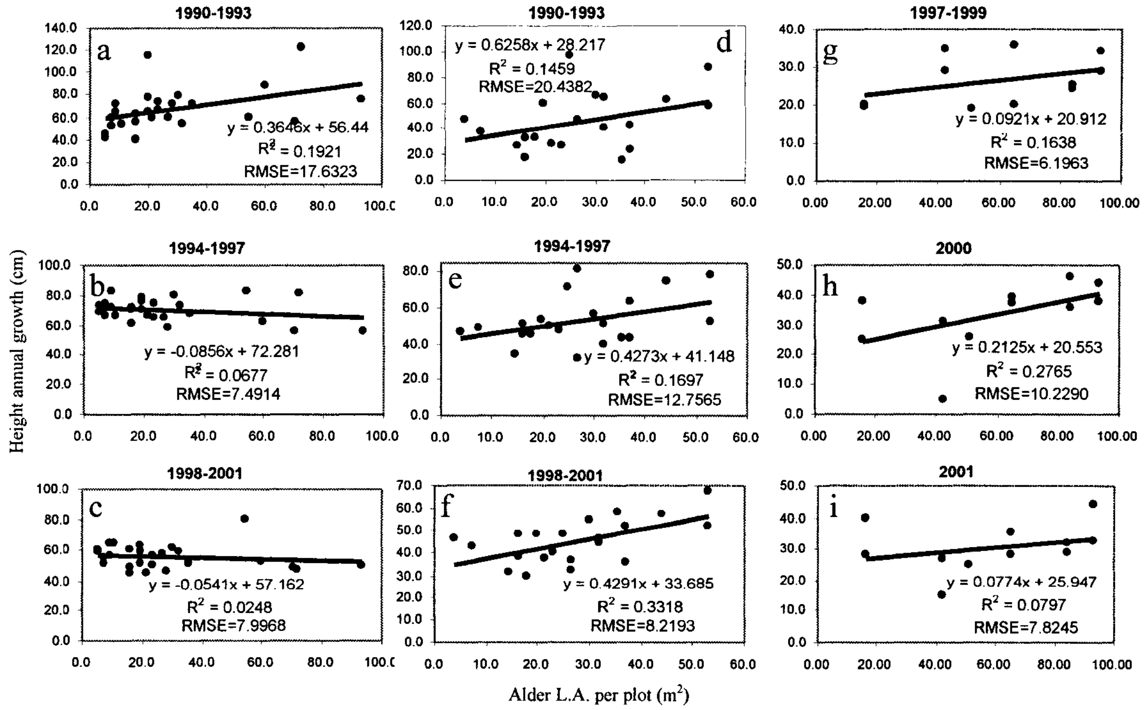


Figure 1.3. Mean height periodic growth with varying pine/alder ratios in the areas of regeneration 1987 (a-c), 1989 (d-f), and 1994 (g-i).

The effect of the interaction between site (area of regeneration) and pine/alder level was not significant for pine height p-value=0.2328), height increment (p-value=0.6542), and stem biomass (p-value=0.9928).

The orthogonal contrast analysis indicated that the pine/alder groups compared (lowest + highest alder versus the sum of the intermediate alder levels) were significantly different for all variables (except stem biomass) in the area of regeneration 1987 (Figure 1.2a-e), being the values from intermediate PARs always higher than those from extreme PARs (height p-value=0.0431, diameter p-value=0.0138, height increment p-value=0.0410, diameter increment p-value=0.0242, and stem biomass p-value=0.7095; Table 1.2).

In the area of regeneration 1994, intermediate pine/alder levels also significantly increased pine height (p-value=0.0023), diameter (p-value=0.0168), height increment (p-value=0.0098), and stem biomass (p-value=0.0160) with respect to extreme PARs (Figure 1.2k-o, Table 1.2).

The effects of alder density on pine growth (height) varied with age. That is especially true for the area 1987, where higher alder densities increased pine height (p-value=0.025) just when pines were 3-6 years of age (Figure 1.3). As pine trees became older, the effect of alder on pine height disappeared. In the area 1989, on the other hand, the effect of alder has been significant and positive during all pine development stages. Regarding the area 1994, alder effects have also been positive during all development stages. However, in this case such effects are not significant (Figure 1.3g-i).

DISCUSSION

Our results concerning the effects of varying pine/alder ratios (PARs) on pine growth indicate that low-to-intermediate densities of alder intermixed with pine trees have positive effects on most growth variables measured in this study (Figure 1.2). In fact, practically all growth variables increase within the PAR range from highest to intermediate indicating that there is a relationship between pine and alder tree density that generally ends up in an advantageous condition for pine trees. Even though there are several reports with similar findings regarding the effects of N-fixing species on crop-tree species growth (Binkley 1983, Dawson *et al.* 1983, Binkley *et al.* 1992, Rothe and Binkley 2001, Tiwari *et al.* 2003), especially referring to North American species, our work suggests several ideas regarding how changes in species densities and sizes may affect their relationships and final effects on the crop species growth rates.

One of our work's suggestions has to do with the shape of the growth response curve. The results indicate that most growth variables measured behaved according to a quadratic curve in the areas of regeneration 1987 and 1994, as judged by the orthogonal contrast analyses. This behavior implies that there exists a PAR that optimizes pine growth and studies should be conducted on a local and temporal basis to precisely determine such PARs, since the optimum PAR greatly varies depending on pine stand age and density, and probably soil fertility among other characteristics (Binkley 1983).

The conversion of the optimum PARs for our areas of regeneration into number of alders per hectare (according to the average stand density, appendix 5) resulted in very divergent values for optimum alder densities between the areas of regeneration 1987 and 1994 (20 Vs. 267 alders ha^{-1} for the areas of regeneration 1987 and 1994, respectively). In some sense, the low alder density in the area of regeneration 1987 may be a reflection of the role alders are playing in that system. Figure 1.3 indicates that alders in this area of regeneration behaved more like a weed shrubs since 1993. In turn, this negative effect of alders in this area of regeneration may be due to the high pine density in this area of regeneration, coupled with the mechanical control of alder growth, which probably caused pine trees to overtop alders by that year, thus reducing alder performance by decreasing the amount of radiation reaching alders.

In contrast, the relatively high alder density that optimizes pine growth in the area of regeneration 1994 indicates that alders have maintained high N-fixation rates and this positive effect dominates over the possible negative effects alders have in the systems as they compete with crop trees for resources other than N (Fisher and Binkley 2000). This behavior of alders should be expected given that the biological N-fixation process is very energy-demanding (Binkley *et al.* 1994) and alders in this area of regeneration are fully exposed to sun.

The maintenance of high N-fixation rates by alder in the area of regeneration 1994 is also supported by the trends of the curves shown in Figure 1.3g-i, which reveals that positive effects of alder on pine growth have dominated over the negative ones along the stand's life.

The curves for pine growth in the area of regeneration 1989 (Figure 1.2f-j) are much more irregular than those in the other areas of regeneration. Even so, it looks like alders had just positive effects on pine growth and that the optimum PARs were not reached in this area of regeneration even though alder densities in this site are quite similar to those in the area of regeneration 1987 (12.9 Vs. 16.6 alders plot^{-1} for the areas of regeneration 1989 and 1987, respectively, appendix 1). The positive effects of alder on pine growth is confirmed by the plots in Figure 1.3d-f, which shows that alders in this area of regeneration have had positive effects on pine growth along the stand's lifespan.

One of the most remarkable differences between stands in the areas of regeneration 1989 and 1987 deals with pine density (31.4 Vs. 56.8 pine trees plot^{-1} for the areas of regeneration 1989 and 1987, respectively, appendix 1). This difference may be determining the role of alders (as beneficial or weed shrubs) in each of the areas of regeneration since the high pine density (and high pine leaf area) in the area of regeneration 1987 may be restricting light reception by alder and, consequently, limiting the N-fixation process as found by Heilman and Stettler (1983, cited by Binkley *et al.* 1994), and Shainsky and Rose (1995).

It is well documented in the literature that most alder species are highly light demanding. Harrington (1990) points out that red alder requires more light than any of the tree species found in association with it. Full sunlight is required for good growth of red alder to be achieved (Hibbs 1996). Heilman and Stettler (1983, cited by Binkley *et al.* 1994) found that red alder trees with intermediate (partially shaded from above) canopies had half the nodule biomass of trees with codominant or dominant (full sunshine) canopies, and the lower nodule biomass was accompanied by a lower rate of acetylene reduction per unit of nodule weight.

In light of the above, our work suggests that alder's shade intolerance may turn alder's function in pine-alder stands from beneficial to detrimental if pine density is not adequately controlled to avoid excessive shading over alder trees. Therefore, studies on radiation reaching understory alders should be periodically carried out if maximum pine growth is to be pursued in pine-alder stands.

The changing behavior of alder (from beneficial to detrimental) in the area of regeneration 1987 (Figure 1.3a-c) indicates that the increase in growth occurring when low-to-intermediate alder densities take place (Figure 1.2a-e) is a reflection of the effects of alder before 1993. If the positive effect shown in Figure 1.3a-c did not occur, inverse curves would be expected (the more the alder density the lower the pine growth) instead of the curves shown in Figure 1.3a-e. In turn, this implies that the maximum pine growth reached according to Figure 1.2a-e (which certainly is significantly higher than that obtained with lowest+highest alder density for most variables) is far below the potential growth obtainable with appropriate alder-and-pine density management strategies tending to get always positive effects of alders.

According to the volume equation developed by Rodriguez and Moreno (1982) for *P. montezumae*, the volume of a tree with the dimensions produced in the plots with the lowest density of alders in the area of regeneration 1987 would be 0.039 m^3. On the other hand, the volume of a tree from the optimal PAR (11.16) would be 0.073 m^3. If we consider a mean pine density of 57 stems per plot (2533 stems per hectare) at age 13, then the differences in size of trees attributable to differences in PARs result in a difference in volume of wood of 86.1 m^3 per hectare. From an economical viewpoint this difference is very important, considering that the price of wood in Mexico is around $ 90.00 U.S. dollars per cubic meter. The extra volume produced as a result of appropriate PARs is also important from the viewpoint of carbon sequestration. Nowadays, one option to decrease CO_2 concentration in the atmosphere is thought to be plantation of forests. However, in order to improve carbon sequestration by these artificial forests, their nutrient status must be optimal and nitrogen is one of the nutrients that most frequently may limit carbon intake by trees (Pritchett 1986).

CONCLUSIONS

The shape of the curves describing pine growth variables as a function of PAR levels (Figure 1.2) corresponds to a quadratic function, indicating that the beneficial effects of alder outweighed the competition effects in alder levels lower than optimum. On the other hand, competition effects from alder became dominant over beneficial effects when the proportion of alder was higher than optimum.

The PARs that optimize pine growth are 11.16 and 0.57 [PARs (number of pine trees per ha/number of alder trees per ha)= 2,666/20 and 711/267] for the areas of regeneration 1987 and 1994, respectively. Maximum pine growth was not reached with the alder densities occurring in the area of regeneration 1989.

The effects of alder on pine growth in pine-alder ecosystems may go from beneficial to detrimental. Our results suggest that beneficial effects of alders on pine growth occurred during the first six or seven years following the regeneration treatment in the area of regeneration 1987. After that time, alders had negative effects impacts on pine growth as they became weed shrubs.

Light limitations for the alder N-fixation process are likely to be responsible for the change of alder's effects from beneficial to detrimental. I would recommend that more detailed studies be carried out to determine the limiting factor for the N-fixation process by alder.

LITERATURE CITED

Aldrete, A. 1990. Evaluación de la regeneración natural de *Pinus montezumae* Lamb. En áreas tratadas con el método de árboles padres. Master of Science Thesis. Programa Forestal, Colegio de Postgraduados. Montecillo, Estado de Mexico. 95 p.

Aldana, B. R., and S. Aureoles C. 1991. Fenología de brotes anuales de *Pinus hartwegii* Lindl. y *Pinus montezumae* Lamb. y su relación con los factores ambientales físicos, en la Estación Forestal Experimental Zoquiapan, México. Bachelor Thesis. Universidad Autónoma Chapingo, Mexico. 80 p.

Arteaga M. B. 1983. Influencia del suelo y las características fisiográficas en el crecimiento de *Pinus radiata* D. Don en Ayotoxtla, Gro. Bachelor Thesis. Universidad Autónoma Chapingo. Chapingo, Mexico.146 p.

Beer , J. 1980. *Alnus acuminata* con pasto. Informe CATIE, Costa Rica. 5 p.

Binkley, D. 1983. Ecosystem production in Douglas-fir plantations: Interactions of red alder and site fertility. *For. Ecol. and Manag.* 5: 215-227.

Binkley, D. 1993. Nutrición forestal: prácticas de manejo. Translation from English by Manuel Guzmán Ortíz. Limusa. México. 340 p.

Binkley, D., R. Bell, and P. Sollins. 1992. Comparison of methods for estimating soil nitrogen transformations in adjacent conifer and alder-conifer forests. *Can. J. For. Res.* 22: 858-863.

Binkley, D. and C. Giardina, 1997. Nitrogen fixation in tropical forest plantations. *In*: Nambiar E.K.S. and A. G. Brown. Management of soil, nutrients and water in tropical plantation forests. ACIAR Monograph No. 43. Pp. 297-337.

Binkley, D., K. Cromack, Jr., and D. D. Baker. 1994. Nitrogen fixation by alder: Biology, rates, and controls. *In*: Hibbs, D., D. DeBell, and R. Tarrant. The biology and management of red alder. Oregon State University Press., Corvallis,OR. Pp. 57-72.

Daniel, P. W., U. E. Helms, and F. S. Baker. 1982. Principios de silvicultura. 2nd ed. Translation from English by: Elizondo, M. R. McGraw Hill. Mexico. 492 p.

Dawson, J. O., P. J. Dzialowy, G. Z. Gertner, and E. A. Hansen. 1983. Changes in soil nitrogen concentration around *Alnus glutinosa* in a mixed, short-rotation plantation with hybrid *Populus*. *Can. J. For. Res.* 13:572-576.

Finck, A. 1988. Fertilizantes y fertilización. Ed. Reverte. Barcelona. 439 p.

Fisher, R. F., and D. Binkley. 2000. Ecology and management of forest soils. 3rd. ed. Wiley. USA. 489 p.

García-Montiel D. C. y D. Binkley, 1998. Effect of *Eucalyptus saligna* and *Albizia falcataria* on soil processes and nitrogen supply in Hawaii. *Oecologia.* 113: 547-556 p.

Garcidueñas M. A. R., M. R. Keyes, G. P. Zárate de Lara, and M. R. Martínez M. 1988. Estimación de biomasa para árboles de *Pinus montezumae* Lamb. *Agrociencia* Num. 72.

Gutierrez, G. B. 1998. Efecto de la asociación de *Pinus patula* con *Alnus firmifolia*. M. C. Thesis. Colegio de Postgraduados. Montecillo, Texcoco, Edo. de Mexico. 96 p.

Harrington, C. A. 1990. *Alnus rubra* Bong.- red alder. *In*: Silvics of North America, Vol. 2. Hardwoods. Coordinated by Burns, R. M. and B. H. Honkala. USDA Forest Service. Agric. Handbook. 654. Washington, D. C. pp.116-123.

Hibbs, D. E. 1996. Managing red alder. The woodland workbook EC1197. Oregon State University Extension Service. 8 p.

Johnson, J. D. 1984. A rapid technique for estimating total surface area of pine needles. *For. Sci.* 30: 913-921.

Pritchett, W. L. 1986. Suelos forestales: propiedades, conservación y mejoramiento. Translation from English by Jose Hurtado Vega. Limusa. Mexico. 634 p.

Rodríguez F. C. and R. Moreno S. 1982. Elaboración de tablas de volúmenes a través de análisis troncales para *Pinus montezumae* Lamb. en el Campo Experimental Forestal San Juán Tetla, Puebla. Boletín Técnico # 90. INIF, Mexico. 37 p.

Rothe, A. abd D. Binkley. 2001. Nutritional interactions in mixed species forests: a synthesis. *Can. J. For. Res.* 31:1855-1870.

Schlesinger, W. H. 1997. Biogeochemistry: An analysis of global change. 2nd Ed. Academic press. U. S. A. 588 P.

Shainsky, L. J. and C. L. Rose. 1995. Effects of competition on the foliar chemistry of young Douglas-fir in monoculture and mixed stands with young red alder. *Can. J. For. Res.* 25:1969-1977.

Silvester, W. B. 1983. Analysis of nitrogen fixation. *In:* Gordon, G. C. and C. T. Wheeler (Eds). Biological nitrogen fixation in forest ecosystems. Foundations and applications. Martinus Nijhoff/Dr. W. Junk publishers. pp. 173-212.

Smith, D. M., B. C. Larson, M. J. Kelty, and P. M. S. Ashtom. 1997. The practice of silviculture: applied forest ecology. 9th ed. Wiley and Sons, Inc. U.S. A. 537 p.

Tiwari, M., S. P. Singh, A. Tiwari, and R. C. Sundriyal. 2003. Effect of symbiotic associations on growth of host *Coriaria nepalensis* and its facilitative impact on oak and pine seedlings in Central Himalaya. *For. Ecol. Manage.* 184:141-147.

UCODEFO No. 1. 1996. Estudio dasonómico del ejido Los Romeros, Municipio de Santiago Tulantepec, Hidalgo. (Unpublished).

CHAPTER 2

PINE NUTRIENT STATUS AS AFFECTED BY ALDER IN YOUNG MIXED STANDS

ABSTRACT

A field study was carried out in the state of Hidalgo, Mexico to determine how varying pine/alder ratios PARs) affect the nitrogen (N), phosphorus (P), and potassium (K) status in pine needles. Five PARs were selected at each of two areas of regeneration (1987 and 1994) and needle samples collected and analyzed for N, P, and K. The results show that even the lowest proportion of alder had negative effects on pine growth and pine N status in the area of regeneration 1987, where alders are in the understory. This effect may have been due to light limitations to the N-fixation process. In contrast, pine N requirements were fully met and surpassed in the area of regeneration 1994, where alders were exposed to full sunlight.

P limitations to pine growth seemed to alternate with N limitations in the area of regeneration 1987. Availability of P, on the other hand, was improved by all alder densities in the regeneration area 1994. K status, as judged by its concentration in pine needles, was improved in both areas of regeneration with increasing proportion of alder.

However, it looks like the increased K concentration in pine needles in the area of regeneration 1987 was the reflection of a concentration effect in pine needles instead of increased availability.

INTRODUCTION

Most plant species modify the properties of the soils where they grow (Binkley and Giardina 1998), thus affecting conditions for co-occurring or subsequently established species. Alder species are well known because of their ability to fix atmospheric nitrogen so that they frequently improve the soil nitrogen status. Nonetheless, because alder's effects are far to deal only with modification of soil nitrogen status, the changes in the status of many other nutrients most be comprehensively studied if improvement of growth of mixed or subsequently established crop species is pursued.

Moreover, even the single ability of alders to fix nitrogen needs to be better understood since there are many knowledge gaps regarding the environmental conditions such as the requirements for radiation, water, fertility, etc., for the nitrogen fixation process to take place.

The present work focuses on studying the effects alders have on pine nitrogen, phosphorus and potassium status in young pine-alder mixed stands in Central Mexico. Additionally, competition for nutrients when alders are found in high proportions in pine-alder stands are also studied in order to determine if nutrient deficiencies occur under these conditions.

REVIEW OF LITERATURE

Effects of alder on N, P, and K status of co-occurring plants

Alder species are able to associate with actinomycetes of the genus *Frankia* to convert molecular nitrogen into reactive forms of nitrogen such as NH_3 or NH_4, which are usable by plants (Hamdi 1985). On the other hand, nitrogen is the nutrient that most commonly limits plant growth (Schlesinger 1997). This combination of facts explains the various efforts that have been made in order to use alder species to improve nitrogen availability to species such as conifers.

Combinations of N-fixing species with crop species have been studied in both ways: species mixtures at a time and temporal species rotations. Provided that species mixtures are more frequently found in natural stands, species interactions in such mixed stands have been more intensively studied than species rotations.

Most of the research work regarding crop species*N-fixing species interactions indicate that N-fixing species generally (but not always) increase soil nitrogen and/or nitrogen status of associated plant species (Binkley 1983, Van Miegroet *et al.* 1990, Binkley *et al.*, 1992, Binkley and Giardina 1998, Kaye *et al.* 2000, Binkley *et al.* 2003). Although studies comparing fertile and infertile sites in terms of crops species and/or soil N status as affected by N-fixing species are rather scarce in the literature. The few existing documents indicate that nitrogen in both soil and non-N-fixing plant tissues are little altered by the presence of alders in fertile soils. This coincides with the generalized idea that N-fixation rates decline with increasing soil N status and also with the idea pointed out by Binkley and Giardina (1998) that N-fixing species reduce the suitability of the soil for supporting their own growth.

Light availability is another factor that may impair the N-fixation process. Like industrial N-fixation, biological N-fixation consumes high amounts of energy to dissociate the N_2 molecule (Brill 1977). While the energy for the industrial N-fixation comes from hydrocarbons, the energy for the biological N-fixation derives primarily from sunlight. This explains why alders are quite intolerant to shaded environments and require high inputs of light (Heilman and Stettler 1985; Harrington 1990, cited by Binkley *et al.* 1994).

Phosphorus nutrition is a key factor to N-fixation processes by N-fixing plants (Binkley *et al.* 1994). In their work in 1997, Binkley and Giardina described several experiments designed to determine responses of N-fixing plant species (mainly tropical ones) to phosphorus fertilization. Results from most experiments show positive responses of the N-fixing plants to the applied nutrient. Radwan (1987), on the other hand, fertilized red alder (*Alnus rubra*) seedlings with several nutrients and found that the greatest responses in growth were obtained when phosphorus was added. Binkley *et al.* (1994) mention an experiment they carried out in British Columbia, where they fertilized a red alder-Douglas fir stand with phosphorus and sulfur. These authors found that phosphorous fertilization allowed red alder seedlings to double in biomass and increase acetylene reduction activity per seedling by fivefold.

Soil phosphorus status is also generally altered by N-fixing species. However, variation of the effects of N-fixing plants on soil phosphorus status is much more variable than that shown on soil nitrogen status (Rothe and Binkley 2001). In fact, some authors have found improved soil P status by N-fixing plants while others have gotten the opposite results.

Zou *et al.* (1995) studied the effects of N-fixing species on soil P in two systems: *Alnus*-conifer and *Albizia-Eucalyptus*, including pure stands of all four taxonomic groups. They found that in both systems, the pure N-fixer and the species mixtures contributed to increase labile P. In this study, these authors suggest that interactions between *Alnus* and conifers, rather than *Alnus*' effect, triggered changes in P geochemical reactions.

Giardina *et al.* (1995) concluded that red alder appears to increase the availability of soil phosphorus in conifer forests of the Pacific Northwest, but they point out that increased P supplies may not prevent a P limitation on productivity for either alder or conifers.

Binkley *et al.* (1992) found that return of phosphorus in fine litterfall in alder-conifer stands in Washington was 1.5 times that in pure conifer stands. Comparatively, a similar study by the same authors in Oregon showed that the ratio alder-conifer/conifer was 2.1 for phosphorus, thus indicating higher phosphorus concentrations in the alder-conifer stands than in the pure conifer stands.

Binkley and Giardina (1997) found higher foliar phosphorus concentrations in *Eucalyptus saligna-Albizia falcataria* stands than in pure stands indicating a beneficial effect of the mixture regarding the availability of this nutrient. It is worth noticing that the effects of N-fixing plants on the nutrient status of foliage of co-occurring plants happens to become more complicated owing to the dilution or concentration effects resulting from changes in biomass production. A dilution effect may explain the reduction in foliar P concentration in Douglas-fir found by Binkley (1983) when Douglas-fir was mixed with red alder in infertile sites.

Shainsky and Rose (1995) carried out an experiment to study the effects of red alder on foliar chemistry of Douglas-fir. They found that increasing alder densities contributed to increase Douglas-fir P concentration when the conifer's density was low. At the highest alder density, percent P in conifer needles declined as Douglas-fir density increased.

In contrast to the positive effects of N-fixing plants on P availability, Binkley *et al.* (2000) found that P supply was about twice as high under *Eucalyptus* as under *Albizia* stands and the effects of mixtures were intermediate between the pure species effects.

Kaye *et al.* (2000) studied an *Albizia-Eucalyptus* replacement series and encountered that soil available P decreased with increasing percentage of *Albizia.* However, foliar P was higher in species mixtures than in monocultures thus suggesting a synergistic effect of species interactions.

In a study carried out in Washington by Van Miegroet *et al.* (1990), a decrease in Brays-extractable P was noted in soils under alder forest cover, suggesting a change in the soil P chemistry (and possibly availability) in the course of site alder occupancy.

The mechanisms at work to explain the effects of N-fixing plants on soil remain poorly understood to date. Several processes, however, have been proposed as possible explanations. Higher P requirements by N-fixing plants coupled with enhanced nutrient cycling because of annual leaf replacement of N-fixing plants (Van Miegroet *et al.* 1990) has been suggested as a possible mechanism. Zou *et al.* (1995) suggest that solubilization of inorganic P and mineralization of organic P are proceses that could be at work.

Solubilization would be enhanced by altered soil chemical characteristics such as pH and concentrations of organic chelates. On the other hand, differences in gross P mineralization rates can result from altering the chemical nature of soil organic matter and the activity of the soil enzymes as well as the dynamics of soil geochemical reactions through litter production.

The effects of N-fixing plants on K soil status have remained even less studied than those on P. Nonetheless, the few documents available indicate that K concentration in soils generally increase under the presence of alder. K concentration may reach so high levels in N-fixing foliage that Van Miegroet *et al.* (1990) suggest that when alder forest conversion is considered, branches should be left on site or harvesting delayed until after leaf fall in order to minimize the loss of base nutrients such as K. Binkley *et al.* (1992) reported a K ratio of 2.0 in organic matter between alder-conifer stands and pure conifer ones. Also, forest floor K was higher in mixed than in pure conifer stands in the same study. However, soil K at 0.0-0.9 m depth was lower in mixed than in the pure conifer stand.

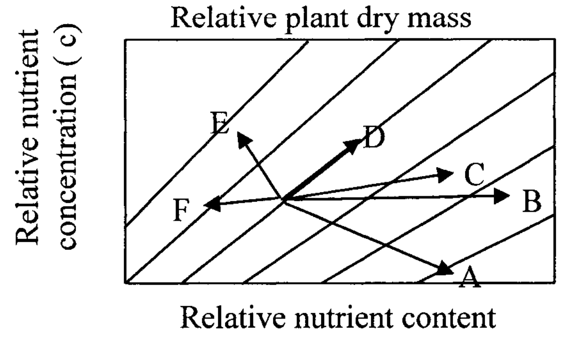
Aber and Melillo (1991) propose the so called cation pumping as one of the possible mechanisms involved in increasing availability of K and other cations by the presence of certain plant species with high requirements for macronutrients. N-fixing plants frequently have higher foliar cation concentrations than other wild non-N-fixing species, so that they could possibly be considered as cation-pumping species.

High nutrient concentrations of N-fixing plants, coupled with high recycling rates due to annual replacement of foliage (Van Miegroet *et al.* 1990) and improved humus quality (because of the low foliar C:N ratio) may be at work in the elevated K frequently found in plants spatially associated with N-fixing species.

Vector analysis technique for foliar analysis interpretation

A useful approach to studying the nutrition of forest species with fixed (determinate) growth is the vector analysis technique (Weetman 1989). This procedure for interpretation of plant nutrient shifts in response to silvicultural treatments (Haase and Rose 1995, Imo and Timmer 1997) is perfectly applicable in cases where treatments are expected to bring about changes in the nutrient status of one or more nutrients (Imo and Timmer 1997). The technique is based on the recognition of the fact that nutrient concentration in plant tissues is a function of nutrient content and dry mass, so that changes from an initial status (control plots) to a final status brought about by one or more treatments may be represented as vectors derived from nutrient concentrations, content and biomass of a fixed number of leaves or needles.

Nomograms may be constructed by plotting treatment values (nutrient content, concentration, and dry weight of a fixed number of needles; X-axis, Y-axis and dry weight isolines, respectively) relative to initial values from a control treatment, as initially proposed by Timmer and Stone (1978). This approach allows one to show several nutrients in a single nomogram. Original values can also be used instead of relative ones. However, in this case, just a nutrient per nomogram can be represented due to scaling differences among nutrients.



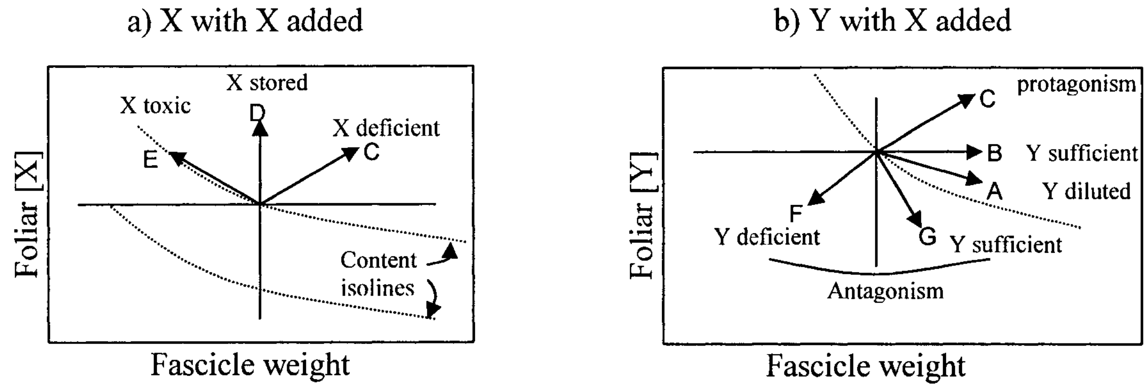
Vector	**Change in**					**Possible**
direction	**m**	**c**	**a**	**Interpretation**	**Change in Conc.**	**diagnosis**
A	+	-	+	Dilution	<0	Growth dilution
B	+	0	+	Sufficiency	=0	Steady state
C	+	+	+	Deficiency	<1, >0	Deficiency
D	0	+	+	Luxury	=1	Accumulation
E	-	++	±	Excess	>1	Toxic accumulation
F	-	-	-	Excess	<0	Antagonism

m=dry mass, c=nutrient concentration, and a=nutrient content

Figure 2.1. Possible directions and interpretation of vectors.

Valentine and Allen (1990) proposed a modification to the original technique, which consisted of plotting just fascicle mass and nutrient concentrations in the X and Y axis, respectively, while showing content-isoline hyperbolic curves.

Vector interpretation is based on shifts in the vector direction (orientation) and magnitude (length), observed as an increase, decrease or lack of change in biomass, nutrient content, and nutrient concentration relative to the initial plant status (Timmer and Stone 1978) as shown below.



a) Diagnoses based on effects of added nutrient (X) on its own status in the leaf.

Shift	Foliar [X]	Fascicle weight	Diagnosis
C	Increases	Increases	X is growth limiting
D	Increases	Constant	X is stored
E	Increases	Decreases	X is toxic

b) Diagnoses based on effects of added nutrient (X) on the foliar status of another nutrient (Y). The protagonism interpretation is ambiguous with regard to sufficiency or deficiency.

Shift	Foliar [Y]	Fascicle weight	Diagnosis (effect of X)
C	Increases	Increases	Y uptake enhanced
B	Constant	Increases	Y is sufficient
A	Decreases (Y content increases)	Increases	Y is sufficient and Y is diluted
G	Decreases (Y content increases)	Increases	Y is sufficient and Y uptake decreased
F	Decreases	Decreases	Y deficiency induced

Figure 2.2. Nomogram interpretation as proposed by Valentine and Allen (1990).

Valentine and Allen (1990) suggested that the nomogram interpretation should take into account not only the nutrient being analyzed, but also the nutrient applied in the treatments if any. For this purpose, these authors constructed a complementary table for nomogram interpretation (Figure 2.2).

MATERIALS AND METHODS

Location of the study area

The present study is a subset of the experiment from chapter 1. Instead of the three areas of regeneration studied in that chapter, this study used data from two regeneration areas (1987 and 1994, Figure 1.1). The area of regeneration 1989 was excluded from this study because a wildfire occurred before needle sampling was carried out. Details on physiography, climate, vegetation, and management history of the study areas were described in chapter 1.

Experimental approach

Plot distribution within each of the two areas of regeneration (1987 and 1994) was done according to a complete random design. Five pine/alder ratios (PARs) were tested in terms of their effects on pine ntrient status. Four replicates per PAR were included in the experiments. The plots selected for this study are marked (†) in appendix 1.

The experimental unit was the 15 X 15-m plot described in chapter 1. The plot selection procedure was described in the materials and methods (experimentsl design) section in chapter 1.

Needle sampling and handling

In every plot, three trees were selected at nearly the center of each of the plots. A sample of needles was taken from each of the trees during the winter 2000-2001. This sample was taken from an East-facing branch in the upper third of the tree crown as suggested by Wells and Allen (1985). The needle samples were put into plastic bags properly labeled, which were put into an icebox for transportation to the laboratory.

In the laboratory, the samples were double rinsed with tap water and distilled water to remove impurities. Thereafter, they were put into paper bags and oven dried at 70 °C for 48 h.

Variables measured

Dry weight of a fixed number of needles

Two sets of composite needle samples were formed in order to comply with the objectives pursued in this study. For the study of pine nutrient status as affected by PARs, just three plots per PAR were further selected (marked ‡ in appendix 1). The needles from all three trees within a plot from the area of regeneration 1987 were pooled and 150 needles were randomly chosen from each plot. Following a similar procedure, 300 needles were obtained from each of the three plots from the area of regeneration 1994. The composite samples were weighed by using a scale 1/10,000 g in precision.

For the study on competition for nutrients, four plots were selected (marked † in appendix 1) from each of the three lowest PARs (highest alder densities), except PAR 5 in the area of regeneration 1994, where just three plots were available. The samples from all three trees within a plot were pooled together and 225 needles were randomly chosen and weighed by using the scale previously mentioned.

Needle nutrient concentration and content

Due to financial limitations, needles from plots within a PAR were further composited for chemical analyses as with the study on nutrient status carried out by Imo and Timmer (1997). In the case of the nutrient competition study, the chemical analyses were done for each of the plots selected.

Chemical analyses were carried out in the Soil Fertility Laboratory at the Colegio de Postgraduados, Montecillo, State of Mexico. N, P, and K concentrations were determined for each of the composite samples. N was determined by the micro-kjeldahl procedure. P was determined colorimetrically by the vanadomolybdophosphoric yellow color method as described by Kalra and Maynard (1991). K was determined by flame spectrophotometry. From nutrient concentrations determined in the laboratory and dry weight, nutrient content was calculated for each of the composite samples (nutrient content = nutrient concentration * sample mass).

Data analysis

The data obtained was processed according to the graphical vector analysis method developed by Timmer and Morrow (1984) for determinations of nutrient status of plants. Graphs were done in EXCEL 2000.

RESULTS

All alder densities induced a decrease in needle dry weight and nitrogen content, and, with the exception of alder density 3 (A3), on nitrogen concentration in the area of regeneration 1987 (Figure 2.3a). According to the criteria defined by Valentine and Allen (1990) for the interpretation of Timmer's nomograms, the trends followed by most vectors in this graph can be interpreted as antagonistic relationships between alder and nitrogen status of pine trees.

Contrarily, most alder densities induced increased concentrations of phosphorus and potassium within pine needles (Figure 2.3b,c) but they still exerted negative effects on needle dry weight and nutrient content. Based on the criteria defined by Imo and Timmer (1997), the status for phosphorus and potassium should be interpreted as excess.

In the area of regeneration 1994 (Figure 2.3d-f), uptake of nitrogen and potassium increased as a result of the increased alder densities according to the criteria proposed by Valentine and Allen (op. cit.), such that even luxury consumption happened to occur (Figure 2.3f, vector A4, Weetman 1989, Imo and Timmer 1997). All alder densities induced a sufficiency level of pine needle phosphorus (Valentine and Allen 1990, Weetman 1989, Imo and Timmer 1997, Salifu and Timmer 2001).

Pine nutrient status is affected by “high” alder densities (“intermediate”, “high” and “very high” alder densities, Figure 2.4). High competition between pine and alder occurs at these densities. Pine nutrient status and growth were affected differently in the two areas of regeneration examined in this study. “High” alder densities (Figure 2.4a-c, vectors 1) increased growth while “very high” alder densities induced a growth reduction

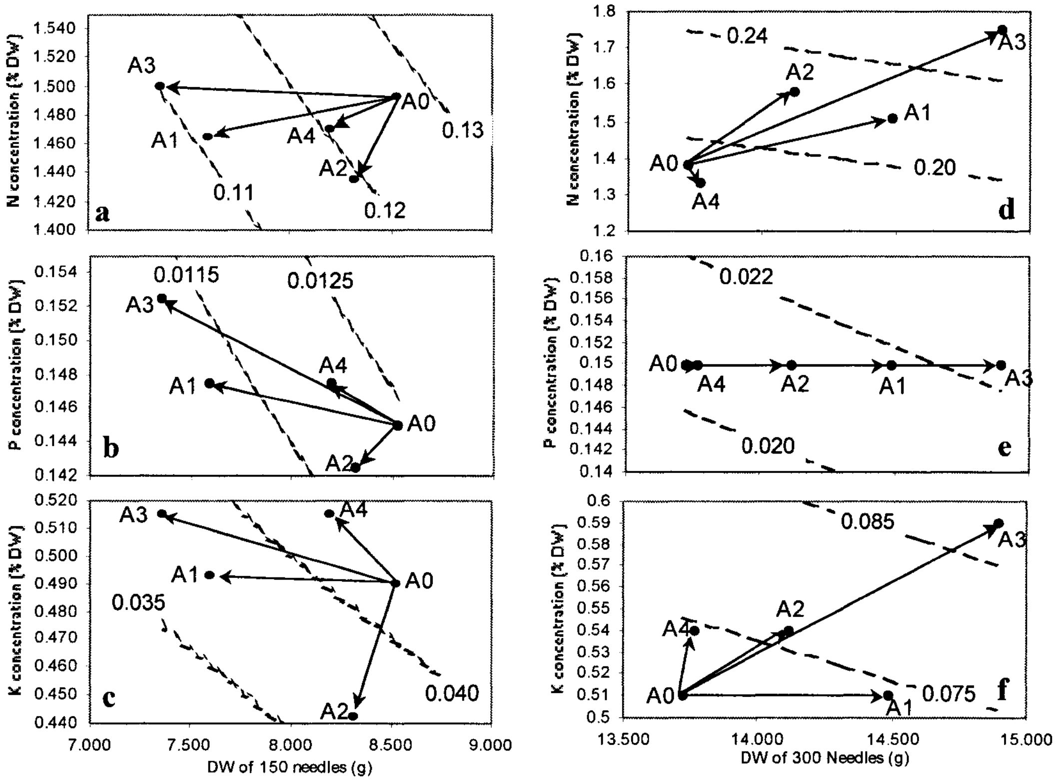


Figure 2.3. Effects of varying pine/alder ratios on needle dry weight and needle nutrient concentration and content in the areas of regeneration 1987 (a-c) and 1994 (d-e). Diagonal dashed curves are nutrient content isolines.

in the area of regeneration 1987 (Figure 2.4a-c, vectors 2). In the area of regeneration 1994 (Figure 2.4d-f), however, “high” alder densities decreased growth and “very high” alder densities increased it.

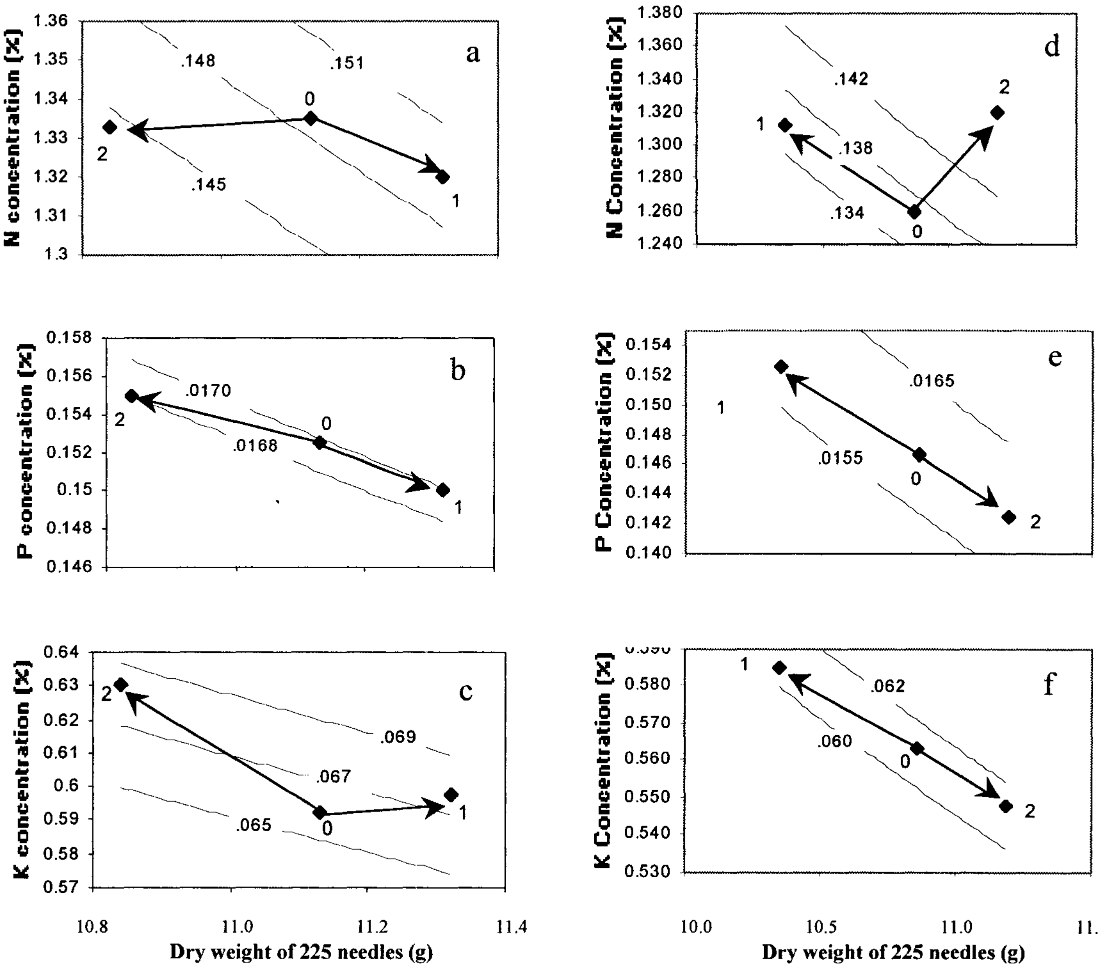


Figure 2.4. Needle nutrient interactions in pine-alder mixed stands under “high” and “very high” alder densities. a-c) Area of regeneration 1987 and d-f) Area of regeneration 1994. 0=Control plots (intermediate alder density); 1=High alder density; 2=Very high alder density. Dashed diagonal lines represent nutrient content.

Dilution processes of both N and P developed in the area of regeneration 1987 when alder density increased from “intermediate” to “high” (Figure 2.4a,b, vectors 1, Imo and Timmer 1997). These nutrients may be limiting pine growth under this alder density. When alder density was “very high” N was the most limiting nutrient, while P and K experienced concentration processes under this condition (Figure 2.4b,c, vectors 2, Imo and Timmer 1997).

In the area of regeneration 1994, on the other hand, no nutrient limitation appeared when alder density increased from intermediate to “high” (Figure 2.4d-f, vectors 1). However, P or K seem to limit pine growth when alder density is “highest”. A dilution of these nutrients occurred when pine growth went up (Figure 2.4e,f, vectors 2), while pine N underwent a concentration process under the same condition of alder density (Figure 2.4d, vector 2).

DISCUSSION

General nutritional effects of alder on pine growth

The antagonistic relationship between most alder densities and the nitrogen (N) status in pine trees in the area of regeneration 1987, should be interpreted rather as a competition effect between alder clumps and pine trees. In fact, even though compensatory or synergistic relationships (Imo and Timmer 1999) between N-fixing species and crop species (where either the crop species or both species in the mixture may be benefited) are expected in mixed stands, our pine species in this specific area of regeneration was negatively affected in terms of its N status.

Diverse changes in the status of nutrients other than nitrogen have been reported to occur in crop trees when in mixture with N-fixing species (Binkley 1983, Van Miegroet *et al.* 1990, Shainsky and Radosevich 1992, Shainsky and Rose 1995, Rothe and Binkley 2001). However, I know of no case in the literature where N status of crop trees has been negatively influenced by this type of plant species as it occurred in this study. Binkley (1983) reported negative effects of red alder on growth of Douglas-fir growing on fertile soils. However, conifer N concentration remained pretty the same as with no alder. The decrease in N concentration and content in pine needles in the present work (Figure 2.3a) indicates that competition for N between alder shrubs and pine trees developed in virtually all alder densities. This finding is in agreement with the results in chapter 1 (Figure 1.3b,c), which indicates that alder had negative effects on pine growth during the last seven years.

As explained in chapter 1, light limitations for alder are likely to be at work in this area of regeneration. Many of the conditions prevailing at this site and some intrinsic alder's characteristics as well, are probably favoring light limitations for alder functioning. First, pine density in this area of regeneration is pretty high (appendix 1) and so is pine leaf area. Second, pine trees are much taller than alder clumps (alders are in the understory). Third, alder requirements for light are known to be high (Harrington 1990, cited by Binkley *et al.* 1994; Binkley *et al.* 1994, Fisher and Binkley 2000) and probably limiting in this site.

In agreement with the situation described above, Heilman and Stettler (1985) found that N-fixation by red alder declined in the fourth growing season, presumably due to increasing shading by the taller black cottonwoods, thus impairing N-fixation by alder.

In 1983, the same authors found that partially shaded red alder trees produced just half the nodule biomass of trees fully exposed to sun. Additionally, they encountered that the lower nodule biomass under shade was accompanied by lower acetylene reduction rates than under full sunlight, as also found in the present work (appendix 3).

The excess of phosphorus (P) and potassium (K) found in the area of regeneration 1987 may have two sources: 1) a concentration process due to limitation of growth by other factor (i.e. nitrogen), or 2) an increase in the availability of these nutrients by alder. Even though there are several reports on increased P availability by alder or other N-fixing species (Binkley *et al.* 1992, Giardina *et al.* 1995, Shainsky and Radosevich 1992, Shainsky and Rose 1995, Zou *et al.* 1995), in this study it appears that the cause for the concentration of P and K was the decreased needle biomass (concentration effect, Koricheva 1999, Domínguez *et al.* 2000). Otherwise, the content of these nutrients would have increased, especially that of K, since this nutrient is widely known to be absorbed by most plants to luxury consumption when its availability in soil is high (Epstein 1972, Rodriguez 1982, Mengel and Kirkby 1982).

The decreased concentration of N within pine needles when alder clumps were present in the stand indicates that alders are currently not fixing N. On the other hand, the deficiency of N could explain the reduction in growth of pine trees occurring during the last years of stand development in the area of regeneration 1987 (see chapter 1, Figure 1.3a-c). Moreover, the integration of these facts suggests that, although somewhat intriguing (since alders fix N), alders competed with pine trees for N. This possibility is highly probable provided that alders are high-N-demanding species as judged by their high foliar N concentrations, and in this case, they were unable to produce their own N due to light limitations.

The accumulation of N and K in pine needles from plots with alder densities higher than A1 in the area of regeneration 1994 (Figure 2.3d,f) indicates that the availability of these nutrients was increased by alder. In the case of N, this effect seems pretty reasonable since appropriate conditions for high N-fixation rates to take place were present in this site. In fact, alder density was quite high in this location (appendix 1) and alders were as tall as or even taller than pine trees, so that they were fully exposed to sunlight.

The accumulation of K, however, is harder to understand, specially if N-fixation rates were high. In fact, literature reports that high N-fixation rates may promote nitrification; a microbiological process through which, H^+ ions are released into the soil solution (Schlesinger 1997). H^+ ions may displace basic cations (such as Ca^{++}, Mg^{++} or K^+) from soil adsorption sites, making them vulnerable to loss to deeper soil horizons (Van Miegroet *et al.* 1990). Nitrification was not measured in this study's site, however, if this process occurred in this area of regeneration, K losses would have also taken place and this would contradict the results from our data.

There are reports indicating that broad leaved species may increase organic matter decomposition rates and nutrient turnover and availability (Van Miegroet 1990, Binkley *et al.* 1992, Prescott *et al.* 2000). Phosphorus is one of the nutrients whose availability has been found to increase in some cases when N-fixing species are mixed with crop trees (Binkley *et al.* 1992, Giardina *et al.* 1995, Shainsky and Rose 1995, Zou *et al.*

1995). If these effects are taking place in this area of regeneration (1994), these processes could explain the increased K availability.

On the other hand, the accumulation of N and K also indicates that there exists some other factor that is limiting pine growth in this area of regeneration. Otherwise, these nutrients would dilute within a greater pine biomass. Figure 2.3e indicates that P status corresponds to the so called steady state (Imo and Timmer 1997, Quoreshi and Timmer 2000, Salifu and Timmer 2001) That is, P availability is just sufficient to keep pace with increase in needle weight (Weetman 1989, Imo and Timmer 1997). However, according to Weetman (1989), vectors with this direction correspond to the Macy's (1936, cited by Weetman 1989) concept of "minimum percentage" level for internal nutrient concentration, and are on the boundary between the dilution zone and the deficiency zone. Accordingly, in this area of regeneration (1994), P was more probably the nutrient that limited pine growth, thus causing N and K to concentrate in pine needles.

It is important to notice that pine growth was a reflection of P availability in this area of regeneration if P was the nutrient limiting pine growth. Therefore, it may be deducted that P availability probably described in quadratic fashion as the growth variables did in the area of regeneration 1994 (Figure 1.2k-o, chapter 1). In turn, this means that P availability was increased by alder densities as reported by several authors for various N-fixing species (Binkley *et al.* 1992, Giardina *et al.* 1995, Shainsky and Rose 1995, Zou *et al.* 1995).

Competition effects of alder on pine growth

Competition effects between alder and pine are expected to become more intense when alder densities are higher. This is why we decided to examine more carefully these effects by taking a greater number of needle samples from the plots with the highest proportions of alder.

The increase in alder density from intermediate to either "high" or "very high" in the area of regeneration 1987 resulted in nitrogen deficiencies in pine trees (Figure 2.4a, Weetman 1989, Valentine and Allen 1990). This is consistent with the negative effects of alder found in this area of regeneration in chapter 1, and with the finding shown in Figure 2.3a. In fact, Figure 2.4a provides additional proof that competition for nitrogen between alder shrubs and pine trees occurs in the area of regeneration 1987, probably due to the alder's high N demand coupled with limitations for the N-fixation process to take place.

Besides promoting N deficiency in pine trees, the increase in alder density from intermediate to "high" also induced P deficiency in the area of regeneration 1987 (Figure 2.4b, vector 1). This induced P deficiency is likely to be a result of the dilution of this nutrient within pine trees when their growth increased probably as a consequence of the remaining N from previous years N-fixation (Figure 1.3a-c, chapter 1). The P sufficiency that occurred when alder density changed from intermediate to "very high", on the other hand, is likely to be due to a concentration process of P brought about by the general N deficiency in this area of regeneration, which induced an increased competition for N between alders and pines.

Summarizing the last paragraphs, it looks like "high" alder densities may be causing N and P deficiencies to alternate in the area of regeneration 1987. However, when alder density increases to "very high", competition for N between pine trees and alders is likely to become more intense, thus causing pine growth to decrease and needle P to concentrate.

The increased concentration and content of K within pine needles in the area of regeneration 1987 are likely to be a reflection of both, increased K availability with increased alder density from intermediate to "high" and 'very high" and a concentration process caused by the negative effects of alder in this area of regeneration probably brought about by competition for N between alders and pine trees, as discussed above.

The effects of alder clumps on pine K in mixed pine-alder stands have not been well documented. However, Binkley *et al.* (1992) reported a K ratio of 2.0 in organic matter between alder-conifer stands and pure conifer ones. This suggests that alders contributed to increase K availability in the mixed stands.

The lack of nutrient (N, P, or K) limitations and the nutrient concentration processes under the "high" alder condition in the area of regeneration 1994 (Figure 2.4d-f, vectors 1) may be a result of the decreased growth of pine trees. This situation, in turn suggests that a factor other than the studied nutrients probably limited pine growth. Moreover, the fact that pine growth increased under the "very high" alder density and it decreased under the "high" alder density indicates that it probably was an alder-density-independent factor that promoted this change in pine growth.

It was previously stated that alders in this area of regeneration were as tall or even taller than pine trees, so that competition for light probably occurred in this site.

However, it is unlikely that competition for light limited pine growth when alder density was “high” since it did not when alder density increased to “very high” in which case a stronger limitation to pine growth would be expected.

Similarly, competition for water between alders and pine trees would be expected to increase with increasing alder density, thus reducing pine growth more strongly under the “very high” alder density than under the “high” alder density. This is why an alder-density-independent factor is believed to have restricted pine growth in this area of regeneration.

Topography in this area of regeneration is more variable than that in the area of regeneration 1987. The position of plots along the slope may have driven some factor (i.e water or a nutrient other than N, P, or K) that limited pine growth in a different way in the “high” than in the “very high” alder density plots. There are several studies that have shown that nutrient and water availability are strongly affected by topography (Garten *et al.* 1994, Rhoades *et al.* 2001, Arteaga 1983).

As mentioned before, unfortunately this area of regeneration was burnt in March 2001, so that several variables that could help explain the competition relationships between alders and pines, could not be evaluated.

CONCLUSIONS

Response of pine N, P, and K to alder densities in pine-alder young stands varies from one site to another depending on site conditions. Height differences between pine and alder seem to be important factors in determining pine nutrient responses to alder densities. In the area of regeneration 1987, where alders are in the understory, N is the limiting nutrient for pine growth. In the area of regeneration 1994, where alders are the same height than pine trees, pine N was non-limiting.

Most alder densities increased N, P, and K availability to pine trees in the area of regeneration 1994, where no apparent light limitations for alder growth exist. However, P seems to be the limiting nutrient for pine growth.

Competition for N between pine trees and alder clumps appeared when alder density changed from either "intermediate" to "high" or "intermediate" to "very high" in the area of regeneration 1987, where light limitations for alder growth existed. In the area of regeneration 1994, on the other hand, no nutrient limitations occurred when alder density was "high", but they did when alder density was "very high". In this case, P or K became limiting for pine growth.

LITERATURE CITED

Aber, J. D., and J. M. Melillo. 1991. Terrestrial ecosystems. Saunders College Publishing. Orlando. 429 p.

Arteaga M. B. 1983. Influencia del suelo y las características fisiográficas en el crecimiento de *Pinus radiata* D. Don en Ayotoxtla, Gro. Bachelor Thesis. Universidad Autónoma Chapingo. Chapingo, Mexico.146 p.

Binkley, D. 1983. Ecosystem production in Douglas-fir plantations: Interactions of red alder and site fertility. *For. Ecol. Manag.* 5:215-227.

Binkley, D., P. Sollins, R. Bell, D. Sachs, and D. Myrold. 1992. Biogeochemistry of adjacent conifer and alder-conifer stands. *Ecology.* 73: 2022-2033.

Binkley, D., K. Cromack, Jr., and D. D. Baker. 1994. Nitrogen fixation by red alder: biology, rates, and controls. *In*: Hibbs, D., D. DeBell, and R. Tarrant (Eds.) . The biology and management of red alder. Oregon State University. Pp. 57-72.

Binkley, D., and C. Giardina. 1997. Nitrogen fixation in tropical forest plantations. *In:* Nambiar, E. K. Sadanandan, and A. G. Brown (Eds.). Management of soil, nutrients, and water in tropical plantation forests. ACIAR. Momograph No. 43.

Binkley, D., and C. Giardina. 1998. Why do tree species affect soils? The warp and woof of tree-soil interactions. *Biogeochemistry* 42:89-106.

Binkley, D., C. Giardina, and M. A. Bashkin. 2000. Soil phosphorus pools and supply under the influence of *Eucalyptus saligna* and nitrogen-fixing *Albizia falcataria.*

Binkley, D., R. Senock, S. Bird, and T. G. Cole. 2003. Twenty years of stand development in pure and mixed stands of *Eucalyptus saligna* and nitrogen-fixing *Falcataria moluccana. For. Ecol. Manag.* (www.elsevier.com/locate/foreso). In Press.

Brill, W. J. 1977. Biological nitrogen fixation. *Scientific American.* March 1977 68-81.

Dominguez, L. S., J. Oliet P., P. Ruiz., I. Carrasco M., J. L. Peñuelas R. y R. Serradas H. 2000. Influencia de la relación N-P-K en el desarrollo en vivero y en campo de planta de *Pinus pinea.* Actas del congreso I Simposio del pino piñonero. Vol. 195-202. Valladolid, Spain.

Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. Wiley. New York. 412 p.

Fisher, R. F., and D. Binkley. 2000. Ecology and management of forest soils. 3rd. ed. Wiley. USA. 489 p.

Garten, C. T., M. A. Huston, and C. A. Thomas. 1994. Topographic variation of soil nitrogen dynamics at Walker Branch Watershed, Tennessee. *For. Sci.* 40:497-512.

Giardina, C. P., S. Huffman, D. Binkley, and B. A. Caldwell. 1995. Alders increase soil phosphorus availability in a Douglas-fir plantation. *Can. J. For. Res.* 25: 1652-1657.

Haase, D. L., and R. Rose. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. *For. Sci.* 41:54-66.

Hamdi, Y. A. 1985. La fijación de nitrógeno en la explotación de los suelos. Boletín de suelos # 49 de la FAO. 188 p.

Heilman, P. and R. F. Stettler. 1985. Mixed, short-rotation culture of red alder and black cottonwood; growth, coppicing, nitrogen fixation, and allelopathy. *For. Sci.* 31:607-616.

Imo, M., and V. R. Timmer. 1997. Vector diagnosis of nutrient dynamics in mesquite seedlings. *For. Sci.* 43:268-273.

Imo, M. and V. R. Timmer. 1999. Vector competition analysis of black spruce seedling responses to nutrient loading and vegetation control. *Can. J. For. Res.* 29:474-486.

Kalra, Y. P., and D. G. Maynard. 1991. Methods manual for forest soil and plant analysis. Forestry Canada, Northwest Region, Northern Forestry Centre. Canada. 116 p.

Kaye, J. P., S. C. Resh, M. W. Kaye, and R. A. Chimner. 2000. Nutrient and carbon dynamics in a replacement series of *Eucalyptus* and *Albizia* trees. *Ecology* 81:3267-3273.

Koricheva, J. 1999. Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. *Oecologia* 119:467-473.

Mengel, K., and A. E. Kirkby. 1982. Principles of plant nutrition. International Potash Institute. Bern. 593 p.

Prescott, C. E., L. M. Zabek, C. L. Staley, and R. Kabzems. 2000. Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures. *Can J. For. Res.* 30: 1742-1750.

Quoreshi, M., and V. R. Timmer. 2000. Growth, nutrient dynamics, and ectomycorrhizal development of container-grown *Picea mariana* seedlings in response to exponential nutrient loading. *Can. J. For. Res.* 30:191-201.

Radwan, M. A. 1987. Effects of fertilization on growth and foliar nutrients of red alder seedlings. Res. Pap. PNW-RP-375. Portland Or: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 14 p.

Rhoades, C., H. Oskarsson, D. Binkley, and B. Stottlemyer. 2001. Alder (*Alnus crispa*) effects on soils in ecosystems of the Agashashok River Valley, northwest Alaska. *Ecoscience* 8:89-95.

Rothe, A. and D. Binkley. 2001. Nutritional interactions in mixed species forests: a synthesis. *Can. J. For. Res.* 31:1855-1870.

Rodriguez, S. F. 1982. Fertilizantes: nutrición vegetal. AGT Editor. Mexico. 157 p.

Salifu, K. F., and V. R. Timmer. 2001. Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. *Soil Sci. Soc. Am. J.* 65:905-913.

Schlesinger, W. H. 1997. Biogeochemistry: An analysis of global change. 2nd Ed. Academic press. U. S. A. 588 p.

Shainsky, L. J. and S. R. Radosevich. 1992. mechanisms of competition between Douglas-fir and red alder seedlings. *Ecology.* 73:30-45.

Shainsky, L. J. and C. L. Rose. 1995. Effects of competition on the foliar chemistry of young Douglas-fir in monoculture and mixed stands with young red alder. *Can. J. For. Res.* 25:1969-1977.

Timmer, V. R., and L. D. Morrow. 1984. Predicting fertilizer growth response and nutrient status of jack pine by foliar diagnosis. *In*: E. L. Stone (Editor). Forest soils and treatment impacts. Forest Soils and Forest Land Management. Proceedings of the 6Th. North American Forest Soils Conference. Pp. 335-351.

Timmer, V. R., and E. L. Stone. 1978. Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. *Soil Sci. Soc. Am. Proc.* 42:125-130.

Valentine, D. W., and H. L. Allen. 1990. Foliar responses to fertilization identify nutrient limitation in loblolly pine. *Can. J. For. Res.* 20:144-151.

Van Miegroet, H., D. W. Cole, and P. S. Homann. 1990. The effect of alder forest cover and alder forest conversion on site fertility and productivity. *In*: Gessel, S.P., D.S. Lacate, G.F. Weetman, and R.F. Powers. Sustained productivity of Forest Soils. Proceedings of the 7th North American Forest Soils Conference, University of British Columbia, Faculty of Forestry Publication, Vancouver, B.C. Canada. pp. 333-354.

Weetman, G. F. 1989. Graphical vector analysis technique for testing stand nutritional status. Pp. 93-109. *In*: W. J. Dyck and C. A. Mees (Ed.). Research strategies for long-term site productivity. Proceedings, IEA/BE A3 Workshop, Seattle, Wa., August 1988. IEA/BE A3 Report No. 8. Forest Research Institute, New Zealand, Bulletin 152.

Wells, C., and L. Allen. 1985. When and where apply fertilizers. A loblolly pine management guide. Gen. Tech. Rep. SE-36. USDA Forest Service. 23 p.

Zou, X., D. Binkley, B. A. Caldwell. 1995. Effects of dinitrogen-fixing trees on phosphorus biogeochemical cycling in contrasting forests. *Soil Sci. Soc. Am. J.* 59: 1452-1458.

CHAPTER 3

PINE NUTRIENT STATUS AND GROWTH AS AFFECTED BY NITROGEN FERTILIZATION AND VARYING PINE/ALDER RATIOS

ABSTRACT

To examine the effects of varying pine/alder ratios (PARs) and N-fertilization on *Pinus montezumae* growth (needle mass) and nutrient status, an experiment was established in each of two areas of regeneration in the Ejido Los Romeros, Hidalgo, Mexico. The experimental design used was a factorial with five PARs and two nitrogen fertilization levels. Data analysis was carried out by using the graphical vector analysis technique. Results indicated that fertilization with nitrogen, generally had positive effects on needle mass. Reductions in needle mass appeared in the area of regeneration 1987 in response to the presence of alders. This finding suggests that no N-fixation by alder was occurring in this area of regeneration, probably due to light limitations for alder growth. The effects of alder on P and K seem to depend to a large extent on the alder's surrounding environment in terms of light availability.

INTRODUCTION

Alder [*Alnus arguta* (Schl.) Spach.] is a non-legume nitrogen-fixing tree that can fix the atmospheric nitrogen in favor not only of itself but also of surrounding vegetation. In pine-alder mixed stands, alder nitrogen fixation greatly improves pine growth. Pine responses to the presence of alders may be modified by a wide variety of factors such as alder and pine densities, and presence of understory vegetation among others. They may also be driven to a large extent by soil factors such as nitrogen availability and may be altered by the levels of other soil nutrients and environmental factors such as soil moisture, radiation, pests, etc.

These types of interactions in the Mexican ecosystems have not been studied. Just very few studies have been conducted to start generating some knowledge on the possible interactions between pines and alder but these research studies have been confined to nursery conditions. Consequently, alder management has been absent from silvicultural programs and large amounts of biologically fixed nitrogen have been spoiled or even prevented as a result of inappropriate alder management practices.

The present investigation is intended to study the interactions between pines and alders in pine-alder mixed young stands in terms of pine growth and foliar nutrients. We hypothesized that either, increased alder density or N-fertilizer increase pine growth and that positive effects of N-fertilizers are stronger when alder levels are lower.

REVIEW OF LITERATURE

Effects of alder on crop tree nutrient status

Alder [*Alnus arguta* (Schl.) Spach] is a tree species of the family Betulaceae, capable of fixing atmospheric N through its association with actinomycetes of the genus *Frankia*. This association generally results in important changes in chemical characteristics of soil. Soil analysis from an experiment conducted by Binkley (1983) indicated that red alder (*Alnus rubra*) trees growing in infertile soils contributed to increase N concentration in soils from 0.09 % to 0.19 %. Nonetheless, alders in a fertile soil showed no significant improvement of soil N status.

In a 5-year old *Pinus patula* (Schl. *et* Cham.) stand, Gutierrez (1998) found that soil N concentration was significantly higher (0.5 Vs. 0.32 % for alder tree and pine tree, respectively) when an alder (*Alnus firmifolia* Fernald.) tree was in the center of a quadrat instead of *a P. patula* tree. However, following the same procedure in a 16-year old stand, no differences were found probably due to pine overtopping.

In other sites in the states of Washington and Oregon, Binkley *et al.* (1992) investigated N accretion by red alder at low and high fertility sites. These authors found that N accretion for the low fertility site was 54 Kg ha^{-1} yr^{-1} for the 52 years since stand establishment. In the fertile soil, N accretion reached 73 Kg ha^{-1} yr^{-1} for 55 years since stand establishment.

The spatial distribution of alders in a given area is likely to have important effects on N fixation rates. Dawson *et al.* (1983) studied the changes in soil N concentration in alder (*Alnus glutinosa* L. (Gaertn.) plantations mixed with hybrid poplar rows. They found that soil N concentrations increased the most with respect to initial levels when alder rows were located between two poplar rows. When an alder row was between a poplar one and a row of alders N accretion was lower. Soil N concentration was lowest, with a significant net loss of N, where alder was associated with rows of alder on either side.

Working with red alder, Van Miegroet *et al.* (1990) found that forest floor N concentration and N in vegetation subsequently established were higher in sites previously occupied by alder. N accumulation both in aboveground biomass components and in the soil was on the order of 34 to 96 Kg ha^{-1} yr^{-1}.

According to Larcher (1995), alder may be able to return amounts of N as large as 150 Kg h^{-1} yr^{-1} as litterfall to soils. These amounts may be considered to be more than sufficient for most conifers to respond (Turvey and Smethurst 1983), especially in N-limited conditions.

The effects of alder, however, not only result in increased soil and adjacent plant N. Alders may also modify concentration of other nutrients and chemical features of soils such as pH and base saturation. Soil processes such as nitrification, denitrification, and nutrient leaching may also be altered by the influence of alders.

The study of the effect of alder on soil P status is of special interest since it is well known that alder has high P requirements for the process of N fixation to take place (Hamdi 1985, Giardina *et al.* 1995, Brown 1999). Even though just a few studies on the P biogeochemistry in alder-crop tree mixtures are available in the literature, the results indicate a positive effect of alder on soil P availability.

Giardina *et al.* (1995) found that inorganic P fractions from soils at 0.0 - 0.15 m were 65 – 225 % greater in plots with alder+Douglas-fir than in plots with just Douglas-fir. Although their results are conclusive in terms of statistical significance, the mechanisms for the increased availability appear to be rather poorly understood. In agreement with these results are the findings of Binkley *et al.* (1992) who determined that the alder-conifer/conifer P ratio including P from both biomass and forest floor was 1.36 times greater than that in the conifer stand. The corresponding P ratio for another site (fertile one), however, was less than unit (0.71) indicating a shortage in the amount of P taken up by vegetation in the alder-conifer stand.

Zou *et al.* (1995) found that red alder stands and alder-conifers [*Pseudotsuga menziesii* (Mirb.) Franco+*Tsuga heterophylla* (Raf.) Sarg.+*Picea sitchensis* (Bong.) Carr.] stands in Oregon presented more organic P and soil phosphatase activity than conifer stands. These authors also found that inorganic P in soils under alder-conifer stands was two-to-five fold greater than that under pure stands, suggesting that interactions between alder and conifers, rather than alder alone, triggered changes in P geochemical reactions.

Regarding other nutrients, results from Binkley *et al.* (1992) indicate that K, Ca, Mg, and S contents in the vegetation+forest-floor at the alder-conifer stands are almost twice as high as those in the conifer forest in the low fertility site. In the fertile site, however, K and Ca content were lower in the alder-conifer stand.

Van Miegroet *et al.* (1990) argues that cation leaching may take place in alder forests as a result of increased N fixation, which may increase nitrification if conditions are appropriate for this process to occur. During nitrification (i.e. the transformation of

NH_4 to NO_3) protons (H^+) are released into the soil solution and eventually some cations adsorbed to soil colloids may be replaced by H^+ ions thus promoting cation losses.

Irrespective of the effects of alder on the adjacent non-N-fixing-tree nutrient status, several authors agree in that the ultimate results in terms of aboveground biomass of non-N-fixing species in mixed stands are more commonly positive, especially when soils are N-poor and no other limitation exists.

In agreement with this statement, the results of Binkley *et al.* (1992) for fertile and infertile soils indicate that at the infertile soils both biomass and aboveground primary production of the alder-conifer stand were much higher than those of the conifer stand. On the contrary, in the fertile soils both biomass and aboveground net primary production were lower in the alder-conifer stand than in the conifer stand.

Hansen and Dawson (1982) report that poplar trees mixed with alders [*Alnus glutinosa* L. (Gaertn.)] exhibited a growth in height comparable to heights obtained from optimal rates of ammonium nitrate fertilization. They also observed that this variable was affected by the spacing between alders and poplars. The higher the spacing between alders and poplars the smaller the poplar height.

In a red alder-Douglas-fir replacement series established in British Columbia (Comeau *et al.* 1997) both height and root collar diameter of Douglas-fir appeared to be maximum at intermediate proportions of alder irrespective of plantation age. These authors also established three additive series with varying amounts of alder per hectare [fixed densities of Douglas-fir and western red cedar (*Tsuga heterophylla*)]. At least in one of the two reported additive series the greatest Douglas-fir and western red cedar values for tree height and root collar diameter were maximum at intermediate red alder densities, coinciding with the results from the replacement series. Even though these authors attributed differences in the variables measured to initial differences in seedling size and site characteristics, it seems somewhat likely that an important part of that trend may be a result of the presence of alders, since these results strongly agree with the results obtained in Chapter 1 in the present research work.

Van Miegroet *et al.* (1990) planted Douglas-fir seedlings in areas previously occupied by red alder or Douglas-fir. They found that Douglas-fir seedlings performed better in the areas that previously had alders than in those with Douglas-fir.

Crop tree nutrient status as affected by alder and N fertilization

When appropriate conditions for nitrification are met in soils, N fertilization may cause soil pH to decrease because of the release of H^+ ions into the soil solution, thus increasing soil aluminum and/or iron concentrations. High aluminum concentrations may induce a decreased root size (Thompson and Medve 1984, cited by Teng and Timmer 1995), thereby decreasing nutrient (especially immobile nutrients) absorption by vegetation including alder.

Under acidic conditions, aluminum and iron react with P to form insoluble phosphate compounds (i.e. aluminum and iron phosphates) which cannot be used by plants. Reduced P availability by this mechanism may bring about P deficiency in plants, especially in soils that previously were P-poor (Teng and Timmer 1995).

Considering that the process of N fixation is very P-demanding (Hamdi 1985), a decrease in the N fixation rates is expected to occur under high N status soils. These interactions could at least partially explain the lack of effects in terms of foliar N and net primary production in the fertile alder-Douglas-fir mixture studied by Binkley (1983).

MATERIALS AND METHODS

Study area

The study was carried out in the Ejido Los Romeros, of Santiago Tulantepec, Hidalgo, Mexico. The sites are located within the same areas of regeneration used for measurements included in chapter 1 (Figure 1.1). The reader should refer to the materials and methods section in chapter 1 for details on the characteristics of the regeneration areas. Unlike Chapter 1, in this study just the areas of regeneration 1987 and 1989 were included.

Selection of experimental plots

The present experiment was based on plots of 15m by 15 m in size, distributed within the areas of regeneration 1987 and 1989. Within each of the areas of regeneration, the plots were located on specific sites that had an average of 60 pine trees per plot. The plots were established by using a compass and a 50-m tape. Wood stakes were put in each corner of the plots. To determine the precise pine/alder ratio (PAR) in the plot, the plot was divided into three sections by using ropes and the amount of pine trees and alders in each section was determined. By using this procedure, a total of 60 plots were established in each of the areas of regeneration.

After the plots were established and the PARs determined, the required PAR series were selected. For doing this, all of the plots within a regeneration area were divided into two sets according to the amount of pine trees per plot. This was done to reduce the variability of pine density in the set of plots. From all of the plots, just 30 plots (those with the highest pine densities) were selected. The selected plots were sorted in descending order according to the PAR and the set was divided into five PAR levels. From each of the PAR levels just three plots were selected to further reduce pine density variation.

Experimental design

The experiment consisted of five PAR levels obtained using the plot selection procedure described above (Figure 3.1). Each PAR level consisted of three plots in the area of regeneration 1987 and two plots in the area of regeneration 1989. Each of the plots were divided into two sections and one of these sections was randomly chosen to receive the fertilization treatment, while the other was left as a control.

Since the alder trees are distributed in small clusters in the areas of regeneration and pine densities are variable across the regeneration areas, and both variables were used to select the plots, the plots within a PAR level are randomly distributed across the area of regeneration. Accordingly, the experimental design nearly looks like a randomized complete design. However, no statistical analyses were carried out on the data. The graphical vector analysis technique (Timmer and Stone 1978) was used instead.

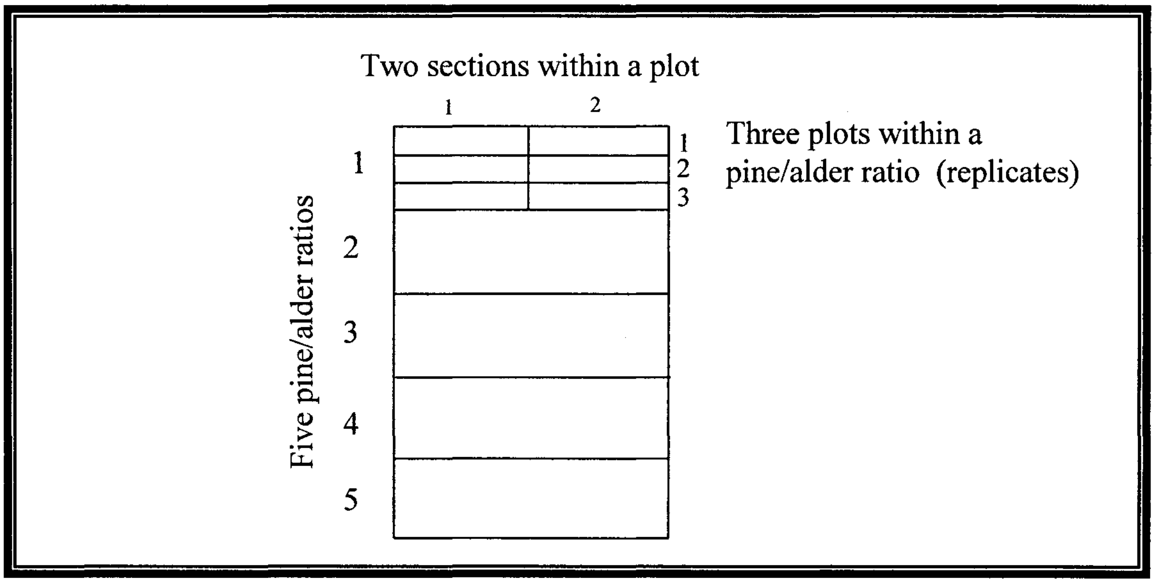


Figure 3.1. Schematic view of the design used in the experiment.

Fertilization treatments

As mentioned before, one of the sections of each plot received a fertilization treatment. This treatment consisted of a single dose of ammonium sulfate [$(NH_4)_2SO_4$] fertilizer, equivalent to 200 Kg N per hectare. According to this figure, an increasing response of pines to the fertilizer was expected as the alder density decreased.

The fertilizer was applied manually close to the base of the tree stems. This was done so in order to avoid absorption of the fertilizer by weeds and facilitate its absorption by trees. The fertilization operations were carried out in both areas of regeneration between July 1st and 20th, 2000. During this period of the year, precipitation events were fairly frequent and fertilizer was dissolved rather rapidly.

Variables measured

The variables considered in this experiment were foliar nutrients and needle dry weight of 105 (area of regeneration 1987) or 375 (area of regeneraation 1989) needles. The nutrients analyzed in the foliage were nitrogen, phosphorus and potassium.

Between January and March 2001, foliage samples were collected using a pair of pruning scissors. Near the center of each of the plot sections, three trees were selected and marked. A foliage sample was collected from each of the trees. The samples were always taken from the upper third of the crown of the tree and in all cases they were taken from the east-facing side of the crown.

After cutting the needles, they were put into plastic bags which in turn were put into a portable cooler as soon as possible to decrease leaf respiration and possible loss of weight. The same day they were slightly washed with tap water and rinsed with distilled water to eliminate dust or any other impurity. After doing that, they were put into paper bags and returned to the laboratory where they were oven dried at 70 °C for 48 h.

To determine the dry weight of a fixed amount of needles, 25 fascicles (5-needle each) from each of the trees were selected and put into a different bag. The veins of the fascicles were separated from the fascicles and the needles were oven dried again and the weight of the 105- or 350-needle groups determined using an analytical scale with 1/10000 g in precision.

For each PAR, needle dry weight was averaged (9 trees) and the corresponding samples were composited for N, P, and K determinations. Nutrient determinations were carried out in the Soil Fertility Laboratory of the Colegio de Postgraduados in the Estado de Mexico. N was determined by the micro-kjeldahl procedure. P was determined colorimetrically by the vanadomolybdophosphoric yellow color method as described by Kalra and Maynard (1991). K was determined by flame spectrophotometry.

The negative results of alder levels on needle weight in the area of regeneration 1987 led us to think that nitrogen fixation, if any, should be low in this area of regeneration. However, financial restrictions prohibited us to estimate N-fixation rates in the different alder levels, so that we opted for measuring variables that are related to nitrogen fixation rates. These variables were photosynthetically active radiation (PhAR) at 1 m from the forest floor and a surrogate for total alder chlorophyll.

PhAR was measured by using a light meter (LI-189) equipped with a quantum sensor. Sixteen measurements were taken along each plot on a systematic basis, during a fully sunny day. All values for a plot were averaged.

The surrogate for alder chlorophyll content was a measure of leaf absorbance by using a SPAD-582 (Minolta, Japan), which has proved useful to get close estimations of total chlorophyll in several plant species (Coleman *et al.* 1998, Rowland *et al.* 2001, Rodriguez 1997).

Data analyses

For each individual PAR level the fertilized sections were averaged for the determination of the dry weight of every group of needles and composited for foliar analyses, as suggested by Imo and Timmer (1997). The same procedure was followed for the non-fertilized sections. This resulted in a single value per PAR per section (fertilized and non-fertilized) for N, one for P, one for K, and one for needle dry weight.

These data were analyzed by means of the graphical method developed by Timmer and Stone (1978), and modified by Valentine and Allen (1990), which consists of plotting in a single nomogram the nutrient content, concentration, and dry weight of a fixed number of needles. Most calculations and graphs were developed using EXCEL 2000, and statistical analyses were done using SAS (v. 6.12).

For a single nutrient, three series of nomograms were developed with the non-fertilized section of the highest PAR plots (no alder) as the control treatment. The first series of plots consisted of plotting all the non-fertilized sections together with the control plot in order to determine the effects of varying PARs. The second series of plots consisted of plotting all the fertilized sections in a single nomogram to determine the effects of fertilization as affected by varying PARs. The last series of plots was developed to analyze the effects of fertilization for every single PAR level, on needle mass and nutrient status.

RESULTS

Effect of varying alder densities and N fertilizer on needle growth and nutrient status

According to the diagrams for nomogram interpretation (Weetman 1989, Haase and Rose 1995, and Quoreshi and Timmer 2000), the trends for the effects of alder density levels on pine nitrogen status in the area of regeneration 1987 should be interpreted as toxicity levels (Figures 3.2a,b).

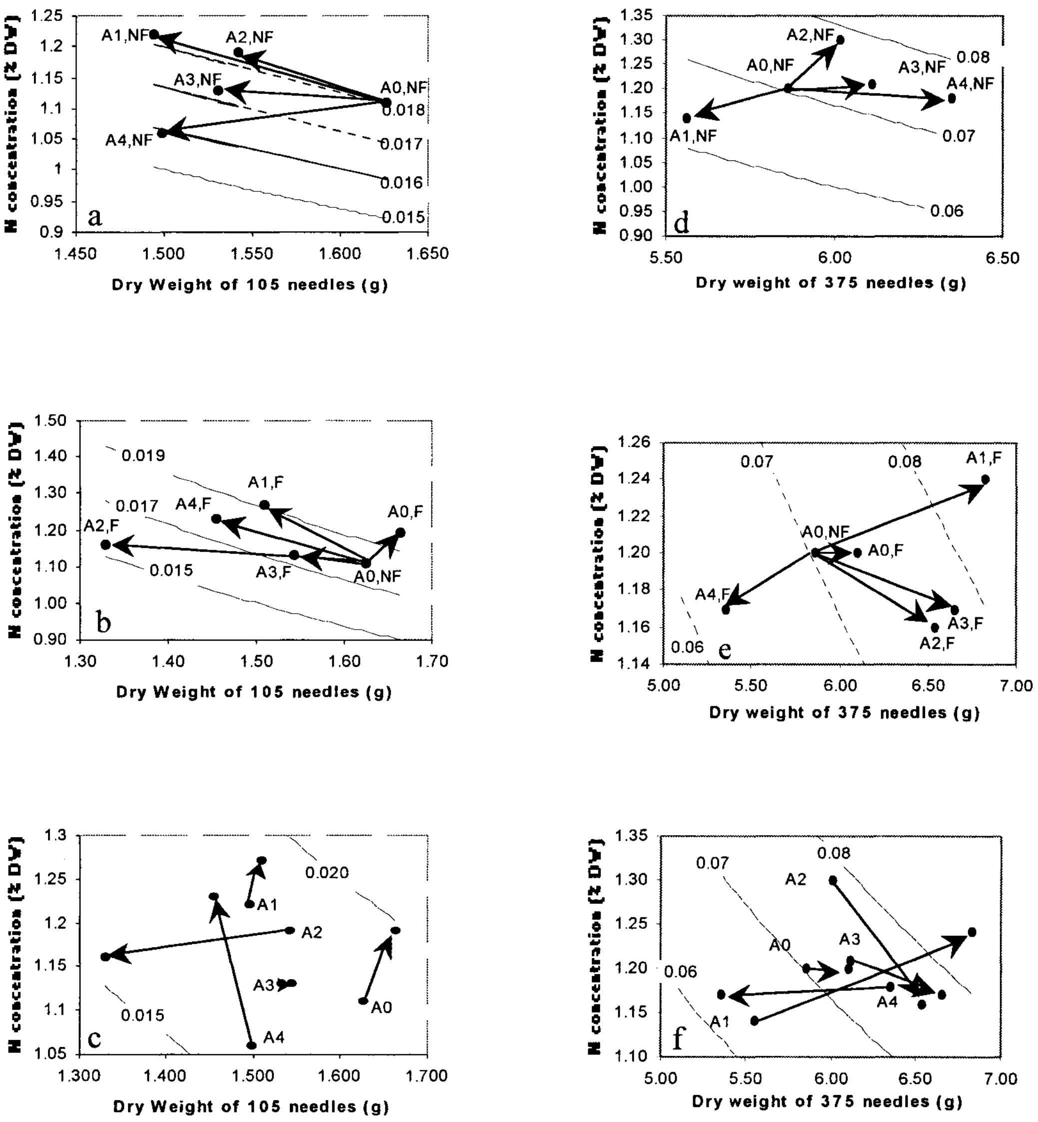


Figure 3.2. Effects of alder level (a and d), N-fertilizer (c and f) and the interaction alder level*N-fertilizer (b and e) on pine needle mass, N concentration and content, in the areas of regeneration 1987 (a-c) and 1989(d-f).

All alder density levels in the absence of fertilization induced reductions in needle mass in the area of regeneration 1987 (Figures 3.2a, 3.3a, and 3.4a). Nitrogen fertilizer application, on the other hand, induced slightly positive effects on needle dry weight in most alder density levels (Figure 3.2c).

Based on the criteria proposed by Valentine and Allen (1990), alder density levels alone (Figure 3.4a) or in combination with nitrogen fertilizer (Figure 3.4b) brought about K deficiency in pine needles in the area of regeneration 1987.

The interpretation of plots for the area of regeneration 1987 shows that alder density levels increased P availability to pine trees (Figure 3.3a) while N fertilization induced P deficiency in pines (Figure 3.3c) from all alder densities except when no alder was present (A0).

Alder densities and N-fertilizer contributed to improve pine needle growth in the area of regeneration 1989 (Figures 3.2d,f). Consequently, the needle mass resulting from the combination of both factors (alder density level and N-fertilizer) was also increased (Figure 3.2e).

N availability to pine trees was improved by alder densities (Figure 3.2d), and, in some cases (A0, A1, and A3), by N-fertilizer. However, at least in alder density level 2, an effect interpreted as depletion or retranslocation (Salifu and Timmer 2001) appeared. On the other hand, the application of N-fertilizer brought about a N toxicity in pine needles in the highest alder level (A4, Figure 3.2f).

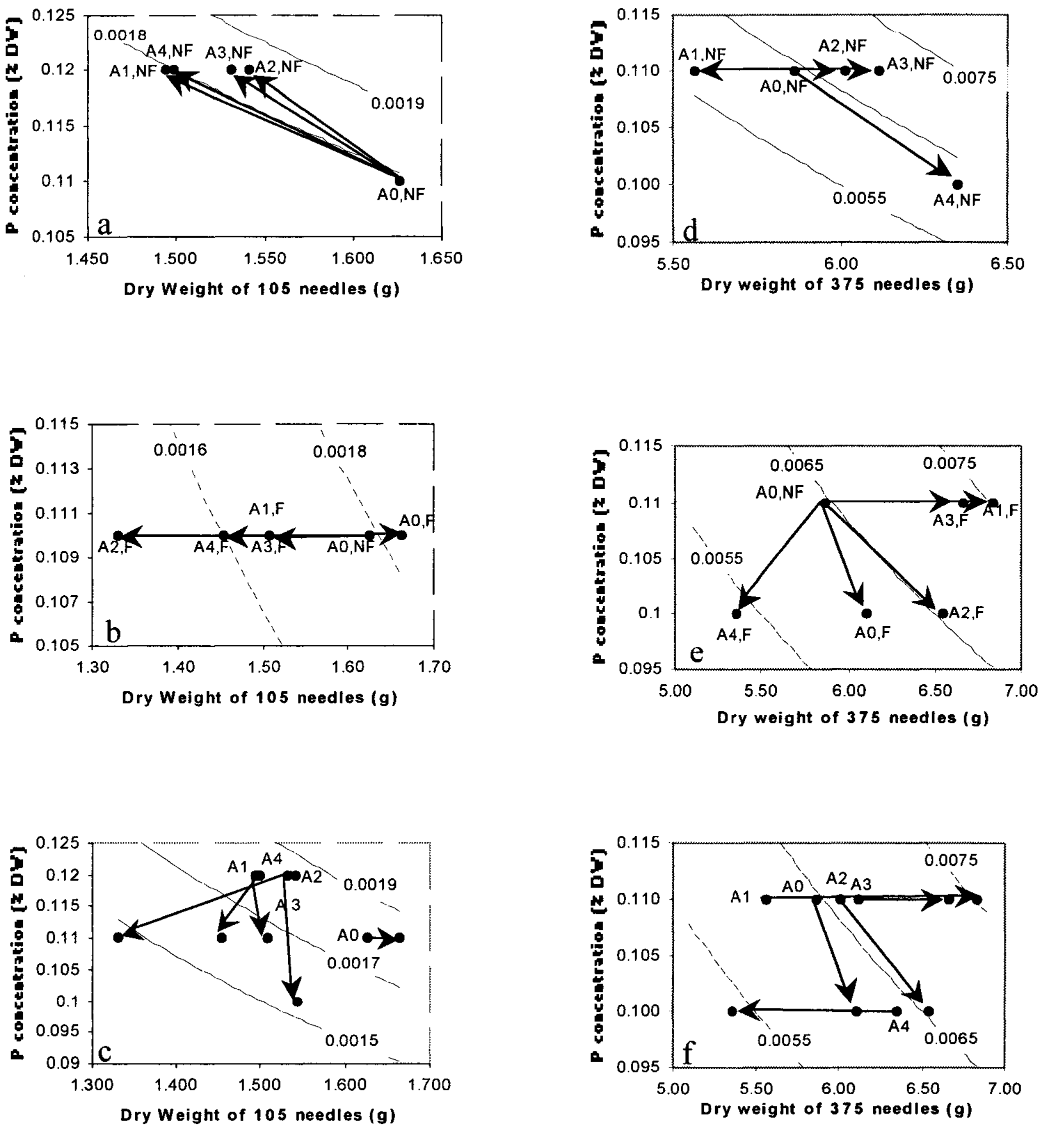


Figure 3.3. Effects of alder level (a and d), N-fertilizer (c and f), and the interaction alder level*N-fertilizer (b and e) on pine needle mass, P concentration and content in the areas of regeneration 1987 (a-c) and 1989 (d-f).

P availability in the area of regeneration 1989 was sufficient to cope with the increased requirement for this nutrients by pine trees after the increased growth promoted by the treatments occurred (Valentine and Allen 1990). When alder density was maximum (A4), however, P availability decreased resulting in P dilution in pine needles

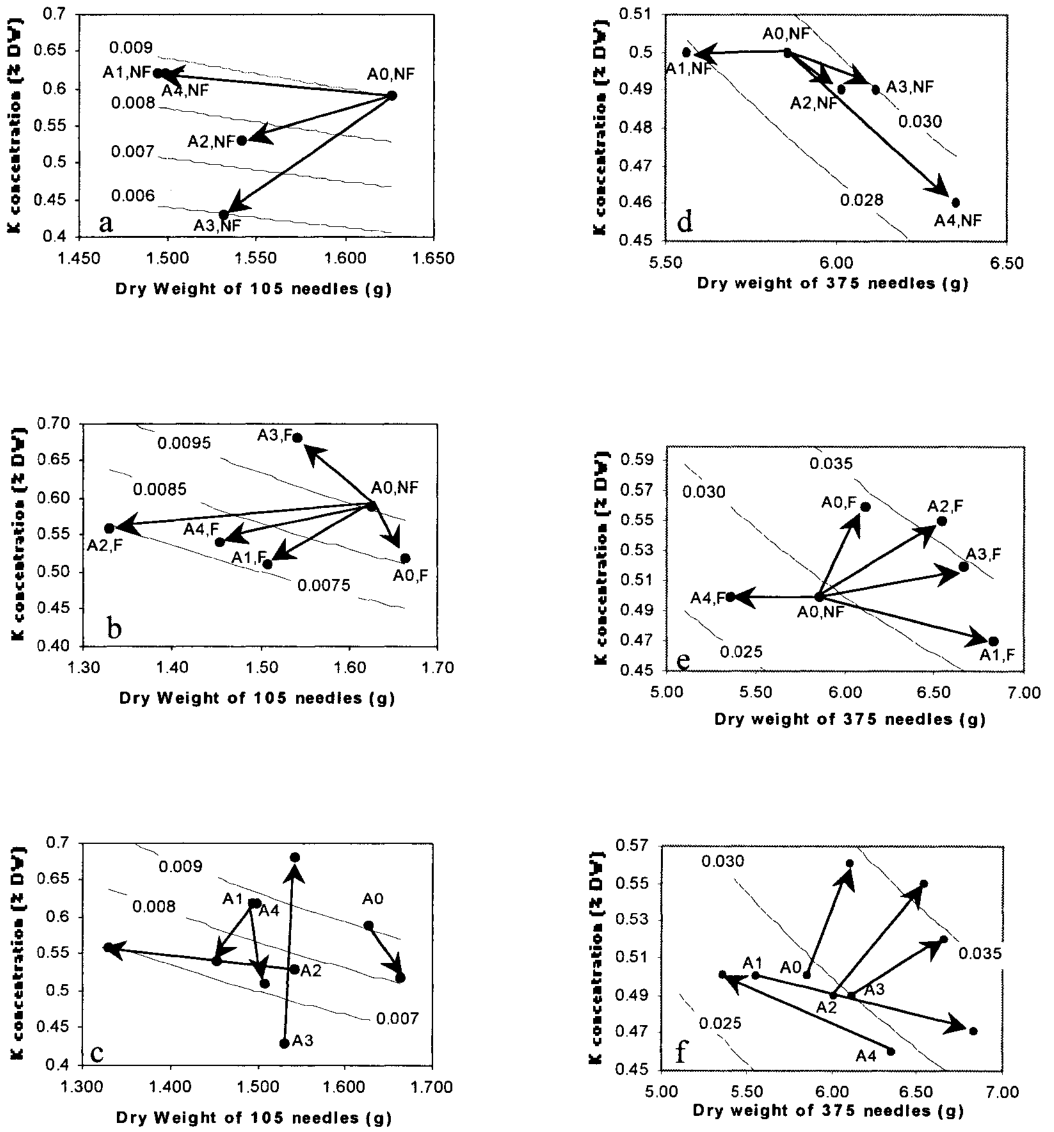


Figure 3.4. Effects of alder level (a and d), N-fertilizer (c and f) and the interaction alder level*N-fertilizer (b and e) on pine needle mass, K concentration and content in the areas of regeneration 1987 (a-c) and 1989 (d-f).

(Figure 3.3d). As pointed out before, the application of N fertilizer (Figure 3.3f) resulted in increased needle growth in most alder densities. P availability was sufficient to meet the extra requirement of pine needles in most alder densities (A1 and A3, A0 and A2; Valentine and Allen 1990), although in the last two alder density levels (A0 and A2), some degree of dilution of this nutrient occurred.

The combination of alder density levels and N-fertilizer in the area of regeneration 1989 resulted in sufficiency levels of P in most alder levels. The exception to this pattern was alder density 4, where P deficiency levels were generated (Figure 3.3e).

Most alder densities caused a dilution of K within pine needles [Valentine and Allen (1990), Figure 3.4d]. Nitrogen fertilizer, on the other hand, improved K nutrition in most alder densities (A0, A2, A3, Figure 3.4f). A K concentration process in pine needles occurred in the alder density four plots (A4).

DISCUSSION

As shown in Figures 3.2c,f, fertilization of pines in lower alder-density plots brought about increases in needle mass (except A2, area of regeneration 1987, Figure 3.2c) in both areas of regeneration (1987 and 1989), while in plots with the highest alder density (A4), N fertilizer resulted in negative effects on needle mass. Although this pattern of the behavior of needle mass is in agreement with our hypotheses, the expectation was to have a stronger growth response in needle mass to N-fertilizer when low alder densities were present, since such densities would imply a poorer pine N nutrition.

Provided that N-fixation rates per plot depend not only on alder density but also on the environment surrounding the N-fixing trees (i.e. light, soil moisture, soil nutrients, etc., Obaton 1985, Binkley *et al.* 1994, Peterson *et al.* 1996), as suggested by Bormann and Gordon (1984), N availability as affected by alder cannot behave so linearly. On the contrary, high variability and inconsistency among plots may frequently appear (Rothe and Binkley 2001) as it did in this study.

The expected relationship between alder densities and the needle mass response to N-fertilizer appears to be weaker in the area of regeneration 1987 (Figure 3.2c) than in the area of regeneration 1989 (Figure 3.2f). The randomness of the response in needle mass to the application of N fertilizer in the area of regeneration 1987 was probably due to the low effectiveness of alders in this site to improve N availability as it will be explained below.

Contrarily, the response in needle mass growth in the area of regeneration 1989 was higher when alder densities were lower (except A0) and negative when the alder density was maximum (A4, Figure 3.2f). According to Weetman (1989), Haase and Rose (1995), Quoreshi and Timmer (2000), and Valentine and Allen (1990), vector A4 in Figure 3.2f should be interpreted as toxicity. However, a competition effect between alder trees and pine trees is more likely to be represented by that vector, since the corresponding needle N concentrations are far below concentrations from other treatments that are not interpreted as toxicity.

The fact that most alder densities plus N-fertilizer had positive effects on needle dry weight (Figure 3.2c) and induced reductions in needle dry weight when no N-fertilizer was applied (Figure 3.2a) suggests that alders were responsible for the reduction in needle mass when both factors (alder density and N fertilizer) interacted (Figure 3.2b). This deduction leads us to think that the supposed N toxicity (Weetman 1989, Haase and Rose 1995, Quoreshi and Timmer 2000) due to the alder levels should be interpreted as competition effects between alder shrubs and pine trees for some soil resource, rather than a N toxicity.

The supposed toxicity shown in Figure 3.2b, which resulted from the interaction of alder densities and N-fertilizer is probably more highly determined by the alder density levels than by N-fertilizer as shown in Figure 3.2c, which indicates that most alder densities induced a slight increase in needle dry weight.

The alder densities alone or in combination with the application of nitrogen fertilizer in the area of regeneration 1987 induced K deficiency (Figures 3.4a,b, Valentine and Allen 1990). This response of pine trees may be due to a competition effect for K between pine trees and alders. However, the behavior of conifer K status in response to conifer*N-fixing species interactions is unclear and much work is needed in this field (Rothe and Binkley 2001).

Increases in P availability by nitrogen fixers have been documented by several authors (Binkley *et al.* 1992, Giardina *et al.* 1995, Shainsky and Rose 1995, Zou *et al.* 1995,). N-induced P deficiency has been much less commonly reported. Teng and Timmer (1995) found P deficiencies in *Picea glauca* (Moench.) Voss., as a consequence of heavy N fertilization. These authors explain that such induced P deficiency was due basically to soil acidification, which promoted aluminum toxicity (bringing about restricted root growth), and formation of aluminum phosphates (decreased P availability). Figure 3.3c shows that P availability in the area of regeneration 1987 decreased just in those plots having alder (not in A0 plots). This means that probably some degree of soil acidification occurred as a consequence of the interacting effects of alder and the addition of N, leading to the formation of aluminum and/or iron phosphate insoluble compounds.

Binkley (1993) describes a case study at Mount Benson, where the presence of alders reduced P availability and concentration in Douglass-fir needles from 0.22 % to 0.12 %. However, in this case, Douglass-fir biomass was increased and the decreased P concentration clearly was due to dilution. This author states that similar results should be expected if N fertilizers were applied instead of intermixing alders.

The positive effects obtained in needle mass in the area of regeneration 1989 when alder was present in mixtures with pine trees (Figure 3.2d) indicate that alder levels contributed to improve pine N nutrition. On the other hand, these positive effects also mean that N probably was the main limiting nutrient in this area of regeneration. The positive effects induced by the application of N-fertilizer (Figure 3.2f) confirm the previous statements and indicate that alder levels did not satisfy the requirements for N by crop trees probably because alder density in this area of regeneration was low (appendix 2), or, more probably, because the N-fixation rates were low, in turn, probably due to light limitations for alder performance.

Although P nutrition was affected by both alder levels and N fertilizer in the area of regeneration 1989, P availability was enough to satisfy the requirements for this nutrient by pine trees (Figures 3.3d-f). This fact may indicate that P availability was improved by alder, as has been documented by several researchers (Binkley *et al.* 1992, Giardina *et al.* 1995, Zou *et al.* 1995).

It is worth pointing out the difference in the behavior of P between the areas of regeneration studied. While alders seemed not to improve P availability in the area of regeneration 1987 they did improve it in the area of regeneration 1989. This differential effect may be due to the difference in light availability between these areas. Alders in the area of regeneration 1987 are much more shaded by pine trees than in the area of regeneration 1989. Shading of alders implies that the N-fixation process probably is low. If so, then P uptake by alders may also be low since it is specifically the N-fixation process within alder trees that demands high amounts of P. If P demands by alder are low, then the influence of this plant species on P cycling may be low too.

Of all three nutrients included in this study, it looks like K became growth limiting as a result of the presence of alder in the area of regeneration 1989 (Figure 3.4d). A competition relationship between pine trees and alders for this nutrient is likely to have developed. Nonetheless, provided the increase in K availability promoted by N fertilization in most alder densities (Figure 3.4f), the interaction effects between alder densities and N fertilizer resulted in a sufficiency K status in pine trees in most alder densities (A0, A2, and A3, Figure 3.2e).

Our overall results indicate that management of mixed pine-alder stands is rather complex. There are many factors that influence the relationships among plant species and their performance as well. In this study, beneficial effects of alders on the crop trees clearly dominated over the negative effects associated to competition in the area of regeneration 1989, while the dominant role of alders in the area of regeneration 1987 was competition.

The major feature determining the difference in the final effect of alder on pine growth between the areas of regeneration studied is likely to be pine canopy density, which is much more dense in the area of regeneration 1987 than in the area of regeneration 1989 (appendix 2).

Density of the upper canopy drastically reduces radiation availability to understory vegetation (Figure 3.5a). This, in turn, is expected to limit photosynthesis of understory species, especially when they are very light demanding as most N-fixers are (Binkley *et al.* 1994, Peterson *et al.* 1996). On the other hand, according to Figure 3.5b there exists a significant relationship between pine density and alder absorbance, which has been considered as a surrogate for chlorophyll content (Coleman *et al.* 1998, Rowland *et al.* 2001, Rodriguez 1997). Figure 3.5b indicates that the higher the pine density the lower the alder chlorophyll content.

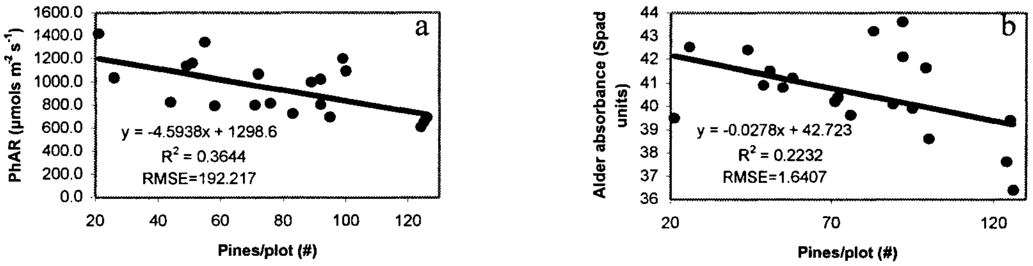


Figure 3.5. Effects of pine density on a) photosynthetically active radiation (PhAR) reaching the understory and b) alder leaf absorbance in the area of regeneration 1987. N = 20

It is likely that the interacting effects of both the reduction in photosynthetically active radiation and the decrease in alder chlorophyll content to have resulted in reduced alder photosynthetic rates and carbohydrate synthesis for nodule construction and maintenance. This, in turn, is likely to have resulted in decreased N-fixation rates. These cascade effects could explain the change of alder from a beneficial species into a weed one in the area of regeneration 1987.

CONCLUSIONS

In most pine/alder ratios (PARs), N fertilization had positive effects on needle mass. However, the hypothesized expectation that lower alder densities would bring about higher pine growth responses to nitrogen fertilizer and vice versa was poorly met. This was probably because N-fixation rates are far from behaving linearly since there is a variety of factors that influence them.

Results suggest that the reductions in needle mass found in the area of regeneration 1987 were due to the presence of alders, and not to nitrogen fertilization treatments. The negative effects of alders were probably due to alder suppression by pines, provided the sharp differences in size and density between alders and pine trees. Suppression of alders probably resulted in reduced N-fixation rates, which in turn, resulted in N-limitations for pine growth.

N-fixation did occur in the area of regeneration 1989. However, alder densities at that site were insufficient to fix the amounts of N required for pine growth.

The effects of alder on P and K depend, to a great extent, on the environment surrounding the alder trees, especially regarding light availability.

LITERATURE CITED

Binkley, D. 1983. Ecosystem production in Douglas-fir plantations: Interactions of red alder and site fertility. *For. Ecol. and Manage.* 5: 215-227.

Binkley, D. 1993. Nutrición forestal: prácticas de manejo. Translation from English by Manuel Guzmán Ortíz. Limusa. Mexico. 340 p.

Binkley, D., K. Cromack, Jr., and D. D. Baker. 1994. Nitrogen fixation by red alder: biology, rates, and controls. Pp 57-72. *In*: Hibbs, D., D. DeBell, and R. Tarrant. The biology and management of red alder. Oregon State University Press. Corvallis, Or.

Binkley, D., P. Sollins, R. Bell, D. Sachs, and D. Myrold. 1992. Biogeochemistry of adjacent conifer and alder-conifer stands. *Ecology*. 73: 2022-2033.

Bormann, B. T., and J. C. Gordon. 1984. Stand density effects in young red alder plantations: productivity, photosynthate partitioning, and nitrogen fixation. *Ecology* 65:394-402.

Brown, K. 1999. First-year growth responses of young red alder stands to fertilization. Extension note No. 36. British Columbia Ministry of Forest Research Program. 6 p.

Coleman, M. D., R. E. Dickson, and J. G. Isehrands. 1998. Growth and physiology of aspen supplied with different fertilizer addition rates. *Physiologia Plantarum* 103:513-526.

Comeau, P. G. Harper, B. Biring, P. Fielder, and B. Reid. 1997. Effects of red alder on stand dynamics and nitrogen availability. Extension Note 09. British Columbia Ministry of Forests Research Program. 6 p.

Dawson, J. O., P. J. Dzialowy, G. Z. Gertner, and E. A. Hansen. 1983. Changes in soil nitrogen concentration around *Alnus glutinosa* in a mixed, short-rotation plantation with hybrid *Populus*. *Can. J. For. Res.* 13:572-576.

Giardina, C. P., S. Huffman, D. Binkley, and B. A. Caldwell. 1995. Alders increase soil phosphorus availability in a Douglas-fir plantation. *Can. J. For. Res.* 25: 1652-1657.

Gutierrez, G. B. 1998. Efecto de la asociación de *Pinus patula* con *Alnus firmifolia.* Master of Science Thesis. Colegio de Postgraduados. Montecillo, Texcoco, Edo. de Mexico. 96 p.

Haase, D. L., and R. Rose. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. *For. Sci.* 41:54-66.

Hamdi, Y. A. 1985. La fijación de nitrógeno en la explotación de los suelos. Boletín de suelos # 49 de la FAO. 188 p.

Hansen, E. A., and J. O. Dawson. 1982. Effect of *Alnus glutinosa* on hybrid *Populus* height growth in a short-rotation intensively cultured plantation. *For. Sci.* 28:49-59.

Imo, M., and V. R. Timmer. 1997. Vector diagnosis of nutrient dynamics in mesquite seedlings. *For. Sci.* 43:268-273.

Kalra, Y. P., and D. G. Maynard. 1991. Methods manual for forest soil and plant analysis. Forestry Canada, Northwest Region, Northern Forestry Centre. Canada. 116 p.

Larcher, W. 1995. Physiological plant ecology. (3rd edition). Springer. Germany. 506 p.

Obaton, M. 1985. Papel de las leguminosas en el ciclo del nitrógeno. 3 pp. *In*: ONU. Manual técnico de la fijación biológica del nitrógeno. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma.

Peterson, E. B., G. R. Ahrens, and N. M. Peterson. 1996. Red alder managers' handbook for British Columbia. Canadian Forest Service and British Columbia Ministry of Forests. Forest Resource Development Agreement Report No. 240. 119 p.

Quoreshi, M., and V. R. Timmer. 2000. Growth, nutrient dynamics, and ectomycorrhizal development of container-grown *Picea mariana* seedlings in response to exponential nutrient loading. *Can. J. For. Res.* 30:191-201.

Rodríguez M. M. N. 1997. Fertilización foliar en el cultivo de tomate en condiciones de invernadero. Doctor of Science Thesis. Colegio de Postgraduados, Montecillo, Mexico. 148 p.

Rothe, A., and D. Binkley. 2001. Nutritional interactions in mixed species forests: a synthesis. *Can. J. For. Res.* 31:1855-1870.

Rowland, D. L., L. Beals, A. A. Chaudhry, A. S. Evans, and L. S. Crodeska. 2001. Physiological, morphological, and environmental variation among geographically isolated cottonwood (*Populus deltoides*) populations in New Mexico. *Western North American Naturalist* 61:452-462.

Salifu, K. F., and V. R. Timmer. 2001. Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. *Soil Sci. Soc. Am. J.* 65:905-913.

Shainsky, L. J., and C. L. Rose. 1995. Effects of competition on the foliar chemistry of young Douglas-fir in monoculture and mixed stands with young red alder. *Can. J. For. Res.* 25:1969-1977.

Teng, Y., and V. R. Timmer. 1995. Rhizosphere phosphorus depletion induced by heavy nitrogen fertilization. *Soil Sci. Soc. Am. J.* 59:227-233.

Timmer, V. R., and E. L. Stone. 1978. Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. *Soil Sci. Soc. Am. Proc.* 42:125-130.

Turvey, N. D. and P. J. Smethurst. 1983. Nitrogen fixing plants in forest plantation management. *In*: Gordon, J. C. and C. T. Wheeler (Eds.). Biological nitrogen fixation in forest ecosystems: Foundations and applications. Martinus Nijhoff/Dr.W. Junk Publishers. The Netherlands. 223-259.

Valentine, D. W., and H. L. Allen. 1990. Foliar responses to fertilization identify nutrient limitation in loblolly pine. *Can. J. For. Res.* 20:144-151.

Van Miegroet, H., D. W. Cole, and P. S. Homann. 1990. The effect of alder forest cover and alder forest conversion on site fertility and productivity. *In*: Gessel, S.P., D.S. Lacate, G.F. Weetman, and R.F. Powers. Sustained productivity of Forest Soils. Proceedings of the 7th North American Forest Soils Conference, University of British Columbia, Faculty of Forestry Publication, Vancouver, B.C. Canada. pp. 333-354.

Weetman, G. F. 1989. Graphical vector analysis technique for testing stand nutritional status. Pp. 93-109. *In*: W. J. Dyck and C. A. Mees (Ed.). Research strategies for long-term site productivity. Proceedings, IEA/BE A3 Workshop, Seattle, Wa., August 1988. IEA/BE A3 Report No. 8. Forest Research Institute, New Zealand, Bulletin 152.

Zou, X., D. Binkley, and B. A. Caldwell. 1995. Effects of dinitrogen-fixing trees on phosphorus biogeochemical cycling in contrasting forests. *Soil Sci. Soc. Am. J.* 59: 1452-1458.

CAPTER 4

ALDER EFFECTS ON SOIL WATER AND PINE WATER POTENTIAL IN PINE-ALDER STANDS

ABSTRACT

To examine how alder affects soil moisture and pine water status in pine-alder mixed stands, a series of field experiments were carried out in Central Mexico. Results from single-tree essays indicated that alder clumps contribute to desiccate shallow soil horizons near around them. Results from a plot-based experiment indicated that alder significantly depleted soil moisture both, during the rainy season and during the dry season of the year. Depletion of soil water by alder, however, did not contribute to significant changes in pine predawn water potentials. Morphological, anatomical, and physiological mechanisms are suggested to be involved in avoiding sharp declines in pine water potential and in inducing drought tolerance.

INTRODUCTION

Plant species modify in one or another way their surrounding environment. In the case of alders, besides of improving soil N status, they demand many resources other than nitrogen to maintain the typical high growth rates of most alder species. If these resources are limited in the ecosystem then competition effects may become apparent in either alders or any other associated species.

The effects of alder trees on associated conifer growth in mixed conifer-alder stands have frequently been found to be positive. So, the management of this type of mixed forests has been viewed as a viable option to increase the productivity of many forest sites. Nonetheless, it is well documented to date that most alder species are high-water demanding (Hibbs 1996). Consequently, competition for water between conifer and alder individuals in pine-alder stands may eventually take place and become a critical factor for the final balance of alder effects on the conifer growth rate.

Water is frequently a limiting resource for conifer growth in Central Mexico, where precipitation varies from 600 to 1000 mm. Under these conditions, competition for water between conifers and associated plant species such as alders may mask the positive effects alders bring about through the N-fixation process.

The effects of alder clumps, on the other hand, are expected to vary spatially. That is, their influence may be restricted by factors such as distance and direction from the alder clump and soil depth as well.

Specific studies on the effects of alders on soil moisture and water status of associated species in mixed conifer-alder stands are scarce, even though interactions between alders and conifers in mixed stands must be better understood if decisions on optimal spacing and timing schemes during management planning of mixed naturally- or artificially-regenerated forests are pursued.

According to these facts, the following objectives were evaluated:

1) To study the effects of alder clumps on soil moisture as affected by distance and direction from alder clumps and soil depth.
2) To determine how varying alder densities affect soil moisture and pine water status in pine-alder stands.

REVIEW OF LITERATURE

Pinus montezumae and *Alnus arguta* are two species that frequently share common habitats in Central Mexico. Although no studies on the ecology of mixed stands in this region have been carried out, strong interactions are supposed to occur between these species as it has been determined for similar systems in other countries. Even though the alder's ability to fix atmospheric nitrogen is well known, its possible benefits have not been taken into account while making management schemes for mixed pine-alder stands in Central Mexico. Instead, greater importance has been devoted to the negative effect alder may exert on pine growth through competition for water or other resources.

In fact, just one report regarding the effects of our alder species on pine growth was found for the study area (Salas and Rivera 2000). Few works dealing with aspects related to competition for water between other alder species and associated crop trees in other countries have been published.

Hibbs (1996) asserts that red alder grows better under moist soils and that south-facing slopes are not good sites to grow alders. This implicitly means that alders intermixed with crop trees may deplete soil water and eventually induce stress in crop trees.

Bond and Kavanagh (1999) reported that red alder (*Alnus rubra* Bong.) was much less sensitive in stomatal conductance to increasing leaf-to-air vapor pressure gradients than conifers such as Douglas-fir [(*Pseudotsuga menziesii* (Mirb.) Franco] and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.]. Being in agreement with Hibbs' statement, this difference among species implies that alder may exhibit higher transpiration and water extraction rates than conifers, since the former species initiate stomatal closure only after the atmosphere's evaporative demand is quite high.

If alder species are high-water demanding, then important competition interactions with commonly associated conifers such as pines and firs most be expected. In fact, Cole and Newton (1987) and Shainsky and Rose (1995) found that red alder inhibited the growth of Douglas-fir through competition for light and water. Cole and Newton (1987) declare that competition effects in conifer-alder mixed stands may mask the positive effects that the N-fixer otherwise could exert on the conifer through the enhancement of the availability of nitrogen. In agreement with this, Brockley and Sanborn (2003) attributed a reduction in *Pinus contorta* growth to supposed competition interactions between this species and individuals of *Alnus viridis*.

In an experiment to study growth and biomass allocation of Douglas-fir and red alder as affected by contrasting light and soil moisture availability, Chan *et al.* (2003) found that red alder growth responded negatively to water limitation. This finding supports the idea that high soil moisture is more important than light for red alder, even though the high requirement for light by this species is also well documented (Harringtin 1990, Hibbs 1996, British Columbia Ministry of Forests 1997, Peterson *et al.* 1996).

Chan *et al.* (2003) also found that alder's strategy to successfully compete for resources seems to consist in allocating more mass to aboveground biomass components in order to increase early growth rates, light capture, and competitive ability.

According to this background on alder species, significant negative effects via competition for water are expected to be exerted by our alder species on *P. montezumae*. However, such negative effects are also expected to be offset to a certain extent by the recognized drought resistance of our conifer species (Rojas *et al.* 1988, Aldana and Aureoles 1991).

MATERIALS AND METHODS

Location of the study area

Because of several practical reasons, the studies on competition for water between pine and alder trees were carried out in the area of regeneration 1990 from the Ejido Sabanetas, in the municipality of Singuilucan, State of Hidalgo.

First, as mentioned in Chapters 1 and 2, two of the areas of regeneration in the Ejido Los Romeros, where most of the research work was carried out were burnt in the early 2001, and most alder clumps were still too small as to significantly compete for soil resources with pine trees. Therefore, the only area available in the year 2002-2003, when these experiments were carried out, was the area of regeneration 1987. However, in this area there were important variations in the density and size between pines and alders, so that effects from competition for water between pine and alders, would have been hard to detect, unless pine density was standardized. However, it was estimated that standardization of pine density would result in large expenses provided that pine trees in that area of regeneration were big and specialized equipment would be needed.

In the area selected (area of regeneration 1990, Ejido Sabanetas), pine density was quite homogeneous and alder clumps more vigorous, so that these conditions were much more appropriate to easily manage both, pine and alder densities within the plots.

The area of regeneration 1990, is located about 1.5 Km south east from the area of regeneration 1987. The management regime, has been similar to that performed in the other regeneration areas. Alders in this area of regeneration were eliminated for the last time in the year 2000. The area of regeneration was not altered since.

Ecological features in this area of regeneration are similar to those in the area of regeneration 1987. Therefore, for details on this type of variables, please refer to the materials and methods section in Chapter 1.

Experimental approaches

Two experiments or essays were done to compliment a one-year plot-based experiment on competition for water. These essays were intended to get some side information that would have otherwise been difficult to be collected from the main plot-based experiment due to financial resource limitations, Both essays were factorial experiments with experimental units distributed according to a complete random design. The factors and levels for both essays are shown in Table 4.1.

Table 4.1. Factors and levels studied in essays # 1 and # 2 in the area of regeneration 1990. ejido Sabanetas, Singuilucan, Hidalgo. Mexico.

	Essay # 1				**Essay # 2**					
Factors	Distance from tree (cm)		Direction from tree		Species		Distance from tree (cm)		Soil depth (cm)	
Levels	50	100	Downslope	Upslope	Pine	Alder	50	100	15-20	25-30

According to the previous chart, the essay # 1 consisted of four treatments in total, whereas the essay # 2 had eight treatments. The experimental unit was a single pine or alder tree, of nearly homogeneous dimensions. Each treatment was replicated four and five times in essay #1 and #2, respectively.

The main experiment was a complete randomized one, where the effect of three alder densities on soil moisture and associated pine water potential was studied. In this experiment, pine leaf area per plot was homogenized to 65 m^2 pine leaf area per plot by cutting some pine trees and branches. Alder leaf area per plot, on the other hand, was manipulated to leave just the desired leaf area-based alder densities. The alder densities tested in this experiment were: 0.0, 5.0, and 9.0 m^2 leaf area/plot. The experimental unit in this experiment was a 7 m by 7 m plot, and each treatment was replicated three times.

The estimation of pine and alder leaf areas within the plots was carried out by using the models generated in chapter 1. such models are:

For pine trees

$PLA = (0.0073963 * NDW) - 0.00014055$, $R^2 = 0.9685$, $RMSE = 3.1562$, $N = 10$

where:

PLA = Pine needle area (m^2)

NDW = Needle dry weight (g)

Needle dry weight was estimated with:

$NDW = 0.0088*DBH^{2.0809}$, $R^2 = 0.8172$, $RMSE = 0.2775$, $N = 9$

where:

NDW = Needle dry weight (Kg)

DBH = Diameter at breast height (cm)

For alder sprouts

$ALA = 0.19113 * SD^{1.5392536}$, $R^2 = 0.9168$, $RMSE = 0.2748$, $N = 33$

where:

ALA = Alder leaf area (m^2)

SD = Sprout collar diameter (cm)

Variables measured

Soil moisture

Within each of the plots, soil moisture and pine predawn water potential were evaluated during a year-round period, starting June 2002. Soil moisture was evaluated from soil samples extracted with a soil corer at a depth of 25-30 cm every week from June to December 2002. From January 2003 through June 2003, soil samples were obtained every other week. From each plot, two composite soil samples were obtained at each sampling date.

Soil samples were put into aluminum cans and transported to the laboratory, where they were weighed using an analytical scale, oven dried at 105 °C until constant weight, and re-weighed for water loss determination.

Predawn pine water potential

Predawn pine water potential was measured using a pressure bomb (Scholander *et al.* 1965). These measurements were made by using fascicle samples from two trees at each plot, selected at the beginning of the experiment. Fascicle samples were always obtained from fixed whorls at each of the selected trees. One needle from each tree was used to measure water potential. Data on water potential were taken on the same dates when soil moisture samples were obtained.

Water retention curve

In order to establish the water retention curve for the study area, a composite 30 cm-depth soil sample was collected in May 2002. The soil sample was returned to the Soil Physics Laboratory at the Colegio de Postgraduados, where the water retention curve was determined.

RESULTS

Soil depth was the only factor that significantly (p-value=0.05) affected soil moisture (Table 4.2). Soil moisture was lower at a depth of 30 cm than at 20 cm.

Table 4.2. Tukey tests (α=0.05) for soil moisture (% W/W) in essays #1 and #2.

Essay #1				Essay #2					
Distance from alder (cm)†		Direction†		Species†		Distance from tree (cm)†		Depth†	
50 ††	100 ††	Downslope††	Upslope††	Pine††	Alder††	50 ††	100 ††	20 cm††	30 cm††
45.168a	47.627a	45.900a	48.895a	42.219a	43.583a	41.814a	43.793a	45.729a	39.879b

†Factors tested
††Factor levels tested
Values with the same letters within a factor are not significantly different (α=0.05).

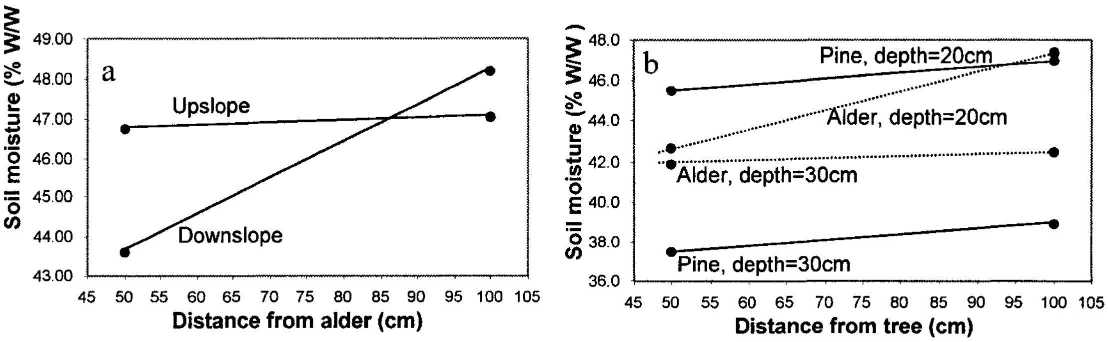


Figure 4.1. Effects of alder and pine trees on soil moisture. a) distance*direction interaction and b) species*distance*depth interaction.

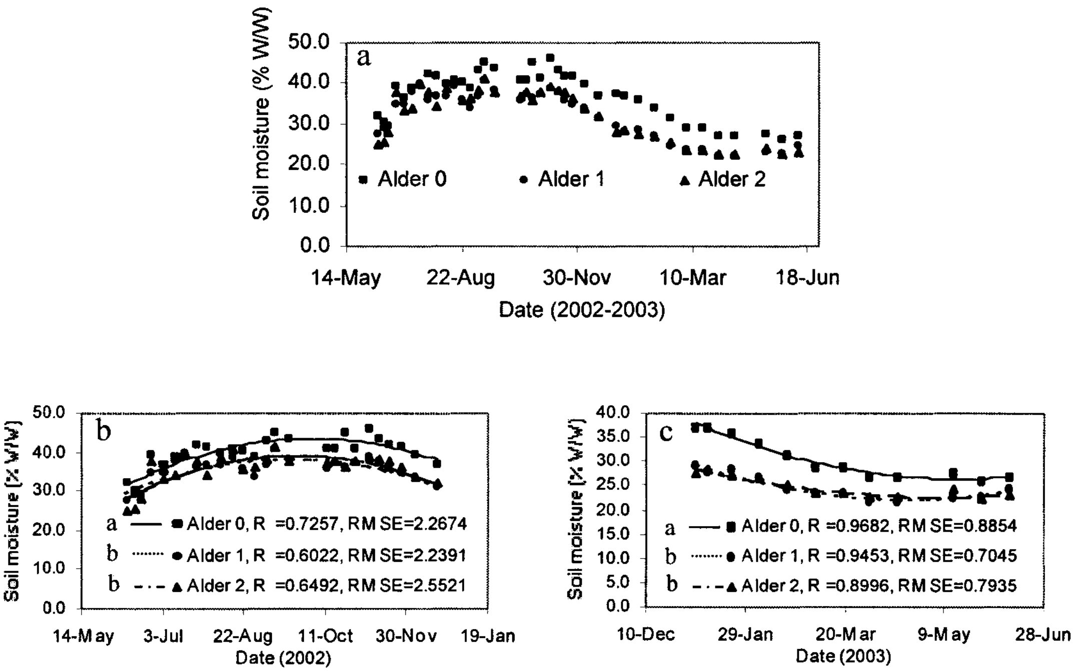


Figure 4.2. Variation of soil moisture as a function of time with varying alder density. a) from June 2002 through June 2003, b) from June to December 2002, and c) from January to June 2003. Curves with different letters indicate soil moistures are significantly different ($\alpha=0.05$).

Soil moisture is lower at 30 cm depth regardless of the distance from pine tree (Figure 4.1b). Also, soil moisture is lower at 50 cm from pine tree than at 100 cm, regardless of soil depth. The same is not true for the case of alder. Soil moisture at 100 cm from alder clumps is higher at 20 cm depth than at 30 cm depth. However, at a distance of 50 cm from alder clumps, soil moisture is nearly the same, regardless of depth (Figure 4.1b). In general, alder tends to be more soil desiccating than pine at a depth of 20 cm (p-value=0.3428) close around the tree but not at 100 cm away. At a depth of 30 cm, however, pine desiccates the soil more than alder clumps do, regardless of distance from tree.

The alder density within plots and the Julian day as well, had significant effects on soil moisture, regardless of the period of the year (Table 4.3).

Table 4.3. Reliance of soil moisture on alder density, with square Julian day as a covariable.

Source of variation	Significance level (α) per period of year	
	June-December 2002	January-June 2003
Alder density	0.0001	0.0001
Julian day^2	0.0022	0.0001

Table 4.4. Tukey tests (α =0.05) for variation in soil moisture (% W/W) with varying alder density, during two periods of year.

Alder density	Average soil moisture (% W/W)	
	June-December 2002	January-June 2003
0 (no alder)	39.8150 a	30.6333 a
1 (5 m^2 L.A. per plot)	35.7350 b	24.8500 b
2 (9 m^2 L.A. per plot)	35.5040 b	24.8250 b

Different letters within a period of year indicate soil moisture is significantly different

No significant differences (α=0.05) in pine water potential existed among plots with varying alder densities (Table 4.6). This is true for both periods of year studied. This is confirmed by the information shown in Table 4.5, which indicates that alder density did not significantly affect pine water potential during either period of year.

Julian day did significantly affect pine water status during the rainy season (p-value=0.0002, Table 4.5).

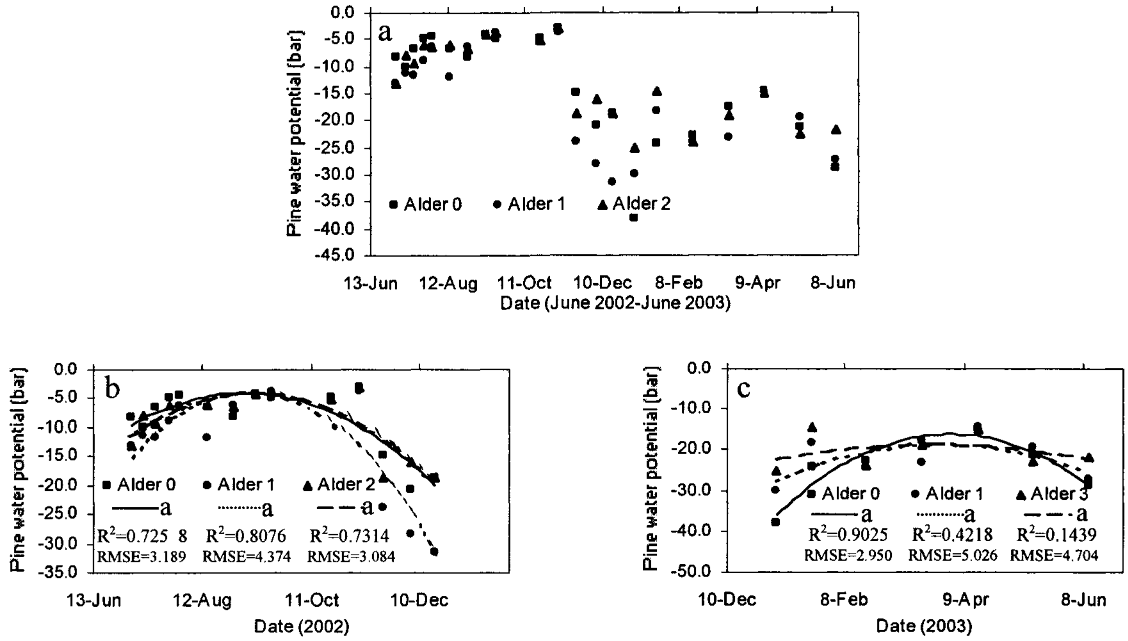


Figure 4.3. Variation of pine water potential as a function of time with varying alder density. a) from June 2002 through June 2003, b) from June to December 2002, and c) from January to June 2003. Curves with different letters indicate pine water potentials are significantly different (α=0.05).

Table 4.5. Reliance of pine water potential on alder density, with square Julian day as a covariable.

Source of variation	Significance level (α) per period of year	
	June-December 2002	January-June 2003
Alder density	0.2160	0.5270
Julian day^2	0.0002	0.8339

A regression analysis of predawn pine water potential on soil moisture indicates that there exists a significant relationship (p-value=0.0371) between these variables.

Nonetheless, variation in soil moisture explains only 12 % of the variation in pine water potential (Figure 4.4).

Table 4.6. Tukey tests (α =0.05) for variation in pine water potential with varying alder density, during two periods of year.

Alder density	Average pine water potential (bar)	
	June-December 2002	January-June 2003
0 (no alder)	-8.650 a	-24.029 a
1 (5 m^2 L.A. per plot)	-12.214 a	-22.414 a
2 (9 m^2 L.A. per plot)	-8.979 a	-20.271 a

Different letters within a period of year indicate alder densities are different

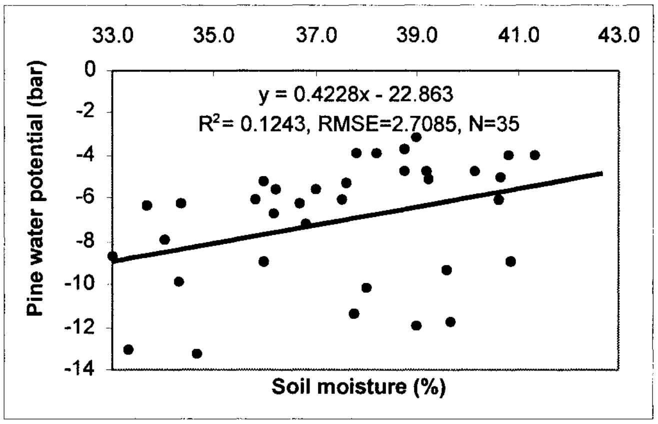


Figure 4.4. Effect of soil moisture on pine water potential in the area of regeneration 1990.

Soil moisture at a soil water potential of 1.5 MPa (15 bar), which conventionally corresponds to the permanent wilting percentage (Hillel 1998, Brady and Weil 2000, Chan *et al.* 2003), was 26.2 % (W/W)). Field capacity of our soil (0.03 MPa in soil tension, Chan *et al.* 2003), on the other hand was 52.6 % (W/W).

A comparison of Figures 4.2 and 4.5 suggests that none of the alder density treatments reached a soil moisture content lower than that corresponding to the permanent wilting percentage of 26.2 % during the rainy season of the year 2002. However, during the dry season, soil moisture in the plots with alder was generally lower than the permanent wilting percentage. Soil moisture in the "no alder" plots was generally higher than wilting point for our soil.

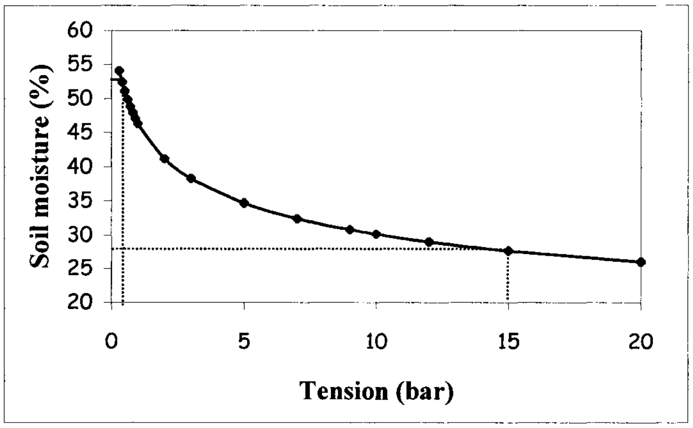


Figure 4.5. Water retention curve for the area of regeneration 1990.

DISCUSSION

Results from essay #1 indicated that soil moisture is lower near (50 cm) alder clumps than at a longer distance (100 cm) from trees. Even though no significant differences in soil moisture between the two distances were found, the consistency of trends (among replicates) indicates that alders contribute in some way to soil desiccation. Few reports related to alder effects on soil moisture were found, and their results support our finding. Shainsky and Radosevich (1992) found that soil moisture limitations were created by increasing density of either alder or Douglas-fir in red alder Douglas-fir stands in Corvallis, Oregon. These effects of alder on soil moisture are consistent with the high demand for water that most alder species present (Chan *et al.* 2003, Hibbs 1996)

It is likely that this type of effects of alder clumps on soil moisture was responsible for the significant differences in soil moisture found between "no alder" plots and plots with any of two levels of alder density, along the whole year (Figure 4.2 b,c).

Even though the differences in soil moisture between "no alder" plots and plots with alder registered in our experiment were significant (Figure 4.2 b,c), pine predawn water potential was not significantly affected by the same alder-density treatments in none of the periods of the year studied (Figure 4.3). Meinzer (1993) states that there exists a coordination between stomatal conductance and soil hydraulic capacity, roots and stems to supply the leaves with water. Such a coordination eventually results in a balance between water loss and water transport capacity to maintain leaf water potential relatively constant over a wide range of growth conditions.

The behavior found in the pine species studied in this investigation indicates that there are some mechanisms that induce tolerance of *P. montezumae* to drought as found by Rojas *et al.* (1988) and Aldana and Aureoles (1991). There exist very few studies on the possible mechanisms that induce drought resistance of *P. montezumae*. It is likely, however, that some interactions among the several mechanisms suggested by these works come into play to generate the tolerance to drought that this species presents.

First, during the rainy season of the year (Figure 4.2b), the reductions in soil moisture occurring in the plots with alder practically never led to soil moistures below that corresponding to the permanent wilting point (26.2 %) that we determined based on our the soil retention curve (Figure 4.5). This suggests that this fact could at least partially explain the lack of differences in pine water potential during this period of the year. During the dry season (Figure 4.2c), however, average soil moisture in plots with alder was lower than the one corresponding to the permanent wilting point (Table 4.4). Therefore, one or more mechanisms for pine trees to avoid reductions in needle water potential even when soil moisture in the surface horizons is low must be in play.

Soil layers explored by alder and pine seem to be different. Figure 4.1b indicates that, at least near the trees (50 cm), alders are more soil desiccating than pines at a depth of 20 cm. However, at a depth of 30 cm, pines extract more water than alders. This means that pine rooting zone is deeper than that of alder, probably allowing the former species to get water from deeper soil horizons and to cope with top soil desiccation brought about by alder, as proposed by Neary *et al.* (1990) and Waring and Schlesinger (1985) for mixed-species stands.

Waring and Schlesinger (1985) point out that many deeply-rooted species explore deeper soil horizons as shallow soil layers become dry, as a mechanism to avoid sharp changes in leaf water potential that could eventually lead to reductions in CO_2 uptake and growth. In agreement with this statement and with our finding, Ewers *et al.* (2000) found a lack of correlation between *Pinus taeda* needle water potential and soil water potential, attributing this behavior to the reliance of pine water status on deep water.

Besides this possible morphology-induced mechanism, other physiological and/or anatomical strategies could also be involved in the buffering of the changes in pine water potential with changing soil moisture. Vargas and Muñoz (1988b) studied several growth variables in four pine species including *P. montezumae*, and found that the resistance of this species to drought was closely linked to growth reductions. This means that this pine species may either, exhibit strategies dealing with stomatal closure under moisture stress, or present a lower stomatal frequency than the other species studied. Any of these features, in turn, would eventually lead to reductions in CO_2 uptake and growth rates as found by Vargas and Muñoz (1988b).

In other work, the same authors (Vargas and Muñoz 1988a) studied the stomatal frequency in four Mexican pine species including *P. montezumae*, finding that this anatomical variable was similar in all the species examined (*P. patula*, *P.leiophylla*, and *P. greggii*), suggesting that the resistance to drought in *P. montezumae* could be a result of a higher stomatal sensitivity.

A high stomatal sensitivity of *P. montezumae* could explain why pine water potential did not significantly vary with decreasing soil moisture in our study, since a high stomatal sensitivity implies that stomata may rapidly close as soil moisture goes down, preventing the tree from water loss, and allowing xylem water potential to remain fairly unchanged.

The lack of significant changes in pine water potential with decreasing soil moisture may also be a reflection of the influence of a variety of factors, besides soil moisture, on pine water potential. In fact, Figure 4.4, shows that even though the relationship between soil moisture and plant water potential is significant (p-value=0.0371), it is easy to notice that there exists a substantial variation in pine water potential with relatively small changes in soil moisture. This means that there are other factors that influence pine water status, apart from soil moisture, which explains just 12

% of the variation in pine water potential. Among these such factors, wind velocity and water pressure deficit are likely to be into play (Landsberg and Gower 1997).

The high variation of pine water potential with small changes in soil moisture (Figure 4.4) also implies that a higher difference in soil moisture than that found among alder density treatments (Figure 4.2b,c) in both periods of the year is needed to induce a significant difference in pine water potential.

Even though no differences in needle water potential among alder density treatments for any of the two periods of the year were found (Table 4.6) large differences in this variable were registered between both periods of the year. From January to June, needle water potentials were too low in comparison with those from June to December. According to an independent experiment (Lopez, unpublished data), it is likely that pine growth during the dry season of the year experienced reductions since water potentials as low as 15 bar induce reductions in transpiration probably because of stomatal closure (Vargas and Muñoz 1988a) in this pine species.

It is not an easy task to investigate, by using simple field experiments, the effects of competition for water between N-fixers and associated crops because the growth of the associated trees is a result of the integration of both positive (from N-fixation) and negative (from competition for water) effects at a time. To precisely evaluate the negative effect from competition for water in such a system, it would be necessary to suppress all possible positive effects that N-fixers have on the growth of associated species. This can be done by optimizing nutrient availability by using chemical fertilization, including not only N, but also P (Binkley 1983, Van Miegroet *et al.* 1990, Shainsky and Radosevich 1992, Shainsky and Rose 1995, Rothe and Binkley 2001) and

K (chapter 2 in this thesis). Optimization of nutrient availability would prevent pine from responding to slight increases in nutrient availability brought about by the presence of the N-fixer.

Water relations in other species-mixture systems frequently indicate that crop tree species performance is negatively influenced by competing species. Ludovici and Morris (1997) studied the water relations in seedlings of *P. taeda* as affected by the presence of crabgrass (*Digitaria* spp.). They found that soil water contents at all depths tested were at least 50 % lower in mixed species treatments compared with pine monocultures during a dry–down cycle. Similar results were found by Shainsky and Radosevich (1986) while studying a replacement series of *Pinus ponderosa* and *Arctostaphylos patula* in California. These results give some light about the different responses that may raise depending on the species combinations in the system.

CONCLUSIONS

According to the points previously discussed, we may conclude the following:

Both the individual-tree and the plot-based experiments indicated that alder clumps contribute to decrease soil moisture around them.

The presence of alders within the plots did not significantly affect pine water potential.

Pinus montezumae is likely to have mechanisms such as an efficient stomatal sensitivity that prevent plant water potential from sharply decreasing in response to drops in soil moisture availability.

Provided that stomatal control of water loss also brings about reductions in CO_2 absorption, pine growth rates are likely to decrease when soil moisture goes down even though needle water potentials remain unchanged. This, however remains to be studied in more detail.

LITERATURE CITED

Aldana, B. R., and S. Aureoles C. 1991. Fenología de brotes anuales de *Pinus hartwegii* Lindl. y *Pinus montezumae* Lamb. y su relación con los factores ambientales físicos, en la Estación Forestal Experimental Zoquiapan, México. Bachelor Thesis. Universidad Autónoma Chapingo, Mexico. 80 p.

Binkley, D. 1983. Ecosystem production in Douglas-fir plantations: Interactions of red alder and site fertility. *For. Ecol. and Manage.* 5: 215-227.

Bond, B. J. and K. L. Kavanagh. 1999. Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiology.* 19:503-510.

Brady, N. C. and R. R. Weil. 2000. Elements of the nature and properties of soils. 12th ed. Prentice Hall. New Jersey. 559 p.

British Columbia Ministry of Forests. 1997. Operational summary for the dry alder complex. *In*: Operational summary for vegetation management. Province of British Columbia. 11 p.

Brockley, R. P., and P. Sanborn. 2003. Effects of sitka alder on the growth and foliar nutrition of young lodgepole pine in central interior of British Columbia. *Can. J. For. R*es. 33:1761-1771.

Chan, S. S., S. R. Radosevich, and A. T. Grotta. 2003. Effects of contrasting light and soil moisture availability on the growth and biomass allocation of Douglas-fir and red alder. Can. *J. For. R*es. 33:106-117.

Cole, E. C., and M. Newton. 1987. Nutrient, moisture, and light relations in 5-year-old Douglas-fir plantations under variable competition. *Can. J. For. Res.* 17:181-186.

Ewers, B. E., R. Oren, and J. S. Sperry. 2000. Influence of nutrients versus water supply on hydraulic architecture and water balance in *Pinus taeda*. *Plant, Cell and Environment*. 23:1055-1066.

Harrington, C. A. 1990. *Alnus rubra* Bong – red alder. *In*: Burns, R. M., and B. H. Honkala. Silvics of North America. Vol. 2., Hardwoods. USDA. For. Serv., Agric. Handbook 654. Washington D. C. pp. 116-123.

Hibbs, D. E. 1996. Managing red alder. The woodland workbook EC1197. Oregon State University Extension Service. 8 p.

Hillel, D. 1998. Environmental soil physics. Academic Press. San Diego. 771 p.

Landsberg, J. J. and S. T Gower. 1997. Applications of physiological ecology to forest management. Academic press. San Diego. 354 p.

Ludovici, K. H. and L. A. Morris. 1997. Competition-induced reductions in soil water availability reduced pine root extension rates. *Soil . Sc. Soc. Amer. J.* 61:1196-1202.

Meinzer, F. C. 1993. Stomatal control of transpiration. *Tree*. 8:289-294.

Neary, D. G., E. J. Jokela, N.B. Comerford, S. R. Colbert, and T. E. Cooksey. 1990. Understanding competition for soil nutrients- the key to site productivity on southeastern coastal plain spodosols. *In*: Gessel, S.P., D.S. Lacate, G.F. Weetman, and R.F. Powers. Sustained productivity of Forest Soils. Proceedings of the 7th North American Forest Soils Conference, University of British Columbia, Faculty of Forestry Publication, Vancouver, B.C. Canada. Pp. 432-449.

Peterson, E. B., G. R. Ahrens, and N. M. Peterson. 1996. Red alder managers' handbook for British Columbia. Canadian Forest Service and British Columbia Ministry of Forests. Forest Resource Development Agreement Report No. 240. 119 p.

Rojas R. F. E., M. R. Keyes, and A. Martínez G. 1988. Susceptibilidad al substrato edáfico y a la sequía de diez especies de pinos. *Agrociencia.* No. 72.

Rothe, A. and D. Binkley. 2001. Nutritional interactions in mixed species forests: a synthesis. *Can. J. For. Res.* 31:1855-1870.

Salas, M. J. J. and J. Rivera R. 2000. Efecto de la proporción entre pino y aile sobre el crecimiento de rodales jóvenes de pino. Superior Technician Thesis. Universidad Tecnológica de la Costa Grande de Guerrero. 22 p.

Scholander, P. F., T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. *Science.* 148:339-345.

Shainsky, L. J. and S. R. Radosevich. 1986. Growth and water relations of *Pinus ponderosa* seedlings in competitive regimes with *Arctostaphylos patula* seedlings. *J. App. Ecol.* 23:957-966.

Shainsky, L. J., and S. R. Radosevich. 1992. Mechanisms of competition between Douglas-fir and alder seedlings. *Ecology.* 73:30-45.

Shainsky, L. J. and C. L. Rose. 1995. Effects of competition on the foliar chemistry of young Douglas-fir in monoculture and mixed stands with young red alder. *Can. J. For. Res.* 25:1969-1977.

Van Miegroet, H., D. W. Cole, and P. S. Homann. 1990. The effect of alder forest cover and alder forest conversion on site fertility and productivity. *In*: Gessel, S.P., D.S.

Lacate, G.F. Weetman, and R.F. Powers. Sustained productivity of Forest Soils. Proceedings of the 7th North American Forest Soils Conference, University of British Columbia, Faculty of Forestry Publication, Vancouver, B.C. Canada. pp. 333-354.

Vargas H. J. and A. Muñoz O. 1988a. Frecuencia estomatal y tamaño del tejido vascular en hojas primarias de cuatro especies de *Pinus*. *Agrociencia* No. 72:173-181

Vargas H. J. and A. Muñoz O. 1988b. Resistencia a sequía: II. Crecimiento y supervivencia en plántulas de cuatro especies de *Pinus*. *Agrociencia* No. 72:197-208.

Waring, R. H., and W. H. Schlesinger. 1985. Forest ecosystems. Concepts and management. Academic Press Inc. New York. 325 p.

APPENDIX 1

Pine and alder densities and pine/alder ratios (PARs) in the experimental plots used in chapter 1.

PAR level	Block	Pine density (# of pine trees per plot)			Alder density (# of alders per plot)			Pine/alder ratio LA/LA		
		Area 1987	Area 1989	Area 1994	Area 1987	Area 1989	Area 1994	Area 1987	Area 1989	Area 1994
1	1	20	15		46	20		Area 1987	Area 1989	Area 1994
1	2	26	21		32	28		1.51	3.2	
1	3	†‡34	26	†‡12	43	18	46	2.47	3.8	
1	4	†‡49	29	†‡17	37	21	69	3.21	3.9	0.20
1	5	†‡69	35	†‡17	38	23	51	3.31	4.2	0.22
1	6	†78	37		51	28		3.43	4.4	0.27
		*46.0	*27.2	*15.3	*41.2	*23.0	*55.3	3.84		0.28
2	1	44	29		16	13		*2.96	*3.9	*0.24
2	2	†‡45	31	†11	14	16	31	5.15	4.7	
2	3	†‡49	33	†‡14	15	19	35	6.05	5.2	0.31
2	4	†‡58	34	†‡16	29	19	36	7.58	5.2	0.33
2	5	†71	37	†‡19	39	16	50	7.67	5.4	0.35
2	6	78	41		18	19		7.72	5.6	0.37
		*57.5	*34.2	*15.0	*21.8	*17.0	*38.0	8.92		
3	1	21	18	†13	4	7	25	*7.18	*5.22	*0.34
3	2	†‡47	19	†‡16	10	8	27	9.25	5.6	0.38
3	3	†‡61	30	†‡18	11	11	30	10.63	5.8	0.44
3	4	†‡68	31	†‡19	13	13	38	11.62	6.4	0.49
3	5	†71	38		13	13		13.42	6.6	0.54
3	6	76	41		11	15		13.55	7.6	
		*57.3	*29.5	*16.5	*10.3	*10.5	*30.0	14.33		
4	1	†‡51	20	†13	7	6	16	*12.13	*6.40	*0.46
4	2	†‡55	29	†‡15	7	8	19	14.39	7.7	0.56
4	3	†‡68	33	†‡18	7	10	23	14.64	7.7	0.73
4	4	†69	34	†‡18	9	9	23	15.42	8.3	0.76
4	5	69	39		9	13		15.81	8.5	0.78
4	6	75	40		9	12		16.19	10.0	
		*64.5	*32.5	*16.0	*8.0	*9.7	*20.2	18.95		
5	1	26	22	†‡13	1	1	15	*15.90	*8.44	*0.71
5	2	†‡42	28	†‡15	1	2	7	26.34	10.7	0.84
5	3	†‡63	33		1	2		27.28	10.8	1.00
5	4	†‡71	38	†‡18	2	9	15	29.92	12.2	1.28
5	5	†72	39		3	7		31.83	14.7	1.44
5	6	79	42		3	6		36.42	25.0	
		*58.8	*33.7	*15.3	*1.8	*4.5	*12.3	40.08		
Area mean		**56.8**	**31.4**	**15.6**	**16.6**	**12.9**	**31.2**	***32.00**	***14.68**	***1.44**

PAR level= Pine/alder level. * Mean values for a given pine/alder-ratio level. † Selected plots for the Chapter 2 experiment

APPENDIX 2

Pine and alder densities and pine/alder ratios in the experimental plots used for the fertilization experiment (chapter 3).

Pine/alder-ratio level	Pine density (# of pine trees per plot)			Alder density (# of alders per plot)			Pine/alder ratio (Number/number)		
	Area 1987	Area 1989	Area 1994	Area 1987	Area 1989	Area 1994	Area 1987	Area 1989	Area 1994
1	84	49		30	35		2.80	1.40	
1	94	77		29	30		3.24	2.57	
1	106			31			3.42		
	*94.7	*63.0		*30.0	*32.5		*3.15	*1.98	
2	95	69		18	21		5.28	3.29	
2	96	72		18	20		5.33	3.60	
2	96			17			5.65		
	*95.7	*70.5		*17.7	*20.5		*5.42	*3.44	
3	99	63		11	6		9.00	3.94	
3	88	64		9	13		9.78	4.92	
3	89			13			6.85		
	*92.0	*63.5		*11.0	*9.5		*8.54	*4.43	
4	89	76		5	11		17.80	6.91	
4	92	72		5	8		18.40	9	
4	98			7			14.00		
	*93.0	*74.0		*5.7	*9.5		*16.73	*7.95	
5	92	78		3	4		30.67	19.5	
5	94	91		1	3		94.00	30.33	
5	115			1			115		
	*100.3	*84.5		*1.7	*3.5		*79.89	*24.91	
Area mean	**95.14**	**71.3**		**13.22**	**15.1**		**22.74**	**8.54**	

* Mean values for a given pine/alder-ratio level

APPENDIX 3

Estimated nitrogen fixation rates for a plot with nine alder trees under two light regimes.

Light condition	Sample #	Nodule DW tree^{-1}	Nitrogenase activity (µmol g-dry nodule^{-1} h^{-1})	N fixation rate * Kg N yr^{-1} alder tree^{-1}
Sun	1	3.9212	204	0.049084
Sun	2	2.4962	183	0.028030
Sun	3	1.6008	91	0.008939
Sun	4	1.8546	84	0.009559
Sun	5	6.4094	185	0.072758
Total sun				*0.168369*
Shade	6	4.8278	198	0.058655
Shade	7	0.6802	74	0.003089
Shade	8	3.2930	175	0.035361
Shade	9	1.1790	89	0.006439
Total shade				*0.103543*
Grand total (9 trees)				***0.271912*****

*N fixation rate was calculated using an Acetylene-Dinitrogen conversion factor of 4 (Hamdi 1985).

** This N fixation rate is equivalent to 12.08 Kg N ha^{-1} yr^{-1}. (for alder density of 400 clumps/ha)

Laboratory method: Acetylene reduction

APPENDIX 4

Some physical and chemical characteristics of two soils with extreme pine/alder ratio levels. Area of regeneration 1987.

P/A Ratio	Bulk density (g.cm^{-1})	pH	O.M. (%)	Total N (%)	P (Bray) (%)	K (%)	CEC (cmol g^{-1}
Lowest (A1)	1.16	5.63	4.71	0.21	0.000747	0.0341	17.06
Highest (A5)	1.06	5.68	4.84	0.20	0.000699	0.0334	17.07

P/A ratio=Pine/alder ratio (Number/number)

Laboratory methods:

Bulk density:	Probete method
PH:	PH-meter using a 1:2 soil:water ratio paste
Organic matter:	Walkley and Black
Total N:	Kjeldahl
P:	Bray
K:	Extraction with ammonium acetate 1.0 N, pH 7. Atomic absorption spectrophotometry
C.E.C	Ammonium acetate 1.0 N, pH 7. Centrifuging

APPENDIX 5

Procedure for calculations of Pine/alder ratio (Number/number) from Pine/alder ratio (Leaf area/Leaf area).

AREA 1987

DATA:
1) Pine/alder ratio (PAR, LA/LA)
2) Average number of pine trees per plot
3) Average DBH per plot
4) Plot area (m^2)

CALCULATIONS:
1) Needle Dry Weight (NDW, g)
2) Pine Leaf Area (PLA, m^2)
3) Alder Leaf Area (ALA, m^2)
4) Number of alders per plot (NA)
5) Pine trees ha^{-1} / alder trees ha^{-1}

EQUATIONS:

1) $NDW=(0.0088*DBH^{2.0809})*1000$
2) $PLA=(0.0073963*NDW) - 0.00014055$
3) $ALA=PLA/PAR(LA/LA)$ (by definition)
4) $ALA=3.9435+(1.7486\ NA)$, therefore, $NA= (ALA-3.9435)/1.7486$

EXAMPLE

DATA:
1) 11.16
2) 60 pine trees per plot
3) Average pine DBH = 25

CALCULATIONS:
1) $NDW=0.0088*(25^{2.0809})*1000 = 7136$ g
2) $PLA=(0.0073963*7136) - 0.00014055 = 52.8$
3) $ALA=52.8/11.16 = 4.73$
4) $NA= (4.73-3.9435)/1.7486 = 0.45$
5) Pine trees ha^{-1} / alder trees ha^{-1} = (60*10000/225) / (0.45*10000/225)
= 2666 / 20

AREA 1994

DATA:

1) 0.57
2) 16 pine trees per plot
3) Average Root Collar Diameter (RCD, cm) = 9

EQUATIONS (Specific for this area of regeneration):

1) $NDW = 0.1882\ RCD^{3.9449}$