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Tree spacings and red:far-red light effects on juvenile *Populus* growth and morphology

by

Sovith Sin

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

**DOCTOR OF PHILOSOPHY**

Major: Forestry (Forest Biology – Wood Science)

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For the Major Program

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For the Graduate College
DEDICATION

I would like to dedicate these works to my mother, wife, son, brothers, and in
memories of my father and mother in law.
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ABSTRACT

Cottonwood stem growth and biomass accumulation are altered by changes in the red to far-red light ratio (R:FR). Genetic variation in this response might be exploited to improve yield. The first objective of this study was to detect the R:FR signals that affect important morphological traits and biomass accumulation under field conditions. The second objective was to compare the effect of R:FR signals on growth traits under greenhouse conditions. The third objective was to compare genotypic variation in responses to R:FR ratio changes. We focused on morphological traits (such as height, diameter, stem taper, and branching), biomass deposition, and non-structural carbohydrate accumulation. Two approaches were used to achieve different R:FR ratios. In the field, two spacings (40 cm and 3 m) were used. In the greenhouse, Plexiglass® chamber filters filled with copper sulfate solution or water were used along with a no-filter control. Six clones (one *Populus deltoids* Bartr., one *P. trichocarpa* Torr. & Gray, and four of their F2 hybrids) were used in the studies. The R:FR ratios inside copper sulfate filters were 1.2, water - 0.6, and no filter - 0.6. The R:FR ratios under both narrow and wide spacings changed dramatically from 1.2 immediately after planting to 0.4 after 42 days from planting at narrow spacing. The results of both greenhouse and field studies showed that trees exposed to low R:FR ratios increased height 10 to 15% (*P* < 0.05) and accumulated 7 to 10% more biomass than trees subjected to high R:FR ratios. However, in the greenhouse trees under the “no-filter” treatment had the shortest stems and least biomass accumulation. They did have increased stem tapers which might be the result of responses to wind flexing. Total leaf areas were lower for trees subjected to high R:FR signals. Clonal effects were significant for most metric traits. Some clones did appear to be less sensitive to the R:FR light.
CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

The dissertation is divided into four chapters. The first chapter is a general introduction, which is divided into two sections: dissertation organization and literature review. The second and third chapters are individual research papers for submission to a scientific journal. Both of those chapters consist of an abstract, introduction, materials and methods, results, discussion, and references. The last chapter is a general summary of the research findings. Literature citations in the General Introduction are listed at the end of the dissertation. The papers in the dissertation present the results of research on *Populus* spp. and their hybrids in response to different ratios of red to far-red (R:FR) light.

Literature Review

Introduction

Wood biomass and bioenergy are widely used on a global basis. People in developing countries depend on wood fuel for household uses and for small industry. Wood bioenergy has become more attractive to many developed countries for environmental reasons such as global warming. Wood biomass is also attractive to the paper industry and other wood processing companies. While global population and the demand for tree biomass is increasing, the land area for growing trees will not increase. Indeed, areas used for wood production have decreased drastically. FAO (1999) reported that 3,350 million m$^3$ of forest were harvested in 1995 and 2,100 million m$^3$ or 63 % of the harvest was used for energy purposes. Among these areas harvested, 10 % were converted into agricultural land.
Therefore, research on increasing biomass production is important: (i) to maintain the balance between biomass production, increasing wood demands, and forestland lost, (ii) to produce clones with better growth rates for biomass plantations, and (iii) to maintain genetic diversity for future tree breeding and tree physiology purposes.

Species background

The genus *Populus* belongs to the *Salicaceae* family. It consists of approximately 30 species (Dickman and Stuart, 1983; Eckenwalder, 1996). This genus has been used to produce biomass energy, pulpwood, and veneer (Dickman and Stuart, 1983). *Populus* is one of the easiest propagating and fastest growing temperate trees. In the current studies, two clones representing two species (*Populus deltoides* Bartr., *P. trichocarpa* Torr. & Gray) and four clones of their hybrids were used. *P. deltoides*, *P. trichocarpa*, and their hybrids are known as commercially important trees (Dickman and Stuart, 1983). *P. trichocarpa* has the common name black cottonwood. It is native to North America and distributed largely in the Pacific coastal states and Canadian provinces (Dickman and Stuart, 1983). *P. trichocarpa* is a moisture-demanding species found mostly on bottomland sites. The annual growth rates are 2.5 cm in diameter and 1 to 1.5 meters in height. The species does well in closely spaced plantations (Dickman and Stuart, 1983). *P. deltoides* has the common name eastern cottonwood. It is distributed over the eastern two-thirds of the United States and southern Canada, and it has been introduced to many parts of Europe (Dickman and Stuart, 1983). Eastern cottonwood is a dominant species on bottomland sites. It is known as being very shade intolerant and competitive (Dickman and Stuart, 1983). Because it is a good species for commercial production (Eckenwalder, 1996), many tree scientists use *Populus* as a model to study tree growth, tree physiology, and genetics. The potential for genetic improvement in
Populus appears promising because of significant genetic variation among morphological and physiological traits of different clones and species (Isebrands et al., 1988). Populations of Populus still offer many opportunities to study natural genetic variation, its magnitude and patterns, and its relationship to major environmental variables.

**Light environment**

Understanding of light measurement and its interpretation is important for studying plant responses to light conditions. Light behaves as a wave phenomenon (λ) with discrete particles of energy called photons. Wavelength is the distance from one peak of a wave to the next. It is typically expressed in units of nanometers (1 nm = 10^-9 m). Light with different wavelengths has different energies and properties (Hopkins, 1998; Taiz and Zeiger, 1998). The energy carried by a photon is called a quantum, which can be expressed as

\[ E = \frac{hc}{\lambda} = hv, \]

where \( h \) is Planck’s constant (6.62 x 10^{-34} J s^{-1} photon^{-1}), \( c \) is the speed of light, and \( \lambda \) is wavelength (Taiz and Zeiger, 1998). There are three parameters of light including, light quantity, light quality, and spectral distribution (Hopkins, 1998). Light quantity is a fluence and can be expressed as the number of photons, quanta (in moles, mol) or the amount of energy (in joules, J) (Hopkins, 1998; Taiz and Zeiger, 1998). Photon fluence is a total number of photons incident on surfaces, and energy fluence is the total amount of energy incident on the surface (Hopkins, 1998). The unit of photon fluence is moles per square meter (mol m^{-2}), whereas the unit of energy fluence rate is joules per square meter (J m^{-2}). Photon fluence rate (mol m^{-2} s^{-1}) and energy fluence rate (J m^{-2} s^{-1} or W m^{-2}) are used often in plant physiological research on plant responses to light conditions (Larcher, 1995). Irradiance is most often used interchangeably with the term energy fluence rate (Hopkins, 1998). The sun’s radiation reaches the biosphere at wavelengths ranging from 290
nm to 3000 nm (Larcher, 1995). Plants utilize radiation energy in wavelengths from 380 nm to 730 nm. This range, often defined as 400 nm – 700 nm, is called photosynthetically active radiation (PAR) (Hopkins, 1998; Larcher, 1995; Taiz and Zeiger, 1998).

**Plant photomorphogenesis**

Several above-ground environmental factor such as light intensity, spectral distribution, air movement, air humidity, and temperature, influence plant growth and development. Plants interact and compete among themselves for limited resource such as light to survive and to dominate their territories. Light conditions provide energy for photosynthesis, which is essential for plant growth and development. Competition for light leads to changes in growth potential and non-structural compounds. It also reduces genetic diversity in mixed stands as many clones are “shaded-out” by their more competitive neighbors. Plants capture and transmit light signals into suitable channels for stem growth and development. These light signals are perceived by photoreceptors that have been found in plants.

Phytochrome is one of these photoreceptors (Bowler, 1997; Smith, 1984), it has been characterized biochemically and physiologically. Phytochrome is an approximately 120-kD soluble protein. It is a photo-convertible chromoprotein, in which the chromophore group is an open-chain tetrapyrrol closely related to phycobilins (Larcher, 1995). Phytochrome is located throughout the cytoplasm and also is found within or associated with plasmalemma and chloroplast membranes (Kozlowski et al., 1991). It regulates a wide range of plant growth response such as seed germination, stem elongation, flowering, and senescence (Reed, et al., 1993). This photoreceptor is able to detect light quality in particular wavelengths and transmit this information about the light environment to alter cellular
metabolism and influence the plant growth process. It exists in two photo-interconvertible forms, Pr (the red light absorbing form, R = 660 nm) and Pfr (far-red light absorbing form, FR=730 nm) (Larcher, 1995; Smith, 1995). Pr can be converted into Pfr form by absorption of red light, and Pfr is converted to Pr by far-red light (Reed et al., 1994, Bowler, 1997, Smith, 1995). Pr form is found in the dark, whereas Pfr form was disappeared or converted to Pr form in the dark by non-photochemical reaction (Hopkins, 1998). In the processes studied to date, the active form of phytochrome is Pfr, and Pr is the inactive form (Reed et al., 1993; Bowler, 1997; Smith, 1995). In studies of a mutant that lacks a gene involved in the Pfr form conversion, seedlings lacking the Pfr form are prevented from responding to FR light, leading to cessation of growth and then death (Reed et al., 1993, Taiz and Zeiger, 1998). Pr is involved in induction of growth and development under low R:FR light (Fankhauser et al. 1997; Taiz and Zeiger, 1998). The expression of a number of phytochrome gene families (PHYA, B, C, D, and E) has modulated through the photo-reversible conversions between a Pr form and Pfr form (Taiz and Zeiger, 1998). It appears that each member of the phytochrome family regulates different aspects of plant development (Adam et al., 1996, Smith, 1995). Among these five phytochrome genes, only PHYA and PHYB have been characterized. PHYA is required for the response to FR light, and PHYB determines the response to R light (Taiz and Zeiger, 1998). PHYA involves in photoperiod control of flowering and PHYB controls the shade avoidance, flowering, and photoperiodically (Smith, 1995; Taiz and Zeiger, 1998). Although, plants show stem elongation under far-red light, the comparison of wild type plant and plant deficiency in PHYA displays no morphological differences when grown under continue white or red light (Sommers and Song, 1996). However, plant deficiency in PHYB showed abnormality when
exposed to continue white or red light, it does not display the stem elongation under far-red light condition. The study of black cottonwood indicated that there has one PHYA locus and two PHYB (PHYB1 and PHYB2) loci presented in the black cottonwood (Glenn et al., 1998). The authors also postulated that black cottonwood lacks of PHYC/F and PHYE subfamily, but PHYB/D is independently occurring. The expression of these three PHY genes are found at the mRNA level (Glenn et al., 1998). The function of other phytochrome genes is still not well studied.

The ratio of photon flux in the R and FR wavelengths is often used to express the light environment quantitatively and in studying plant responses to the light environment. The ratio can be formulated as follows:

\[
\text{R:FR ratio} = \frac{\text{Photon flux in R region}}{\text{Photon flux in FR region}}
\]

The calculation of R:FR ratios is based on wavelengths centered around the peak absorption of Pr (665-670 nm) and Pfr (730-735 nm) (Smith, 1984). R:FR light signals carry critical information to plants competing with each other to colonize a site (Ballaré, 1994; Smith et al., 1990). Light signals also influence the life cycle and growth ability of plants to ensure that resources are utilized effectively and to allow for appropriate reaction to competition with neighbors (Smith, 1995). Several studies have shown that the R:FR signal changes relative to tree height, canopy distances, canopy densities, and leaf areas within a canopy. Plant density, leaf, and branch angle of inclination are also major determinants of light perception (Gilbert et al., 1995). In open-grown trees, the R:FR ratio in the upper crown is 1.1-1.2, and within canopies it is 0.4-0.8. Low levels of blue (B) and red (R) light are due mostly to absorption by chlorophyll, while near-ambient levels of FR light remain in reflected and transmitted light within plant stands (Gilbert et al., 1995; Ballaré et al., 1990).
Light has significant effects on morphological and physiological structures of plants. The response is the result of photomorphogenesis, which activates enzymes and regulates gene activity (Larcher, 1995). The response may occur before intense competition for light is established. Some of these responses are simple changes in growth rate or increases in mortality caused by variation in the supply of light imposed by neighboring individuals (Ballaré et al. 1987). The responses can be seen as a result of small reductions in the R:FR light below the values provided by full sunlight.

Denser canopies allow less photosynthetically active radiation (PAR) to penetrate to the base of the crowns, which affects leaf number and leaf area production. The biomass production in agricultural crops is directly related to the radiant energy interception by foliage (Montieth, 1994). The quantity of leaf area is a critical determinant of biomass production. Reductions in R:FR may be due to leaf area indices (LAI) (the ratio of leaf area of a plant to the ground area) and the degree of canopy shading as leaf canopy increases. Shifts in R:FR ratios over the range of tree heights that occur in the natural environment result in large changes in the phytochrome photoequilibrium (Pfr/Ptot, where Ptot is the total phytochrome quantity) in different plant tissues, and plants respond with morphological changes (Ballaré et al., 1987; Smith, 1995).

**Tree morphological characteristics related to competition**

In general, competition is the interaction between individual plants as they try to capture limited resources to colonize a location; it often leads to the elimination of less competitive genotypes under close spacing (Lemaire and Millard, 1999). Trees compete with each other for light, water, and nutrients. The result of tree competition leads to changes in growth characteristics such as leaf structure, stem form, stem taper, branching, leaf and
branch angle of inclination, root growth, and biomass allocations (Larcher, 1995; Ritchie, 1997; Kozlowski et al., 1991).

**Competition affect on leaf, branch, and root**

Tree leaves are involved significantly in competition during stand development. Leaves have a capacity to capture light signals, synthesize growth and storage compounds, and produce their own dry matter. Leaves can adjust their position with respect to wind and incident radiation to capture maximum light energy for photosynthesis and also to avoid excessive irradiance and overheating (Larcher, 1995; Ceulemans, 1990). Dry matter production is closely related to the leaf area index, and high dry weight production is found under optimum LAI (Kozlowski et al., 1991). In *Populus*, the number of branches and branch angles are influenced by competition and clone (Heilman et al., 1993; Ceulemans, 1990). This interaction then influences dry weight production. Plants with more branches appear to dominate during the early stages of competition (Ceulement, 1990). According to Ballaré (1994), reduction in the R:FR ratios initiates an increase in apical dominance and reduces branching. The impact on height growth is greater than the actual shading effect. The allocation of photosynthetic products to the branches shifts from multi-layered to mono-layered branches. The remaining photosynthate is shifted into height growth and biomass accumulation in the main stem (Givnesh, 1995). Low R:FR ratios reduce nutrient uptake in grasses and reduce the development of root density (Casper et al., 1997). Plants grown under high FR light increase shoot dry weight (Kasperbauer et al., 1992; Ritchie, 1997). Kozlowski et al. (1991) demonstrated that under high competition for light, plant roots are reduced extension, configuration, and density. Above-ground and below-ground competition is
interactive. It is not easy to separate the effects of different types of competition on whole plant growth (Casper et al., 1997).

Wind also has significant impacts on plant growth and development. Plant growth in the field and in the greenhouse are subjected to the effects of wind movement. Cleugh et al. (1998) indicated that plants exposed to 30 sec. of shaking by the wind each day had reduced stem height, premature terminal nodes, a decreased number of lateral branches, decreased internode length, and shorter and thinner xylem vessels. Different species are affected differently by wind (Cleung et al., 1998).

**Hormone effects**

Light signals appear to control the phytohormone signals at some stage of development. Studies in pea (*Pisum sativum* L.) and beans (*Phaseolus vulgaris*) showed that phytochrome (Pfr) alters the levels of gibberellins. Low R:FR ratios seem to increase levels of gibberellins and lead to the increase of internode elongation, cell extension and division, and leaf development (Weller et al., 1994; Beall et al., 1996). Potter et al. (1999) demonstrated that gibberellins were strongly correlated with cell expansion and stem elongation, but dry weight deposition had no relationship with gibberellin concentration. Auxin also is involved in cell division, cell expansion, and cell differentiation (Hopkins, 1998). Phytochrome regulates stem elongation and also changes the level of auxin within the stem (Morelli and Ruberti, 2000). Auxin accumulates mostly in the apical meristem of stems and roots, and young leaves under low R:FR ratios (Morelli and Ruberti, 2000; Taiz and Zeiger, 1998). Auxin accumulation causes plant stems to elongate faster under low R:FR signals. In contrast, the study of cytokinins in beans (*Phaseolus vulgaris*) showed that cytokinins were involved in dry matter production, rather than stem elongation. Cytokinin
concentrations in stems, leaf, petioles, and roots are high under high R:FR ratios and low in concentration under low R:FR (Hammerton et al., 1998). Therefore, phytochrome and R:FR signals are involved in controlling hormone level. A study of aspen confirms such control occurs in *Populus*. *PhyA* over-expression in aspen (*Populus tremula x tremuloides*) reduced levels of gibberellins (GA) and idole-3-acetic acid (IAA) in apical leaf and stem tissues (Olsen et al., 1997). This study also indicated that photoperiod responses of trees are regulated by amount of *PhyA* gene expression, and that the metabolism of GAs, and IAA can be regulated by the amount of phytochrome A.

**Non-structural carbohydrate contents in relation to competition**

Carbohydrate accumulates in the branch, stem, and root prior to winter dormancy when day length and temperature changes lead to slow or ceased vegetative growth. These storage carbohydrates are used in the next growing season for initiating growth, conversion to defense mechanisms against biotic and abiotic stress, and competing to colonize additional growing areas (Kozlowski et al., 1991). Plants under low-light regimes have decreased non-structural carbohydrates and that leads to decreased leaf growth after winter dormancy (Wyka, 1999). This leads to less competitive ability during the next growing season. Coleman et al. (1991) postulated that the initial dormancy of woody plants in early winter is mediated by phytochrome when the changes of day length in early winter alter numerous metabolic components and storage compounds. Irradiance within a *Populus* canopy decreases exponentially with depth of canopy, resulting in a decline in photosynthetic capacity and leading to poor growth rates low production of storage compounds and death of heavily shaded branches and whole plants provide plants a better opportunities to compete with other plants during the next growing season. Conversely, good light availability
supports organic reserve accumulations that lead to faster initial shoot growth rates increases in total shoot regrowth, more dry weight accumulation larger overall plant size (Avice et al., 1997; Kalengamaliro et al., 1997).

**Current and future research needed**

Therefore, the mechanisms of competition between plants in dense stands should be a major consideration in tree improvement. Hall (1994) has provided an outline of plant competition based on spacing management, and suggested that row spacings have their initial effects mediated by the phytochrome shade avoidance response. Recently, several researchers have found that light (R:FR) regulation through phytochrome is a major factor in tree competition and growth. Studies on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and red alder (*Alnus rubra*) indicated that stand density and the abundance of other vegetation influences potential growth through effects on intra and inter-specific competition as well as other kinds of species interaction (Shainsky et al, 1992). Trees respond to radiation reflected and transmitted from neighbors before canopy closure and actual shading occurs, and the responses were eliminated if reflected radiation was filtered through copper sulfate solution (CuSO₄), which absorbs FR (Smith, 1995; Ballaré et al., 1994). A dilute solution of CuSO₄ can be used to reduce the fluence rate of FR radiation with little effect on the fluence rate of photosynthetically active radiation (PAR) and maintains values of R:FR ratios close to open sky conditions (Ballere et al, 1990). By using CuSO₄ solutions as filters, individual plants can be isolated from other competing sources. Comparing plants in CuSO₄ filters with control plants can provide the best measurement of plant competition responses to light regimes. In some circumstances, productivity measurement in terms of plant biomass was increased under conditions in which shade-avoidance reactions were induced (Smith,
1984; Smith, 1995; Ballaré et al., 1987), but the long-term consequences of the shade-avoidance response in trees have not been adequately studied.

Reduction in the R:FR ratio depends on canopy densities, structures, and orientation of leaves. Different *Populus* clone respond differently to spacing, resulting in changing biomass allocations (Panetsos, 1980). Therefore, field spacing experiments comparing different clones and light regimes should provide a good opportunity to test the competitive ability of clones. Good measurements are needed to determine how much of the growth dynamics of tree height and canopy development under plantation can be accounted for on the basis of phytochrome-mediated proximity perception at different plant densities. The impacts on stand self-thinning also need to be studied. Ballaré et al. (1987) have suggested that the shade-avoidance response is a major factor in reducing genetic diversity in stands of mixed genotypes. We further speculate that the shade avoidance response hastens the loss of stems from monoclonal plantations when environmental variation triggers initial size differences. The work of Gilber et al. (1995) show how R:FR effects can change canopy development and then biomass allocation. Information describing the importance of accumulation of glucose and starch in stems due to light competition in *Populus* has not been available. Our hypothesis is that different accumulations of stem organic reserves occur in relation to R:FR under different spacings. Plants with high storage reserves should grow faster at the beginning of the next season and therefore, be more successful in stem growth as a stand develops. It would be useful to know whether differences are stem organic storage (starch and sugar contents) during light competition is a factor contributing to differences in shoot yield and plant mortality.
Therefore, gaining further information about phytochrome action and R:FR ratio influences in trees under field and greenhouse conditions based on tree morphological characteristics, biomass, and storage compound accumulation is very important. Such information will provide useful tools for forest geneticists and physiologists to improve tree yield.

It will be very important to investigate R:FR signaling effects on different clones and species and in different locations of adaptation. Field and greenhouse observations on genetic and morphological characteristics relating to plant responses to light qualities are important in developing new stand management and genetic selection techniques for the best growth response in plantations.
CHAPTER 2. TREE SPACINGS AND LIGHT QUALITY AFFECT PHENOTYPIC CHARACTERISTICS AND BIOMASS ALLOCATION OF COTTONWOOD

A paper to be submitted to the journal Tree Physiology

Sovith Sin and Richard B Hall

Abstract

Cottonwood stem growth and biomass yield are influenced by red:far-red (R:FR) signal changes under different spacing in young plantations. The purpose of this study was to identify the relative importance of tree spacings on light quality, tree morphology, non-structural carbohydrate, and biomass accumulation in six Populus clones. Two spacings (narrow spacing - 40 cm and wide spacing - 3 m) were used for the study. Six clones (one Populus deltoides Bartr., one P. trichocarpa Torr. & Gray, and four of their F2 hybrids) were measured to determine total stem height, stem diameter, stem taper, number of branches, non-structural carbohydrate, and dry weight. Red:far-red light ratios were determined at two-week intervals for two growing seasons. Stem height was recorded weekly. Red:far-red ratios dramatically decreased (from 1.2 to 0.4) as the trees increased in height and size. Average stem height was affected by spacing ($P = 0.005$), but not by clone ($P = 0.63$). Accumulations of biomass showed trends between clones ($P = 0.08$) and spacings ($P = 0.12$). Narrow spacing tended to result in more stem biomass accumulation (39.4 g) and more stem height elongation (65 cm) than stem biomass (30.4 g) and stem height (41.0 cm) under wider spacing. Weekly stem elongation showed no differences at 7 days and 42 days after planting ($P = 0.22$ and $P = 0.98$); however, stem elongation showed differences at 14, 21, 28, and 35 days after planting ($P = 0.001$). The height induction under narrow spacing
corresponds to reduced R:FR signals, and the light ratios (R:FR = 1.2 to 0.4) were related to spacing effects. Leaf areas were different among clones ($P < 0.001$), and showed a trend by spacing ($P = 0.14$). Trees under narrow spacing tended to produce more leaf area (1222 cm$^2$) compared to wide spacing (1037 cm$^2$). Stem taper was affected by spacing ($P = 0.02$), but was not different among clones ($P = 0.67$). Higher average stem tapers were found with wide spacing (taper = 0.078) when compared to narrow spacing (taper = 0.064), which suggested that plants under wide spacing allocate more photosynthate into stem diameter growth and might have the ability to compete more efficiently over time compared to plants under narrow spacing. Stem height and biomass accumulation were different in 1998 and 1999. In 1999, trees were severely damaged by cottonwood leaf beetles, septoria leaf spot, and septoria stem cankers. In general, clone ILL-129 showed more biomass accumulation (40.9 g) and greater stem elongation (70 cm), whereas F$_2$ clone 1096 showed low biomass accumulation (20 g) and shorter stems (47 cm). Although stem growth and biomass accumulation was greater in narrow spacing than wide spacing in the juvenile stage, stem elongation in response to low R:FR signals may not be the best long-term strategy for the accumulation of harvestable biomass in plantations.

Introduction

Competition among plants for resources leads to altered morphological characteristics and biomass allocation. Morphological responses of plants to their light environment are critical to the outcome of this competition under natural condition. Understanding responses to competition in managed plantations is critical to improving harvestable biomass yield.
The annual production of a forest stand depends on canopy architecture, interception of radiation, leaf area index (Ballaré et al., 1994; Niinemets and Kull, 1995), and stand density (Hall, 1994). The effects of light quality on stem elongation and biomass production have been studied in several plant species. Incident light is reflected, absorbed, or transmitted, depending on wavelength and leaf canopy orientation. Leaf pigments (e.g. chlorophyll) are high in absorption of red light (R). They are also high in reflectance and transmittance of far-red light (FR), which leads to a low R:FR ratio in the developing canopy zone and results in altering growth rates and allocation patterns of plants (Aphalo, 1999; Ballaré et al., 1994). Size differences result from changes in light regime (R:FR ratios) that relate to the different plant population densities, growing space, and relative shade tolerance of the species involved. Under low R:FR ratios, an increased stem elongation rate is found (Alphalo, 1999; Ballaré et al., 1994; Ritchie, 1997). Phytochrome is one of photoreceptor that involves in sensing the change of light R:FR rations. It regulates a wide range of plant growth response such as seed germination, stem elongation, flowering, and senescence (Reed, et al., 1993). This photoreceptor is able to detect light quality in particular wavelengths and transmit this information about the light environment to alter cellular metabolism and influence the plant growth process. Studies in rice (Oryza sativa L.) and lettuce (Lactuca sativa M.) indicate that the phytochrome signaling system under high R:FR conditions blocks the production of gibberellins (GA) and reduces stem elongation (Behringer et al., 1990). Alternatively, phytochrome might act by increasing GA sensitivity of the tissues under high far-red radiation (Nick et al., 1993; Thomas et al., 1980). In a study of aspen (Populus tremula x tremuloides), the over production of phytochrome A was involved in regulating both GA and indole-3-acetic acid (IAA). These plants had reduced
capacity to respond to the low R:FR light when it was over expression of PhyA. The amount of PhyA can regulate the apical growth and dormancy induction (Olsen et al., 1997). In Avena, both auxin and light quality act together to control coleoptiles and mesocotyl growth, which induces stimulation of coleoptile elongation (Shinkle et al., 1985). Plants responding to light competition also express changes in morphological and developmental components, which result from changing receptor proteins and pigments between PSI and PSII (Aphalo et al., 1999). Cuomo, et al. (1998) also has indicated that changes in plant densities of corn lead to change in plant morphology and dry matter production. Dry matter production is directly related to shoot architecture, because the ability to change shoot structure with respect to shading within the canopy correlates closely with the efficiency of light interception and leaf area (Niinemets and Kull, 1995; Leverenz and Hinckley, 1990). Tree and crop scientists have used plant spacings to evaluate plant competition for underground resources (Casper and Jackson, 1997). However, above ground competition such as competition for light in Populus has not been adequately studied. Hall (1994) has proposed studies of clones based on the crown competition factor concept, and Panetsos (1980) found that different clones differ in the abilities to adapt to different spacings. Based on these findings, we hypothesized that changes in light quality due to different spacings are the signals that change morphological traits and biomass accumulation.

Another factor of plant development that may be influenced by spacing and light quality is storage compound accumulation. Irradiance within a Populus canopy decreases exponentially with depth of canopy, resulting in a decline in photosynthetic capacity and leads to poor growth rates for some clones. Kalengamaliro, et al. (1997) indicated that high organic reserves led to faster initial shoot growth rate and provided improved ability for plant
growth under competition. The effect of competition on organic reserve accumulation
(starch content) and subsequent shoot regrowth, leaf area development, and overall plant size
has been studied in perennial crops like alfalfa (*Medicago sativa*) (Avice et al., 1997).
However, information describing the importance of accumulation of stem storage compounds
due to light competition in *Populus* has not been available. The functional details of
morphological responses to the changes in light environment have not received enough
attention in *Populus*.

The major objective for this research was to study tree growth, biomass allocation,
and genetic variation in response to the R:FR signals provided by two different tree spacings
under field conditions. The study evaluated spacing effects on light quality, stem height, leaf
area, stem taper, stem storage compounds, and biomass accumulation in young *Populus*
plantations.

**Materials and Methods**

Research plots were located at the Iowa State University Lower Reactor farm
northwest of campus (latitude 42° 02' 46" N and longitude 93° 39' 46" W). The site has a
Terrill loam soil with about a 2% slope (Thomas, 1984). Total rainfall in 1998, was 405 mm
falling from June to September (Fig. 2.1). In 1999, precipitation was 648 mm falling from
May to August (data adapted from National Climatic Data Center, Ames 5 SE, Ames, IA).
Weekly rainfall for each growing season is presented in Fig. 2.1; average daily temperature is
shown in Fig. 2.2.

Six clones were selected for study. These clones represented one *P. trichocarpa*
selection (93-968), one *P. deltoides* selection (ILL-129) and four of their F₂ hybrids (1068,
1096, 1125, and 1579). The clones were obtained from the University of Washington (Bradshaw et al., 1994). The clones were chosen based on different stem tapers (1068 = 0.053, 1096 = 0.049, 1125 = 0.036, 1579 = 0.029, 93-968 = 0.056, ILL-129 = 0.029) from three-year old field experiment (Lin et al., 1998). The hardwood stem cutting method was used to propagate the clones. One-year-old branches were harvested during fall 1997 and stored in a cold storage room at 5 °C. In early April 1998, the stem cuttings were placed inside book planters with a soilless mixture of 1:1:1 of peat:perlite:vermiculite (one stem per hole) for rooting before transfer to the field in May 1998. On 27 May 1998, the six clones were planted in the two spacings. Soon after planting, all trees were cut back to 10 cm above the ground. The field was fenced with electric wire to reduce damage from deer.

The experiment was conducted with a narrow spacing (40 cm x 40 cm) and a wider spacing (3 m x 3 m) using the field design that was considered most appropriate to each type of spacing and the plant and land resources available. The narrow-spacing component was replicated 4 times in a randomized complete block design. Each replication of a clone consisted of a 12-tree plot, three rows with four trees per row. The wider spacing (3 m x 3 m) component was conducted in a Latin Square design with 6 replications of one tree-plots.

The field was weeded weekly to avoid weed competition. When severe damage by cottonwood leaf beetles occurred, the plot was sprayed with Novodor (Abbott Laboratories, North Chicago, IL), a formulation of the bacteria Bacillus thuringiensis subspecies tenebrionis. It was sprayed at a rate of 9.3 L/ha with a Solo backpack sprayer. The field plot was not fertilized. Trees were cut back to 10 cm from the ground at the end of the first growing season, when all trees reached full dormancy. The tree stumps were allowed to regrow in summer 1999. The regrowth was thinned to a single stem for each tree. The plots
were hand weeded weekly to reduce competition with weeds. The plots were again sprayed with Novodor (Abbott Laboratories, North Chicago, IL) to control cottonwood leaf beetles.

**Light measurement**

R:FR ratios were measured with a portable light spectroradiometer LI-1800 (LI-COR, Inc., Lincoln, NE) using an integrating cylinder (Ballaré et al., 1987; Ritchie, 1997). The integrating cylinder was able to collect a ±10° acceptance angle (Ritchie, 1997), which was set up to collect only light propagated horizontally in the wavelength range from 400 to 800 nm at 5 nm bandwidth intervals. The ratios were determined every 14 days from 14 to 56 days after planting in 1998. In 1999, trees were severely damaged by cottonwood leaf beetles despite of control efforts. This resulted in only three light measurements being taken from 14 to 42 days after regrowth. On each measurement date the integrating cylinder was placed at the mid height of the tree being evaluated. The stems were physically bent to allow the sensor head to detect the surrounding light quality. In the wide spacing treatment, the light measurement was conducted at each single tree plot, whereas in the narrow spacing, the light measurement was taken at the central tree within the central row of a clonal plot. Triple scans were made at each plot and averaged. The R:FR ratios were calculated as the ratio of the photon fluence rate in the 10-nm band centered on 660 nm divided by the photon fluence rate in 10-nm band centered on 730 nm of light (Taiz and Zieger, 1997).

**Data collection**

All data for the wide spacing treatment were based on each single-tree plot. For the narrow spacing treatment, the average of the four-tree central row within each clonal plot was used. All leaf and stem position were identified using the Leaf Plastochron Index (LPI) system (Ceuleman, 1990) with LPI-0 defined as the youngest leaf ≥ 3 cm. The
morphological data used to study the spacing effect included weekly height, stem taper, leaf area in the developing leaf zone (LPI 0–10), number of branches, and shoot dry weight.

Stem tapers were calculated as follow:

\[ \text{Taper} = \frac{(D2 - D1)}{L} \]

Where:

\[ D1 = \text{Diameter at the midpoint between LPI 3 and 4, mm} \]
\[ D2 = \text{Diameter at the mid point of the interval nearest to 60 cm basipetal from D1, mm} \]
\[ L = \text{Actual stem distance between D1 and D2 with all measurements converted to mm}. \]

Leaf area at the end of growing season was determined based on the leaves from LPI 0-10 on the main stem with a LI-3000A (LI-COR, Inc. Lincoln, NE). Trees were cut at 10 cm from the ground by hand after leaf-fall. The total plant height at the end of growing season was measured. Biomass was measured as the combined stem and branch dry weight.

**Non-structural carbohydrate analysis**

Five grams of fresh tissue were taken at the base of each stem for non-structural carbohydrate analysis at the time of the fall harvest. The tissues were dried at 70 °C for 72 hours. The dried stems were ground to pass an 80-mesh screen. The ground tissues were stored at room temperature until analyzed for sugar content and carbohydrate content. The procedure consisted of the three following steps: carbohydrate extraction, sugar analysis, and non-structural carbohydrate analysis. The extraction and non-structural carbohydrate analysis followed the procedures previously used with Populus (Dickson, 1979; Haissing and Dickson, 1979; Reichenbaker, 1994). Phenolic compounds, pigments, and soluble
carbohydrate were removed by using 2 ml methanol:chloroform:water mix (12:5:3 by volume) on weighed tissue samples. The extraction procedure was modified in amount of stem tissue sampled (0.10 mg) and starch hydrolyzing enzyme Amyloglucosidase (from Aspergillus niger) (Sigma, St. Louis, MO) from the procedure described by Reichenbacker (1994).

Statistical analyses

Due to shortages of field space and plant material, two different plot designs were used for the spacing treatments. The combined analysis of the two experiments was based on the average values of measured data and preceded with a simple two-way factorial statistical analysis. Analysis of variance was used to evaluate spacings and clone effects on biomass allocation by light regime. A separate analysis of variance was performed on the data from each year and measurement date using the SAS statistical package (SAS Institute, 1989). Due to the severe damage by leaf beetles and Septoria canker, in 1999, combined year analyses were not performed.

Results

Trees grown under narrow spacing elongated more and accumulated more above ground dry weight than those at wide spacing.

Weather conditions in 1998 were quite different from those in 1999. The 1998 total rainfall was low, only 405 mm, from June to September. The least rainfall occurred between 21 and 49 days after planting (Fig. 2.1). The total rainfall in 1999 was 648 mm from May to
Figure 2.1. Weekly cumulative rainfall in 1998 (from 27 May to 23 September) and in 1999 (from 30 April to 5 August) growing seasons. Each bar is a total for that seven day periods. The white bar represents rainfall in 1998 and the solid bar represents the rainfall in 1999.
Figure 2.2. Average daily temperature for seven day intervals in 1998 (from 27 May to 23 September) and 1999 (from 30 April to 5 August) growing season. Each bar was an average of seven-day periods. - • - represents the temperature in 1998 and - ■ - represents the temperature in the 1999 growing season.
August, and rainfall reached a peak 63 days after resprouting began (Fig. 2.1). The average rainfall the first part of 1999 was 49 % more than 1998. The 1998 average daily temperature was constant at 23 °C between 7 and 42 days after planting (Fig. 2.2) and gradually decreased to 14 °C 56 days after planting. The temperature in 1999 reached a peak of 29 °C at 35 days after resprouting. It leveled off between 49 and 63 days after resprouting.

R:FR ratios varied within spacings and time of the growing season. In 1998, R:FR ratios between narrow and wide spacing were not statistically different ($P < 0.05$) at 14 days after planting, but highly significant differences ($P < 0.001$) were found at 28 days after planting through to the end of growing season. High R:FR ratios were seen under wide spacing and low R:FR were found in the narrow spacing. Maximum R:FR ratios were found at 14 days after planting and then declined until the last measurement at 56 days after planting (Fig. 2.3). When trees reached 28, 42, and 56 days after planting, the R:FR ratio in the wide spacing was 30.9 %, 32.1 %, and 35.4 % higher than in the narrow spacing (Fig. 2.3). In 1999, the resprouting trees suffered severe leaf and stem damage from cottonwood leaf beetle (*Chrymela scripta* E0 and Septoria canker disease. There were no differences ($P > 0.05$) for R:FR ratios 14 days and 42 days after regrowth for both wide and narrow spacing, but there were statistical differences ($P < 0.001$) between spacings 28 days after resprouting. The R:FR ratio under wide spacing was 21.8% higher than narrow spacing after 28 days from resprouting in 1999 (Fig 2.4).

In 1998, there was no strong statistical difference among clones ($P = 0.12$) and between spacings ($P = 0.20$) for branches at the end of growing season. However, the number of branches on average was low (averaged 8 branches) in the narrow spacing and higher (averaged 11 braches) in wide spacing. In 1999, the number of branches at the end of
Figure 2.3. R:FR ratios during the growing season in 1998. Each point is a mean of 6 replications in wide spacing and 4 replications in narrow spacing. The short line on the top left hand side represents ± 0.05 standard error. -●- represents the narrow spacing and -■- represents wide spacing.
Figure 2.4. R:FR ratios during 1999 growing season. Each point is a mean of 6 replication scans in wide spacing and 4 replication scans in narrow spacing. The short line on the top left hand side represents ± 0.08 pooled standard error. -○- represents the narrow spacing and -■- represents wide spacing.
growing season was statistically different \((P = 0.05)\) between spacings and not different among clones \((P = 0.26)\). There were a 30.8 \% more branches in the wider spacings (averaged 12 branches) compared to narrow spacing (averaged 8 branches).

Weekly height growth rates were no different between spacings at 7, 42, and 49 days after planting in 1998 \((P > 0.05)\). However, weekly height growth rates, at 14, 21, 28, and 35 days after planting were highly statistically different \((P < 0.001)\). Total height at the end of the growing season was also significantly different \((P < 0.001)\) between spacings, but not among clones \((P > 0.05)\). Trees increased in height approximately 2-18 cm per week. All clones exhibited increased leaf size and internode elongation in response to the presence of low R:FR ratios at the narrow spacing. Greater weekly height growth rates were found in narrow spacing (averaged 9.3 cm per week) than in wide spacing (averaged 5.8 cm per week). The highest growth rate (18.2 cm) for narrow spacing was 21 days after planting, when the rainfall was also high (Fig. 2.1, 2.2, and 2.5).

The height growth for narrow and wide spacing decreased when day-length was shorter; rainfall and temperature were also less (Fig. 2.1, 2.2, 2.5, and 2.6). The growth rates were almost the same at 49 days after planting when the day length was short.

Plant to plant interactions within the 40 cm spacing treatment rows were sensitive to declines in R:FR, which resulted in elongation of trees within plots. Leaf areas produced under narrow and wide spacing were statistically different \((P = 0.01)\). Leaf area under the narrow spacing was 15.1 \% higher than under the wider spacing (Table 2.1). The leaf area and dry weight data were used to measure the relative growth rate under both narrow and wide spacings. Means foliage dry weight was statistically different among clones \((P = 0.02)\) and between spacings \((P = 0.04)\). The foliage dry weight was greater at narrow (3 g) than at
Table 2.1. Effect of tree spacing (wide and narrow) on tree morphological characteristics. Total leaf areas, dry weight, and stem tapers in both narrow and wide spacing in 1998. The least Significant differences (LSD) at $P = 0.05$ was used to differentiate the means

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leaf area, (cm$^2$)</th>
<th>Leaf dry weight, (g)</th>
<th>Stem dry weight, (g)</th>
<th>Stem taper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wide</td>
<td>Narrow</td>
<td>Wide</td>
<td>Narrow</td>
</tr>
<tr>
<td>1068</td>
<td>121</td>
<td>232</td>
<td>0.99</td>
<td>2.41</td>
</tr>
<tr>
<td>1096</td>
<td>106</td>
<td>123</td>
<td>1.03</td>
<td>1.43</td>
</tr>
<tr>
<td>1125</td>
<td>113</td>
<td>319</td>
<td>1.00</td>
<td>2.83</td>
</tr>
<tr>
<td>1579</td>
<td>92</td>
<td>225</td>
<td>0.75</td>
<td>2.04</td>
</tr>
<tr>
<td>93-968</td>
<td>127</td>
<td>215</td>
<td>1.04</td>
<td>1.87</td>
</tr>
<tr>
<td>ILL-129</td>
<td>477</td>
<td>719</td>
<td>4.93</td>
<td>9.74</td>
</tr>
<tr>
<td>LSD</td>
<td>85</td>
<td></td>
<td>1.65</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.5. Weekly height growth in 1998. Each point was an average of six replications in wide spacing and four replications in narrow spacing. The measurement started 14 days after planting in June 1998. The short line on the top left hand site represents ± 1.8 pooled standard error. The - ● - represents the wide spacing and - ■ - represents the narrow spacing.
Figure 2.6. Weekly height growth in 1999. Each point was an average of six replications of wide spacing and four replications of narrow spacing. The short line on the top left hand site represents ± 1.5 pooled standard error. The -●- represents wide spacing and -■- represents the narrow spacing. Measurements started 7 days after resprouting in May 1999.
wider spacings (2 g) (Table 2.1). Stem dry matter was statistically different only at \( P = 0.12 \) between spacings and \( P = 0.08 \) among clones. Higher stem dry weight was found under narrow spacing averaged with an average of 182 g compared to an average of 158 g at wide spacing (Table 2.1). The average stem dry weight in narrow spacing was 13.5 % higher than at wide spacing. Two clones (1068 and 1096) produced more leaf areas, but reduced stem dry weight under narrow spacing. The F\(_2\) progeny clones (clones 1068, 1096, 1125, and 1579) showed intermediate dry weight production between both parents. The most dry weight was found in the \( P. \) deltoides (clone ILL-129) parent and lowest dry weight found in the \( P. \) trichocarpa (clone 93-968) parent.

Stem tapers were significantly different between spacings \((P = 0.02)\), but not between clones \((P = 0.68)\). Average stem taper was 0.078 under wide spacing and 0.064 stem taper under narrow spacing. Although stem taper was not different among clones, the results suggested that larger leaf clones (ILL-129) have larger stem tapers (Table 2.1) in a young stand.

In the 1999 growing season, data on dry weight were incomplete due to heavy pest damage. Damage to the plants led to a decline in height growth and biomass accumulation, and results were not consistent with the 1998 data. Tree growth also seemed to be related to other factors. One week after rainfall, trees increased height growth rapidly compared to a week without rain (Fig. 2.5 and 2.6).

There were no significant differences between spacings and between clones for both stem sugar \((P = 0.62)\) and starch contents \((P = 0.19)\). This may be the result of stem and leaf damage by insects and disease before the plants reached full dormancy. No sugar or starch measurements were made for the 1999 growing season due to the severe pest damage.
Discussion

Spacing treatments affected the R:FR ratio, tree growth, and biomass allocation in new plantations. Light is one of the most limiting resources to plant growth in a plantation and under natural stand conditions. The ability of trees to capture and utilize light efficiently is important in competition and for plasticity under limited-resource conditions. At wide spacing the tree canopy environment is low in far-red and high in red irradiance, whereas at narrow spacing the predominant effect was a decrease in red irradiance caused by leaf absorption and high reflection of far-red wavelengths (Fig. 2.3 and 2.4). The field spacing data suggested that an increased leaf area per plant would result in increased stem height. This may be the result of high hormone production due to spacing effects. The study of plant hormone signals has indicated that hormone level regulates the cell elaboration and expansion. Increase leaf areas result in an increasing hormone level (Weller et al., 1994; Beall et al., 1996). So is anticipated that with continued stand development, there will even more reflection of far-red light resulting in even lower R:FR ratios (Figs. 2.3, 2.4, 2.5, and 2.6). In 1998, the differences in height growth started 21 days after planting with the gradual reduction of the R:FR ratio (Figs. 2.3, and 2.4), which suggested that the plants responded to the changing R:FR ratio during very early stages (Fig. 2.1, 2.2, 2.5, and 2.6). There are statistical differences among clones for leaf dry weight ($P = 0.01$) and leaf area ($P = 0.002$), which indicated that these clones differ in growth response to the changes in R:FR ratio. Stem taper was different only between spacings ($P = 0.02$) with more accumulation to lower stems under high R:FR condition. However, the altered allocation indicated by stem taper differences did not seem to be reflected in biomass accumulation or non-structural carbohydrate storage at least in the juvenile stage of development. However, trees subjected
to wide spacing have larger stem taper than trees subjected to narrow spacing, which indicated that size inequality of these trees occurred as R:FR ratios changes and this could be important to later stand development as shown by the increased rate of leaf turn-over under low R:FR conditions studied by Gilbert et al. (1995). The stem dry weight showed the trend of differences only at $P = 0.12$ between spacings and $P = 0.08$ among clones and tended to have more stem biomass (Table 2.1). The biomass allocation appeared to be utilized more for leaf production and stem height growth under the narrow spacing, while fewer branches were produced. The trees subjected to narrow spacing seemed to display more leaves on the top of the main stem and that provided a greater light harvest ability to convert photosynthetic products into above-ground biomass for most clones (Table 2.1). The data of this study were consistent with the results of Wu et al. (1997) who postulated that poplar hybrids (Populus) with large leaves would outgrow their parents. Thus, it appears that in the early stages of growth under narrow spacing, stem elongation responses produce more leaf area and a marginal increase in stem dry weight. This finding was also consistent with the results of Maliakal et al. (1999) and Ritchie (1997) who showed that under high densities Impatiens capensis and Pseudotsuga menziesii allocated more dry weight to stem and reduced allocation to roots. Increased stem elongation under narrow spacing was also consistent with the responses of some grasses to decreasing of R:FR ratios. The phytochrome pigment system provides the first signal for detection of neighbor plants before actual shading starts (Ballaré et al., 1990; Casal et al. 1990; Goldberg, 1987). Under wide spacing, the leaf areas were distributed more evenly through the growing canopy. The R:FR ratios were high and elongation was less because there was less competition among individuals for light resources and the partitioning of biomass into stem, root, and leaves.
might have been more balanced. The relationship between an initial spacing effect and initial plant size is not surprising because total capturing of light resources obviously increases with size. This effect might be expected to continue until stand closure and the shading-out of lower foliage offsets the greater production of leaf area. The response of some clones that allocated more biomass to leaf growth seems to have been at the expense of allocation, into stem dry weight (Table 2.1 for F2 clones 1068 and 1096), this result suggests that different clones have different abilities to convert carbon gain into biomass in different tissues.

A comparison of taper in the interval of 60 cm from the LPI-0 to the base of the stem showed different responses of each clone to the spacings. Taper is an important characteristic for the mechanical support of the plant. It also reflects the relative allocations made to height and diameter growth. Trees subject to wide spacing showed lager stem tapers in the juvenile stage of development. This might be useful in the long term tree growth and in the production of harvestable yield. It may also help a plant to compete better for resources and allocate more biomass in the long term growth. Briand et al. (1998) indicated that stem taper is mechanically advantageous as maximum stress occurs at the base of a stem during bending by adverse environmental factors.

Under wide spacing, light intensities and water losses per plant may be higher and cause earlier stomata closure. If that is the case, there would be less carbon gain less carbon resulting in less biomass as observed in this study (Table 2.1). Plants under narrow spacing seem to elongate faster after a week of high rainfall and temperature (Figs. 2.1, 2.5, and 2.6). This suggests that the water was more limiting under narrow spacing as might be expected.
Stem non-structural carbohydrate was not affected by spacings \( (P = 0.52) \) and by clones \( (P = 64) \) in this study, contrary to our expectation. Whether this is the actual behavior or an artifact of pest impact on storage compounds will need further study.

Although studies on plant response to light regime (R:FR ratios) have not been intensively conducted in *Populus*, there have been consistent observations in other tree species, agriculture crops, and horticulture plants. Ballard et al. (1990) indicated that under low R:FR ratios, *Datura ferox* L. and *Sinapsis alba* L. respond considerably with increased height and leaf areas. A *Pseudotsuga menziesii* spacing experiment also showed that plant height, leaf areas, and crown biomass increased with increasing plant density (Ritchie, 1997). However, the results of this study indicate that trees need to be grown to older ages before final conclusions can be drawn. This study showed consistency with the results of studies in annual plants and the early development of perennial plants.

The results of this study provide clear evidence that spacings and light regime can alter tree biomass allocation. However, it is difficult to assess the generality of the results because there were very strong effects by insect and disease disturbances. The 1999 data were not consistent with the 1998 data. This removed the opportunity to study longer-term effects of competition and to more fully complete the test of the hypothesis of genotypic variation in response to R:FR signals. The efficient utilization of tree spacing needs to be further investigated in older plantations. Gas exchange and water use efficiency in different spacings also need further investigation in relation to R:FR ratios effects.
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CHAPTER 3. ALLOMETRIC RESPONSES OF COTTONWOOD TO RED:FERAL-RED IRRADIANCE

A paper to be submitted to the journal Tree Physiology

Sovith Sin and Richard B Hall

Abstract

Red:far-red (R:FR) signals passing through different light filter chambers can alter stem elongation and biomass allocation of Populus species. This study is a first step designed to test the hypothesis that R:FR signaling regulates tree height and biomass accumulation in Populus spp in a commercially important way. Low red to far-red light ratios increased stem elongation and biomass accumulation. Differences in mean phenotype responses to light environment were measured in representative clones of Populus deltoides, P. trichocarpa, and their hybrids. Two filters (CuSO₄ solution and distilled water) were used to provide different R:FR light signal environments along with a completely open treatment (no-filter). One Populus deltoides clone, one Populus trichocarpa clone and four of their F₂ hybrid clones were grown for an average of 21 days under the three light conditions. Trees exposed to high R:FR signals were 5% shorter than trees exposed to the low R:FR signal found under light competition conditions. However, the no-filter trees were even shorter (7%) indicating that responses to air movement also play a major role in stem morphology. There were no significant differences in stem dry weight between light filter treatments, but there were significant differences among clones. There was no interaction between light filter and clones for leaf, stem, and root dry weight. The change in stem height was modulated by R:FR light signaling and likely, by air movement around the no-filter trees. Although air movement data were not collected at the time of study, this factor seems to be a
major cause of shortening stem height and increasing stem tapers. Differences in internode length, taper, and leaf number ($P < 0.05$) provide an explanation for the responses of each clone to the R:FR ratios. The clones with large leaves accumulated more biomass than clones with smaller leaves. Clones 1125 and ILL-129 appeared to have a greater sensitivity to changes in R:FR signaling and showed more shifts in biomass allocations. The significant differences among leaf areas, and leaf and stem dry weights at the leaf plastochron index (LPI) intervals of LPI-0 to LPI-3, suggest that this section of internodes is probably the primary R:FR response site. Therefore, shifts in R:FR signaling altered stem elongation, stem taper, and leaf area. Low R:FR signals during early stand development increased stem elongation and leaf areas and tended to also increase biomass accumulation. These results indicate the R:FR response provides a clear competitive advantage for young trees in stands. However, the greater allocation of biomass to the lower stem when the competition signal is blocked, suggests that this may be a strategy to pursue for yield improvement in older plantations.

**Introduction**

A response of plants to irradiance quality affects their morphological structure and physiological processes. Irradiance signals may be utilized throughout the life cycle of a tree to synchronize plant growth and development with the status of surrounding competition. Some signal responses are simple changes in growth rate caused by a variation in the quality of light reflected from neighboring individuals (Ballaré et al., 1987). The process of light response relates to photoreceptors, which perceive and transduce the light signal and lead to shifts in biomass allocation and accumulation. These photoreceptors are molecular devices
that translate electronic excitations caused by light into specific cellular signals. The original signals send information about the light signals through a variety of signal pathways and lead to an altered cellular metabolism and consequently influence plant growth and development (Ballaré et al., 1987; Smith, 1995). Thus, by recognition of R:FR changes in the spectral composition of reflected light by phytochrome pigments, the plant can remotely detect close neighbor plants and respond with biomass changes (Ballaré et al., 1987; Smith, 1995). Stand density and the abundance of other vegetation influence potential growth through effects of intra- and inter-specific competition as well as other kinds of species interactions (Hall, 1994). One of the major factors in this response is that R:FR signaling responds to plant competition and alters growth (Ballaré et al., 1990). The signals can also alter the biomass and morphological characteristics, i.e., increased stem elongation, number of leaves, and leaf area (Gilbert et al. 1995). Under some circumstances, plants exposed to low R:FR enhance stem elongation, increase biomass production, and assimilate more photosynthate into leaf area production for better light harvesting (Ballaré et al., 1990; Gilbert et al., 1995; Ritchie, 1997).

Many techniques have been used to study responses to light spectrum distributions within plant canopies, and these include plant spacings, light solution filters (e.g., water and copper sulfate), and green leaf filters. Plant height is increased under conditions in which shade-avoidance reactions were induced (Ballaré et al., 1987; Smith, 1984). Shade-avoidance responses occur due to radiation reflected from neighboring plants before canopy closure and shading occurs. This response can be eliminated if reflected radiation is filtered through a copper sulfate (CuSO₄) solution, which absorbs far-red (FR) radiation (Ballaré et al., 1994; Smith, 1995). By using different filter types, the R:FR spectral composition of
reflected light can be regulated. Plants can detect near-by neighbor plants and respond with morphological changes such as increased leaf area and stem length (Ballaré et al., 1987; Smith, 1995).

Although considerable effort has been expended on growth and stand evaluation, the most important traits in response to R:FR light signaling in the genus Populus have not been identified. Many previous studies seem to neglect the underlying physiological or physical effects related to the influence of light signals on the morphological structure and development of trees. The objective of this research was to study R:FR controls over morphological traits, biomass alteration, and growth response sites of two Populus species and their F2 hybrids.

Materials and Methods

Clone selection

One clone each of Populus deltoides Bartr. clone (ILL-129) and Populus trichocarpa (G&T) clone (93-968) and four clones of their inbred F2 hybrid offspring clones (1068, 1096, 1125, and 1579) were used for this study. Clonal material was obtained from the University of Washington (Bradshaw et al., 1994). The four hybrids were chosen from the F2 family based on their range in stem taper under field conditions. The clones were chosen based on different stem tapers (1068 = 0.053, 1096 = 0.049, 1125 = 0.036, 1579 = 0.029, 93-968 = 0.056, ILL-129 = 0.029) from a three-year-old field experiment (Lin et al. 1998).

Plant propagation

Procedures of Faltonson et al. (1983) were used. Shoots on greenhouse-grown stock plants were cut = 20 cm from the tip. Then, a two-internode length was cut and dipped in a
1000 mg·L⁻¹ solution of indole 3-butyric acid (IBA). The stem segments were placed in standard Jiffy-7 Peat Pellets (Jiffy Products of America, Inc.) for rooting under a mist system. The mist system was run at intervals of 16 seconds every 4-8 minutes. The stem segments rooted within two weeks of cutting. Propagules were potted in a soilless mixture of 1:1:1 of peat : perlite : vermiculite (one propagule per pot). High-density polyethylene pots (one-L volume) were used. Propagation procedures were repeated every 21 days to provide a sufficient supply of plants for the experiments.

**Greenhouse environment**

The experiments were conducted at 25 °C ± 5 °C (Fig. 4.3) in a greenhouse at Iowa State University under a 16-hour daylength. All pots were watered daily. Plants were fertilized once weekly with a Scotts® Miracle-Gro™ Excel All Purpose (21-5-20) fertilizer.

**Experimental design**

A two-way factorial design was used for this experiment with six replications. Each clone was grouped into sets of three trees of uniform size and then each tree was randomly assigned to one of three treatments: growth inside a Plexiglass® chamber filled with a CuSO₄ solution to reduce far-red light, growth inside a Plexiglass® chamber filled with water as one control, and growth with no chamber as a second control. The filters were modified in size (30 cm diameters and 50 cm height) from the design of Ballere et al. (1987). A CuSO₄ concentration of 1.4g /L of water was used to achieve a R:FR ratio of approximately 1.2, and distilled water was used to achieve uniform distribution of reflected of far-red light, uniform intensity, and a R:FR ratio of 0.6. The R:FR ratio in the treatment without a filter was also 0.6. All treatment trees were surrounded on all sides (20 cm spacing) by border trees (Fig.3.1), which were used to create the reflected light environmental effects. Plants were
placed under treatment when they reached a height of 14 to 18 cm. String was tied on the fully expanded leaf of each plant that was just inside the filtered chamber to mark the starting point of growth under treatment.

Data collection

Each set of trees of a clone was harvested and measured when the first tree of a set reached the top of its chamber. Growth duration (number of days in the filters) was recorded. The following traits were determined: height growth during treatments, number of leaves (LPI – Leaf Plastochron Index), stem diameter at every internode position, leaf angle from the main stem, and leaf, shoot and root dry weight. The internode length was measured from LPI-3 to the string on the oldest leaf in the chamber environment. Leaves from LPI 0 (first leaf ≥ 3.0 cm) down to the starting string marker were collected and subjected to area measurement and dry weight analyses. Stem tapers were calculated as follows:

\[ \text{Taper} = \frac{(D2 - D1)}{L} \]

Where:

- \( D1 \) = Diameter at the midpoint between LPI 3 and 4, mm
- \( D2 \) = Diameter at the midpoint of the interval nearest to 60 cm basipetal from \( D1 \), mm
- \( L \) = Actual stem distance between \( D1 \) and \( D2 \) with all measurements converted to mm.

Roots were soaked in tap water and placed inside a cold storage room at 4 °C overnight to facilitate removing soil medium from the root system. Roots were washed free of potting mix and dried in an oven at 70 °C for dry weight determination. Leaf, stem, and root dry weights were determined after 72 h of drying.
Figure 3.1. Design of light filter chambers inside greenhouse experiment showing the position of three filter chambers. From left to right, A-copper sulfate solution filter, B- no filter, and C- water filter.
Light condition and measurement

R:FR ratios were measured using a spectroradiometer (LI-COR-1800, LI-COR Corp., Lincoln, Nebr.). An integrated cylinder head (Ballaré et al., 1987; Ritchie, 1997) was positioned at mid-height in the center of each filter chamber. The R:FR light ratios and photosynthetically active radiation (PAR) were determined at the beginning and end of each experiment with a set of new plants.

Statistical analysis

Analysis of variance followed the procedures of Steel and Torrie (1980) and Gomez and Gomez (1984). Least significant difference (LSD) comparisons ($P = 0.05$) were used to compare treatment means. The SAS statistical package program version 6.12 was used for analysis of variance (SAS Institute, 1996).

Results

Photon flux densities ranged from 18 to 125 $\mu$mol m$^{-2}$ s$^{-1}$. Inside the CuSO$_4$ filters, the R:FR ratio averaged 1.2, whereas inside the water filters and with no filter the R:FR averaged 0.6. Temperatures inside the chambers were not significant different between light chambers (Fig. 3.2). Results indicated that plants under low R:FR responded to the competition signal by elongating their internodes and increasing their leaf areas at the top of the main stem (LPI-0 to LPI-3). Growth duration inside the filter chambers differed among clones ($P < 0.05$). The shortest duration was 18 days (clones 1096 and 1125) after trees were placed inside the light chambers. The longest average duration was 24 days for clones ILL-129 and 1579 (Table 3.1)
Total leaf area showed no statistical difference among light filter treatments ($P = 0.63$), but highly significant differences among clones ($P < 0.0001$). The *P. deltoides* clone ILL-129 had the largest leaf area, averaging $1025 \text{ cm}^2$, and the *P. trichocarpa* clone 93-968 had the lowest leaf area, averaging $485 \text{ cm}^2$. The leaf areas of hybrid clones ranged from $676 \text{ cm}^2$ (clone 1125) to $503 \text{ cm}^2$ (clone 1096) (Table 3.1). Leaf areas for LPI-0 to LPI-3 were significantly different ($P < 0.05$) between light filter treatments and also between clones ($P < 0.05$) (Table 3.2).

Total leaf dry weight showed no significant differences among light filter chambers ($P = 0.27$), but there were significant differences for the stem segments between LPI-0 to LPI-3 ($P = 0.01$) and highly significant differences among clones ($P = 0.001$). There was a trend for interactions between clones and light filters ($P = 0.08$). Clone ILL-129 had the largest leaf dry weight (Table 3.1) compared to the other genotypes.

Trees exposed to different R:FR levels showed highly significant responses in height growth ($P = 0.001$) for both clones and light filter treatments. Averaged over all clones, trees under water filters were $6.5 \text{ cm}$ taller than trees grown with no-filters, and $4.0 \text{ cm}$ taller than trees grown under copper sulfate filters (Fig. 3.3). There were no significant interactions between clones and light chamber treatments.

Stem taper showed highly significant differences ($P < 0.001$) among clones and chamber treatments. It has also interaction between clones and light chamber treatments ($P = 0.009$). Clone ILL-129 had higher stem taper (0.07) than other clones (Fig. 3.6), especially under the copper sulfate filters. Lower stem tapers were seen in clones 1068 and 1096.
Figure 3.2. Average temperature inside filter chambers over a 24-h period averaged over a 10 day recording period. -○- air temperature in the greenhouse, -■- temperature of chamber filled with copper sulfate solution, -▲- temperature of chamber filled with water, and -◊- temperature in the plant canopy with no filter.
Figure 3.3. Average stem elongation during treatment period (height between LPI-0 to the starting points of experiment) of six clones exposed to different light R:FR ratio treatments under three light filter chambers, copper sulfate filter (white bar), no filter (solid bar), and water filter (hatch bar). Each bar was an average of six trees. The short line on the top left hand site represents the ± 2.3 polled standard error ($P < 0.05$).
Table 3.1. Average total leaf area, leaf dry weight, stem dry weight, root dry weight, and growth duration by clone. The least significance different ($P = 0.05$) was used to differentiate the means.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leaf area, (cm²)</th>
<th>Leaf dry weight, (g)</th>
<th>Stem dry weight, (g)</th>
<th>Root dry weight, (g)</th>
<th>Growing duration, (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1068</td>
<td>643</td>
<td>2.22</td>
<td>1.31</td>
<td>0.34</td>
<td>23</td>
</tr>
<tr>
<td>1096</td>
<td>503</td>
<td>2.38</td>
<td>1.25</td>
<td>0.27</td>
<td>18</td>
</tr>
<tr>
<td>1125</td>
<td>676</td>
<td>3.24</td>
<td>1.54</td>
<td>0.39</td>
<td>18</td>
</tr>
<tr>
<td>1579</td>
<td>647</td>
<td>3.24</td>
<td>1.45</td>
<td>0.40</td>
<td>24</td>
</tr>
<tr>
<td>93-968</td>
<td>485</td>
<td>2.14</td>
<td>1.14</td>
<td>0.24</td>
<td>22</td>
</tr>
<tr>
<td>ILL-129</td>
<td>1026</td>
<td>6.30</td>
<td>2.31</td>
<td>0.76</td>
<td>24</td>
</tr>
<tr>
<td>LSD</td>
<td>110</td>
<td>0.66</td>
<td>0.16</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2. Effects of light spectral filters on means of leaf areas, leaf dry weight, and stem dry weight between LPI-0 to LPI-3 of six poplar genotypes. The least significant difference ($P = 0.05$) was used to differentiate the means.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leaf Area (LPI-0 to 3), (cm²)</th>
<th>Leaf Dry weight (LPI-0 to 3), (g)</th>
<th>Stem dry weight (LPI-0 to 3), (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CuSO₄</td>
<td>No-filter</td>
<td>Water</td>
</tr>
<tr>
<td>1068</td>
<td>23</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>1096</td>
<td>22</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>1125</td>
<td>19</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>1579</td>
<td>26</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>93-968</td>
<td>19</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>ILL-129</td>
<td>29</td>
<td>55</td>
<td>34</td>
</tr>
<tr>
<td>LSD</td>
<td>9</td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>
which averaged 0.04. Stem tapers were 0.06 under no-filter treatments and lower (0.05 and 0.04) under copper sulfate and water filters, respectively (Fig. 3.4).

Number of leaves varied among clones and chamber treatments ($P < 0.001$). There were no interactions between light chamber treatments and clones. In general, trees under copper sulfate filters produced more leaves (averaged 15 leaves) compared to other filters, which had 14 leaves. Trees exposed to water and no-filter treatments showed the same trend of average leaf number. Clone 1068 produced more leaves (17 leaves) under copper sulfate filters and fewer (15 leaves) under no-filters (Fig. 3.5). Clone ILL-129 had fewer leaves and averaged only 13 leaves under copper sulfate filters, 12 and 14 leaves under no filters and water filters.

The total biomass above and below ground was not significantly different among chambers ($P > 0.05$), but was highly significant between clones ($P < 0.001$). The total above ground dry weights per clone are shown in Table 3.1 and Figure 3.3. Above ground biomass was statistically different among clones ($P = 0.001$) but not among light filters ($P = 0.47$). The highest biomass producing clone was ILL-129, which averaged 8.3 g per plant, and the lowest biomass producing clone was 93-968, which averaged 3.8 g per plant. There were no statistical differences for root dry weight among light filters ($P = 0.35$), but there were highly significant differences among clones ($P = 0.001$). The clone ILL-129 had higher root dry weight (averaged 0.76 g per trees), whereas clone 93-968 had the lowest root dry weight (averaged 0.24 g per trees) (Table 3.1).

Internode length of the tree stem showed statistical differences ($P = 0.001$) among clones and chamber treatments between LPI-2 to LPI-9. From LPI-0 to LPI-6, trees under
Figure 3.4. Average stem tapers of six clones inside three different light chamber treatments: copper sulfate (white bar), no filter (solid bar), and water (hatch bar). Each bar was an average of six trees. The short line on the top left hand side represents ± 0.005 pooled standard error ($P < 0.05$) used for separate the mean differences.
Figure 3.5. Average number of leaves six clones grown under different R:FR ratios in three light filter chambers: copper sulfate chamber (white bar), no filter chamber (solid bar), and water chamber (hatch bar). Each bar was an average of six trees. The short line on the top left hand side represents ± 1.4 pooled standard error ($P < 0.05$) that used for separation of means differences.
water filters elongated more than trees under no filters and copper sulfate chambers (Fig. 3.7). Trees under water chambers showed longer internode length within LPI-0 to 9 followed by trees with no-filters, and the shortest internode lengths were found under the copper sulfate filters. However, at LPI-7 to LPI-12, trees showed no statistical differences in internode length. The internode length of trees under water filters ranged from 0.87 cm (at LPI-0) to 3.95 cm (at older LPI); under copper sulfate filters, internode length varied from 0.54 cm (at LPI-0) to 3.36 cm (at older PLI); and under no-filters, internode length ranged from 0.77 cm to 3.97 cm.

There was no statistical difference among chamber treatments for diameters of the internodes from LPI-0 to LPI-9 (P > 0.05), but there were highly significant differences among clones (P < 0.001). Internode diameters were statistically different at P < 0.05 after LPI-10. These are the internodes that fully developed after the chamber treatments were applied.

Therefore, growth and biomass allocation were affected by R:FR changes. Table 3.1 presents the means of the main effects. Variation was found in leaf area, dry weight, root dry mass, growth duration, and stem diameters. Stem dry weight was different (P < 0.001) among clones, but they did not differ among chamber treatments (Table 3.1). There were no interactions of clones and light filter for height, LPI number, and leaf area (P > 0.05). In general, average stem height among clones was taller in the clone with larger leaf area than in the clone with smaller leaf area. But, the growth rates at each measurement did not always show the same trend. Plants under no-filters for clone ILL-129 had larger leaf areas (1112 cm²), but the stem length was shorter than plants under CuSO₄ (45.80 cm) and water (56.20 cm) filters (Table 1). Mean leaf areas of clone ILL-129 and 1125 are 45% and 30% larger
Figure 3.6. Average internode length inside light filter chambers of six clones 1068, 1096, 1125, 1579, 93-968, and ILL-129 across three treatments, copper sulfate filter (-♦-), no-filter (-■-), and water filter (-▲-).
Figure 3.6. (Continued).
Figure 3.6. (Continued)
than the means of the rest of the clones. Clone 1125 and ILL-129 showed a sensitivity to the altering of R:FR. Clones ILL-129 and 1125 performed well in the different light treatments.

Stem height increased with enlarging leaf areas. Treatment means for leaf area were 699 (CuSO₄), 645 (water filter), and 622 cm² (no filter), respectively, and plant height were 56 (CuSO₄), 54 (water), and 47 cm (no filter). Number of leaves per plant was different among clones and filter treatments (Fig. 5). There were interactions between light chambers and clones (P = 0.03) on overall biomass (the average whole plant dry weight including stems, leaves, and roots).

Discussion

Red:far-red signaling altered stem elongation, stem taper, leaf area, and stem dry weight in young *Populus* trees. Light filter treatments were used to measure the relative growth rate and growth components affected by the R:FR ratios. The growth duration (days inside the chambers until harvested) responses varied among clones. This result demonstrated that each clone has different capacity to elongate in short periods of time. In other words, the shade-avoidance response differed significantly between clones. The results of the experiment supported the hypothesis that R:FR is a critical component in morphological responses. The growth traits such as stem elongation, internode length, stem taper, and number of leaves, were the most critically influenced by R:FR. Inhibition of internode elongation depended on the R:FR ratios existing inside the light filters. After several days of exposure to high R:FR ratios, inhibition of elongation of the stem began. Stem elongation inhibition under copper sulfate FR filters was about a 6% reduction compared to water filters. In this case, R:FR ratios may affect cell elongation and cell
divisions, which contributed to the growth of internodes. Some clones did not show much
difference in stem elongation between low and high R:FR ratios. This suggests there may be
genetic variation in signal recognition or subsequent physiological responses. Auxin and
gibberellin production do appear to be under some control of the phytochrome system
(Weller et al., 1994; Beall et al., 1996; Olsen et al., 1997).

Most clones under no filter treatment showed the shortest stem length and higher
stem tapers compared to stem length and tapers under water and CuSO₄ filter treatments.
This suppression of growth may be the result of the influence of different air movement
under the no filter treatments causing the plants to allocate photosynthate to mechanical
support systems to strengthen the stems. In one study of air movement, plants exposed to 30
sec. of shaking by wind (6 m per hour) each day had reduced stem height, premature terminal
nodes, decreased number of lateral branches, decreased internode length, and shortened and
thinner xylem vessels (Cleugh et al. 1998). Data on air movement were not collected in this
experiment, but are planned for future studies.

Although there were no statistical differences for leaf area and leaf dry weight among
light filters, some clones such as clone ILL-129, increased leaf areas and leaf dry weight
when R:FR was decreased. In contrast, there was an interactions between clones and light
filter chambers for overall total biomass ($P < 0.03$). This result suggests that total biomass
production was under the influence of R:FR signal changes. Most clones increased leaf areas
and leaf dry weight under high R:FR ratios, suggesting that different clones had different
ability to respond to R:FR, and the relative elongation response to R:FR was larger in the
larger stemmed-plants (Fig. 3.4 and table 3.1). The larger plants seemed to respond more to
changes in the light environment than smaller stemmed plants. The study also indicated that
larger clones had a higher fraction of total dry weight allocated to stem dry weight, which might be the result of leaf and stem respiration differences and/or light use efficiency. Therefore, the relationship between leaf area and the relative R:FR ratios mostly resulted in more stem and leaf dry weight. Our results are consistent with the result of Hikosaka (1999), which indicated that plants compete for light more efficiently with an increased leaf area index. Ceulemans, (1990) postulated that total leaf areas controlled poplar biomass accumulation and trees with larger leaf area accumulated more biomass. ILL-129 had a shorter stem height (43 cm) and less leaf area (1120 cm²) under high R:FR (CuSO₄ filter) and a longer stem (57 cm) and more leaf area (1204 cm²) under water and open filters (Table 3.1), suggesting that the phytochrome photoreceptors are involved in detecting critical information to alter plant elongation. In experiments where the phytochrome photoreceptor was partially knocked out, responses to R:FR ratios showed reduced morphological plasticity in *Datura ferox* L. and *Sinapsis alba* L. (Ballere et al., 1994; Bowler, 1997; Smith, 1984). Changes in R:FR result in large shifts in the phytochrome photoequilibrium that modulate tree development and change biomass allocation (Kasperbauer, 1987; Gilbert et al., 1995). The other possibility is that FR light and phytochrome pigment might trigger cell elongation. Le Noir (1967) indicated in a study of FR effects on *Phaseolus* that increased cellular elongation was found after FR treatment in the internodes. As illustrated in Figure 3.6, internode length showed great variation in response to R:FR signals. Significant differences in internode length and upper leaf areas (LPI-0 to LPI-3) were observed between light chambers and all clones. Internode length decreased dramatically from base to the top of the trees, but the most variation and fluctuation are between LPI-4 to LPI-12. This might be the site that received the influence from R:FR changes, resulting in the variation of upper
internode and leaf areas (LPI-0 to LPI-3). These sections (LPI-0 to LPI-3) of internode length and leaf area were young and still undergoing development, and they appear to be more sensitive to the R:FR signal. Therefore, these LPI intervals might be the primary light response site.

Clones differ in ability to respond to R:FR signals and in altering biomass accumulation (i.e. stem dry weight and root dry weight) (Table 3.1). The results of F2 hybrids and their parents demonstrate that the variations in physiological response of these clones are high. Other evidence of the high variability within cottonwood supports these results. Isebrands et al. (1988) indicated that cottonwood appears promising in terms of genetic variation among morphological and physiological traits.

The results of R:FR responses of clone 1068, 93-968, and ILL-129 are consistent with those obtained by others who studied Lilium multiflorum and Pseudotsuga menziesii, which showed increased stem length and reduced rates of tillering under low R:FR irradiance (Casal et al., 1990; Ritchie, 1997). The physiological processes alter the internal growth and increase stem elongation when induced by the R:FR signal, resulting in gaining greater light-harvesting ability (i.e. greater leaf areas and height) and improving their competitive ability (Gilbert et al., 1995; Ritchie, 1997). The results of this study also agree with Ballaré et al. (1990) which indicated that highly elongated plants under low R:FR signaling have greater light conversion into biomass, provide maximum photosynthetic rates, and more efficient capture of light per unit area compared to plants grown under copper sulfate.

Clones ILL-129, 1579, and 1125 were considered the best clones for growth under natural light conditions. Clone ILL-129 had greater stem elongation and larger leaf area expansion (Table 3.1) under low R:FR signals which is important in a high density tree
plantation. Clone 1125 exhibited normal growth under low R:FR light conditions. This clone might be well adapted to use high-density tree plantations. These two clones might represent the best clones for tree biomass production and silvicultural management. This experiment was conducted with young trees and produced some results on dry weight accumulation that are contrary to results of other people studying annual crops over a full production cycle. Therefore, further investigation based on age differences of *Populus spp.* responding to R:FR signals needs to be continued.

References


CHAPTER 4. GENERAL CONCLUSIONS

General Discussion

The results of this study provide a new foundation for future tree breeding and tree physiology work, not only for genus *Populus*, but also for other genera in the plant kingdom. The study of light competition has important implications concerning priorities for research and finding ways to increase woody biomass production. The results of this study showed that trees subjected to R:FR signal competition have an average 10% faster growth in height and produce 7% more dry weight. These findings agree with many studies of woody tree species, but are less consistent with some annual crops. These findings provide a new approach for improving tree biomass production. The significant responses of trees subjected to R:FR signals indeed indicates the possibility for improving and incorporating a new strategy for plant selection.

Under field conditions, leaf area and stem growth kept increasing throughout the growing season for all clones. The leaf area increase resulted in changes in R:FR ratios within the juvenile tree stand. The R:FR light signals began to differ 14 days after planting, indicating the R:FR signals start to play a crucial role very early in the response to plant competition. Trees subjected to narrow spacing reacted immediately when the R:FR light environment changed 14 days after planting by increasing stem height and leaf area, while the wider spacing still had a slower response. Dry weight biomass showed a trend to vary by spacing ($P = 0.12$) and by clone ($P = 0.08$). Trees exposed to the narrow spacing accumulated more biomass because of increased in stem height and accumulation of more leaves on the top of the stem. This resulted in increased ability to capture light. Storage
compounds are a part of the survival capacity and eventual dominance in stand developments. Numerous biotic and abiotic factors affect the absolute concentration of storage compounds. However, under this study, non-structural carbohydrate seemed to not be affected by spacing. Our starch and sugar measurements showed that spacing did not influence the starch accumulation at the end of growing season. This might be the effect of leaf damage due to insects, diseases, and deer before trees completely reached dormancy, with some amount of starch and sugar content used to rebuild mechanical support and provide defense. Unfortunately, there was no opportunity for verification in the second year (in 1999) because pest damage was even heavier.

In the greenhouse study, the trees subjected to low R:FR ratios accumulated more dry weight and elongated faster than trees exposed to high R:FR ratio. The total stem and root dry weight was not affected by the R:FR ratio treatment, but it differed among clones. However, stem height and internode length were different among light treatments and also among clones. This finding indicates that plants subjected to competition for R:FR signaling tended to increase their height and internode length, but were less affected in dry weight accumulation. The results also showed that the R:FR signals influenced stem internode length, leaf areas, and leaf dry weight between LPI-0 to LPI-3. The top leaves seemed to be the most responsive to the R:FR signals and may react by sending the signal to the rest of stem internodes below.

Therefore, we can conclude that in the juvenile stage, the tendency of trees to develop large size and accumulate more biomass was under low R:FR light signals. Trees respond to low R:FR signals at an early stage of development as a result of phytochrome involvement when they detect the low red light. The response of different clones is not the same, and very
high variation was seen among clones in the early stages of stem development. Plants will react to the low R:FR ratios by changing height and size in response to light competition. Our data suggest that R:FR signals play a role in developing the size inequality structure of plant populations.

**Recommendation for future research**

The investigation of physiological and genetic variations in tree competition and biomass allocation is a very important aspect for modifying tree growth and keeping viable genetic diversity. The alteration of phytochrome sensor systems needs to be investigated as a genetic improvement strategy. This might allow tree scientists to produce clones with better ability to adapt to low R:FR signals and to have better growth in stand development.

Information concerning stem elongation, levels and patterns of genetic diversity among clones and within clones in response to R:FR light signals may be useful for identifying diverse parental combinations. These might then be used to create segregating progenies with maximum genetic variability for tree improvement and silvicultural management under plantation conditions. There are many questions related to R:FR signals and plant competition that still need to be answered. Further research should be focused on the integrated R:FR signals, wind speed effect, storage compounds in the roots, cell elaboration in different parts of stems, and hormone signaling in responses to R:FR signals. The involvement of storage compounds as a defense mechanism or in biomass accumulation should be studied because storage proteins seem to serve as reserves for future growth, competition or resistance mechanisms. Water use efficiency in response to R:FR ratios also should be investigated.
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