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INTERSPECIFIC VARIATION IN ADAPTIVE TRAITS

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Grant Edward Jones

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INTERSPECIFIC VARIATION IN ADAPTIVE TRAITS OF TRUE FIRS (*ABIES SPP.*)

By

Grant Edward Jones

A THESIS

Submitted to
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ABSTRACT

INTERSPECIFIC VARIATION IN ADAPTIVE TRAITS OF TRUE FIRS (*ABIES* SPP.)

By

Grant Edward Jones

Recent efforts to increase conifer diversity for Christmas tree and landscape use have

sparked increased interested in planting true firs and their hybrids. Expanded use of firs

has been limited by their perceived intolerance of many site conditions; however, recent

research shows firs are more tolerant of environmental conditions than originally thought.

In this project we studied adaptive traits of 17 species and interspecific hybrids of firs at

four locations in the Lower Peninsula of Michigan. Project goals include characterizing

species difference in: 1) budbreak and cold hardiness, 2) the influences of soil pH on

foliar nutrition, physiological processes, and growth, and 3) the influence of needle

morphology and shoot architecture on net photosynthesis and drought tolerance. Mean

date of budbreak and growing degree days differed among species and location in both

years. Fir species that broke bud early were more prone to late spring frost damage than

species with late budbreak. Maximum mid-winter cold hardiness was negatively

correlated with date of budbreak. Soil pH influenced nutrient availability of several

important nutrients necessary for physiological processes. Net photosynthesis decreased

with increased soil pH and response differed among species. Needle morphology

differed among species and needle thickness was correlated with increased net

photosynthesis. Needle carbon isotope discrimination was related to water use efficiency

and varied among species. Continued improvement of stress tolerance of firs for the

upper Midwest is possible through selection for late budbreak and tolerance to soil pH.

ACKNOWLEDGMENTS

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LITERATURE REVIEW

Introduction

Michigan has a large nursery industry of which conifers represent a sizable portion. In the year 2000, coniferous trees produced in Michigan resulted in sales of over $35 million (USDA, 2001). Colorado blue spruce (*Picea pungens* var. *glauca* Engelm.), Norway spruce (*Picea abies* (L.) Karstens), white pine (*Pinus strobus* L.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) are used extensively in the landscape, to the point where they are overplanted. This lack of diversity has resulted in increased disease problems and insect pressures (McCullough et al., 1999; McCullough et al., 1998).

True firs (*Abies spp.* Mill.) are generally underutilized in landscapes. In general, *Abies* prefer cool, moist, well-drained sites with acidic soil. They have been used primarily for Christmas trees, with *A. fraseri* grown in the eastern United States, while *A. procera* and *A. nordmanniana* are grown in the Pacific Northwest. In cooler regions of the United States, *A. concolor* is commonly planted in the landscape. However, the use of additional species and varieties has been limited due to their intolerance to varying site conditions.

The number of *Abies* species has been debated, reportedly ranging from 39 (Liu, 1971) to 46 (Farjon, 1990) to 55 (Rushforth, 1987). *Abies* species are found only in the northern hemisphere at higher latitudes or at lower latitudes at higher elevations. In their natural habitat, *Abies* are considered late-successional trees (Kohyama, 1984) as they are not among of the first plant community that successfully colonizes disturbed sites. Rather, they are slow growing and shade tolerant replacing pioneer species as shade levels increase. *Abies* have been fragmented by glaciation and have adapted to a wide

range of site conditions. As a result, species ranges vary greatly. *Abies nebrodensis*, for example, consists of only several dozen trees spread over a few square kilometers (Parducci et al., 2001). In contrast, the range of *A. sibirica* stretches for thousands of square miles. Species such as *A. balsamea* and *A. fraseri* are thought to be closely related but isolated due to glaciation (Jacobs et al., 1984; Myers Jr. and Bormann, 1963).

Farjon (1991) clustered the species into 10 sections based on cone characteristics, flower color, needle structure, pollen grains, geography, and fossil records (Table 1). *Abies* naturally hybridize easily within and between groups (Liu, 1971) and where native ranges overlap (Isoda et al., 2000). Hybrids often display increased vigor (Klaehn, and Winieski, 1962). Crichfield (1988), however, suggests that artificial crosses between sections are more difficult than crosses within sections. Perhaps genetic relationships need to be investigated and considered in future classification efforts.

In past introductions, adaptive traits varied greatly among species and provenances. These different characteristics and can be used to screen future introductions. The following literature review will focus on the different adaptive characteristics that are important in Michigan and the upper Midwest with respect to temperature, water relations, nutrition, and light response in the genus *Abies*.

Temperature

Tolerance to temperature extremes are an important adaptive characteristic for future introductions. In Michigan, temperatures vary considerably. During winter, temperatures below -20 °C are common and have reached -45 °C. Temperatures ranging from 25 to 30 °C are not unusual during summer.

Cold Hardiness

In the Upper Midwest, cold hardiness is an important requirement for quality trees. Cold hardiness develops as trees pass through the following three stages: 1) short days cause the cessation of growth, 2) freezing temperatures cue a metabolic reorganization of macromolecules resistant to severe dehydration, and 3) extreme low temperatures increase cold hardiness through dehydration resistance or supercooling (Weisner, 1970). When days become shorter and temperatures drop in the fall, trees accumulate sugars and starches in their cells, lower their freezing point (Sutinen et al., 2001; Weisner, 1970). Trees reach their maximum cold hardiness in mid-winter and then gradually become less cold hardy as temperatures warm in the spring (Ritchie, 2003). Gradually rising temperatures in the spring lead to decreasing cold hardiness. Trees can regain cold hardiness when colder temperatures return; however, cold hardiness is still lost more quickly than it is regained (Strimbeck et al., 1995). Temperature fluctuation in the spring can lead to late winter injury and damage to buds, needles, roots, and cambial tissue (van der Kamp and Worrall, 1990).

Conditioning to low temperatures effects the physiological responses to temperature extremes. *Abies procera* seedlings exposed to temperatures ranging from 24 to 35 °C experienced more damage when exposed to sub-freezing temperatures than those grown at warm (18 to 27 °C) or cool (12 to 19 °C) temperatures (Owston and Kozlowski, 1981). Branches of field-grown *A. lasiocarpa* trees were cold hardy to temperatures below -60 °C in mid-winter. Branches from the same tree exposed to 20 °C for 132 hours and were cold hardy only to temperatures near -20 °C (Gordon-Kamm, 1980).

Under field conditions, snow cover often insulates plant material from colder air temperatures. In cold regions receiving heavy snowfall, snow can keep needle temperatures near 0 °C and prevent them from acclimating to colder conditions. *Abies sachaliensis* grown in areas with heavy snow cover were more prone to frost injury than trees grown in areas with less snow cover (Eiga and Sakai, 1987). These results support the concept that trees can lose cold hardiness when exposed to prolonged warming periods.

Trees adapt to different temperature extremes found in their native ranges. *Abies spectabilis*, native to Nepal, is cold hardy to -25 °C while *A. balsamea*, native to Canada and *A. sibirica*, native to Siberia, are cold hardy to -70 °C (Sakai, 1982). Christmas tree growers found *A. fraseri*, native to North Carolina, and *A. concolor*, native to the Rocky Mountains, were less cold hardy than *A. balsamea* (Nicholls and Palmer, 1985). Likewise, varying levels of cold hardiness are also found within species. A 10-year provenance test in British Columbia showed *A. grandis* from coastal areas were more susceptible to frost damage than trees from inland seed sources (Xie and Ying, 1993).

Average air temperatures can influence the maximum cold hardiness of trees. Air temperatures in dry air cool at the adiabatic lapse rate of 3 °C per 300 m increase in elevation. *Abies* are common to many mountainous regions in the higher latitudes of the northern hemisphere. *Abies sachalinensis* is native to the Hokkaido islands in Japan where their maximum cold hardiness increases as elevation increases (Eiga and Sakai, 1984; Xie and Ying, 1993). Snow cover keeps branches covered by more snow closer to freezing and insulates them from extreme freezing temperatures.

In trees a chilling requirement and a thermal time requirement exists during winter and restricts tree growth (Howe et al., 2003; Campbell and Sugano, 1979). Cold hardiness can be lost and growth can occur once the thermal time requirement is met (Gordon-Kamm, 1980; Perry and Wu, 1960). Prolonged warming spells followed by dramatic temperature drops result in the loss of cold hardiness and damage to trees. In British Columbia, temperatures were above normal for much of December 1988 and January 1989. This warm period was followed by a sudden drop in temperatures to -30° C. In *A. amabilis* and *A. lasiocarpa* buds on lateral branches above the snow level were killed following a sudden drop to temperatures to -30 °C; however terminal buds remained undamaged (van der Kamp and Worrall, 1990).

Once the temperature threshold has been met, trees accumulate growing degree days (GDD). Growing degree days are used to quantify temperatures above a threshold specific to the tree and their duration. In *Abies,* GDD influence budbreak. Frequently a base temperature of 50 °C is used to calculate growing degree days (Dickson et al., 2000). Trees growing at higher elevations are exposed to fewer warm days. Because trees at higher elevations are exposed to cooler temperatures, they take longer to surpass their GDD requirement than trees at lower elevations or coastal regions. In response, trees grown at higher elevations have a reduced GDD requirement and break bud only after sufficient warming has occurred. For example, Worrall (1983) found *A. lasiocarpa* growing at high elevations have a shorter temperature threshold than provenances growing at lower elevations or coastal areas, suggesting adaptation to a shorter growing season. In provenance tests and species trials, trees are often grown in areas with climates different from their native regions. Worrall (1983) found that *A. amabilis* broke

bud later than *A. lasiocarpa* and thus species differ in their GDD requirement for bud break. Trade-offs exist as trees breaking bud early are at increased risk from late spring frosts (Hansen and Larsen, 2004).

Different tree organs have varying levels of cold hardiness. Roots are insulated from extreme winter temperatures by the soil. However, soil temperatures can still fall below freezing and injure roots during winter months (Bigras et al., 2001). Maritime species (*A. amabilis*) and continental species (*A. lasiocarpa*) did not differ at the temperature in which root damage was 50% (Coleman et al., 1992). While different species may have similar cold hardiness levels in their roots, they can differ in the degree of cold hardiness present in other organs. Coleman et al. (1992) found *A. lasiocarpa* withstood needle injury at lower temperatures than *A. amabilis*. In *A. koreana*, primordial shoots were cold hardy to -40 °C while needles and twigs were cold hardy to -70 °C (Sakai, 1982).

High Temperature

Temperature is an important environmental condition affecting net carbon gain. Gross photosynthetic increases with increasing temperatures, plateaus and declines rapidly as high temperatures begin to degrade cell processes (Berry and Björkman, 1980). Net photosynthesis is the difference between gross photosynthesis and respiration. As temperatures rise, respiration increases exponentially and eventually causes net photosynthesis to decline. The temperatures where net photosynthesis is greatest represents a peak range of optimal temperatures. Plants have an optimal temperature range where photosynthesis occurs at maximum rates. Maximal quantum yield of

photosystem II (PSII) increased in *A. alba* with increasing temperature reaching a maximum at 26 °C and declining steadily after 32 °C (Robakowski et al., 2002).

In *Abies*, species differ in their optimal temperature range. In cooler regions, plants have evolved methods to maximize their leaf temperature and reach their optimal temperature range more quickly. In conifers needle packing is an adaptation that results in increased needle temperature to levels higher than air temperature. Needle temperatures rise as the number of needles·cm^{-1} of shoot length increase due to boundary layer effects (Smith and Carter, 1988; Martin et al., 1999, Smith, 1980). In *A. lasiocarpa*, needle packing increased morning temperatures by 8 °C to temperatures near the optimal temperature range for photosynthesis resulting in increased carbon gain (Smith and Carter, 1988).

The boundary layer is a layer of relatively calm air that exists due to the friction above a surface. This layer of calm air can help insulate needles and keep them warmer than surrounding air temperatures. Needles of conifers are much coarser than broadleaves and hence create more friction with air. This increased friction results in enhanced boundary conductance layer and increases needle temperatures in *A. amabilis* (Martin et al., 1999) and *A. lasiocarpa* (Smith and Carter, 1988). Temperature gain from needle packing in *A. lasiocarpa* decreased in the afternoon as wind speed increased (Smith and Carter, 1988). Increases in wind speed decreases the boundary layer existing above the needle surface, reducing the insulating effect. In contrast, elevated needle temperature resulted in no increased photosynthetic response in *A. amabilis*, which has a much larger range of optimal photosynthetic temperatures, and thus reached its optimal temperature range more easily (Martin et al., 1999).

Populations adapt and function with lower optimal photosynthetic temperatures in cooler regions. In New Hampshire, the optimal temperature for photosynthetic CO_2 uptake declined by 2.7 °C for a 305 m increase in elevation in *A. balsamea* (Fryer and Ledig, 1972). This suggests adaptations occurring at high and low elevations and resulting in changes to optimum temperature.

Water Relations

In Michigan, periods of water stress are common throughout the growing season and can limit tree growth. Identifying species capable of tolerating drought conditions is an important criterion for future tree introduction in Michigan.

Drought tolerance

Conifers respond to both soil drought and atmospheric drought. *Abies* species and provenances differ in their response to drought. Drought reduced the number of shoot internodes and internode elongation in *A. magnifica* and *A. concolor* (Hallgren and Helms, 1998). In Switzerland, *A. alba* shed needles in response to drought conditions (Webster et al., 1996). Inland and higher elevation provenances of *A. grandis* survived planned dry-down treatments better than coastal provenances (Scholz and Stephan, 1982).

Stomata regulate water lost during transpiration and are particularly important as humidity decreases. At low humidity, the water vapor pressure deficit increases and results in more plant water loss if not regulated. Increases in water vapor pressure deficit reduced photosynthetic gain in *A. nordmanniana* (Guehl et al., 1989). Stomatal conductance decreases as humidity decreases and results in lower photosynthetic rates in several Mediterranean *Abies* species (Guehl et al., 1991). Stomatal response to increased

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water vapor pressure deficits was delayed in *A. alba* and resulted in excess transpiration loss and eventually decreased photosynthetic gain. However, *A. alba* provenances differed in their photosynthetic gain and transpiration levels under favorable and drought conditions (Guehl and Aussenac, 1987).

Species also respond differently to soil drought conditions. In *A. bornmulleriana*, stomata closed quickly and the photosynthetic rate was reduced at low soil water potentials. Under the same soil conditions, *A. cephalonica* exhibited a higher photosynthetic rate than *A. bornmulleriana*. Guehl et al. (1991) suggest *A. bornmulleriana* adapts to avoid internal water deficits while *A. cephalonica* adapts to tolerate drought.

In mountainous areas, lower temperatures can reduce moisture stress. During winter and spring months, colder temperatures increase snowfall, limit snowmelt, and improve summer soil-moisture levels at higher elevations. Adequate snowfall improved summer soil moisture levels and increased growth in *A. lasiocarpa*. Furthermore, cooler temperatures reduce evapotranspiration in the summer and reduce moisture stress in *A. lasiocarpa* (Peterson et al., 2002).

Water use efficiency and Carbon isotope discrimination

Water use efficiency (WUE) is the amount of CO_2 fixed per unit of water lost and is often used to compare drought tolerance between species or provenances. Other factors being equal, high WUE indicates better drought tolerance. Under normal growing conditions, *A. alba* provenances from southern Italy (warmer, drier regions) maintained higher photosynthetic values in one- and two-year-old needles than provenances from central and eastern Europe (cooler, wetter regions) in which 2-year-old needles declined

in photosynthetic gain (Larsen and Mekic, 1990). This resulted in higher WUE in the

provenances from southern Italy indicating increased drought tolerance.

Carbon isotope discrimination (Δ) may be used to investigate WUE and drought

stress. ^{12}C and ^{13}C represent 98.9% and 1.1% of atmospheric carbon respectively. C_3

plants, which include conifers, discriminate against ^{13}C while fixing carbon and thus have

a lower ratio of $^{13}C/^{12}C$ ($\delta^{13}C$) than the atmosphere (Farquhar et al., 1989). However, as

stomata close, intercellular CO_2 levels decline due to photosynthetic assimilation. As a

result, discrimination against ^{13}C decreases resulting in a higher $\delta^{13}C$ ratio. Tissues with

lower Δ (higher $\delta^{13}C$) values indicate periods of reduced stomatal conductance.

Increased water use efficiency is frequently correlated with reduced Δ (Farquhar et al.,

1989; Masle and Farquhar, 1988).

Several studies have documented environmental response of Δ in *Abies*. In *A.*

spectabilis, increased relative humidity and precipitation were positively correlated with

Δ in tree rings (Xiaohong et al., 2003). Similarly Guehl et al. (1991) showed Δ increased

(lower $\delta^{13}C$) due to more conductance with increased rainfall. Other environmental

factors such as pollution reduce stomatal conductance and lower Δ (increase $\delta^{13}C$) values

in tree rings (Sakata and Suzuki, 2000).

In the mountainous areas of the western United States, precipitation patterns can

vary greatly. Alexander et al. (1990) describe the native range of *A. lasiocarpa* to include

regions receiving less precipitation than *A. magnifica* (Laacke, 1990a) and *A. procera*

(Franklin, 1990) and more precipitation than *A. concolor* (Laacke, 1990b) and *A. grandis*

(Foiles et al., 1990). Instantaneous WUE measurements in *A. lasiocarpa* were higher

early and late in the day than another subalpine conifer, *Pinus albicaulis* Engelm., which

shares a similar native range; however, WUE levels declined and were lower than *P. albicaulis* at midday. Conversely, needles from the previous year show *A. lasiocarpa* had a lower $\delta^{13}C$ than *P. albicaulis* and thus a lower WUE over time (Sala et al., 2001). When compared to five other conifers native to the northern Rocky Mountains, Δ values were significantly higher (lower WUE) in *A. lasiocarpa* (Piñol and Sala, 2000) suggesting that while *A. lasiocarpa* may be moderately drought tolerant among other *Abies* species in the Western United States; it still less tolerant of dry sites when compared to other conifers sharing its native range.

Soil Conditions

Different nutrients are required for many physiological processes by trees. Even if soil nutrient levels are high, the availability of these nutrients may be regulated by soil pH (Lucas and Davis, 1961). In New Hampshire, *A. balsamea* showed little correlation between soil and foliar nutrient levels, suggesting that another factor such as soil pH influenced nutrient availability (Bruns, 1973). In Michigan, soil pH levels vary greatly from more acidic in coniferous forests to more alkaline in grassland regions. Therefore, tolerance to varying soil pH and nutrient availability is necessary in future plant introductions in Michigan.

Nutrition

Abies from around the world have adapted to survive under varying soil pH levels, although they generally prefer acidic soils. As a result, species differ in their soil pH tolerances. Soil pH levels influence the plant nutrients available to the plant and can lead to nutrient deficiencies (Lucas and Davis, 1961), which can ultimately hinder physiological processes. Seedling growth in *A. fraseri* was greatest in soil pH ranging

from 4.2 to 4.5 (Bryan et al., 1989) while *A. balsamea* grows best on sites with soil pH levels between 6.5 and 7.0 (Bakuzis and Hansen, 1965). In *A. nordmanniana*, needle chlorosis developed in higher pH soils (Khalil et al., 1989).

Nutrient deficiencies disrupt tree physiological processes, which can lead to abnormalities such as needle chlorosis and stunted growth. Decreasing soil pH levels tend to increase the Mn, Fe, Mg, and P that is available to the tree (Lucas and Davis, 1961). Increasing soil pH in *A. alba* led to Mn deficiencies and needle chlorosis ultimately resulting in tree decline (Hiltbrunner and Flückiger, 1996). In several *Abies* species, photosynthetic efficiency and foliar Mn, B, K, Zn, and Cu levels declined with increased soil pH levels (Cregg et al., 2004). Decreasing levels of these nutrients were also strongly correlated with a decreased variable fluorescence to maximum fluorescence (F_v/F_m) ratio and decreased chlorophyll concentration in the needles.

Nitrogen, Mg, Mn, and Cu are several nutrients that are important to the photosynthetic process. In *A. fraseri*, higher foliar N and P levels were characteristic of increased visual quality while higher foliar Ca, Mg, and Fe levels were found in trees with lower visual quality. In the same study, foliar nutrient ratios (eg. N:Ca, N:Mg, N:Fe, P:Ca, P:Mg, and P:Fe) were important indicators of increased visual quality in *A. fraseri* (Rothstein and Lisuzzo, 2003). Needle chlorosis developed in *A. nordmanniana* with decreased foliar Fe, Mg, Mn, N, and S levels (Khalil et al., 1989). However, other site factors can influence foliar nutrient levels. Foliar P and Ca levels were highly variable in *A. grandis*, possibly in response to shade conditions (Moore et al., 2004). Chlorophyll concentration increased following NPK fertilization in *A. balsamea* (Lavigne

et al., 2001). In *A. balsamea* var. *phanerolepis* Fern. N, K, and Ca foliar nutrient levels were significantly correlated with tree height and needle length (Brown, 2000).

Nitrogen is frequently applied in conifer nurseries to improve tree color (Bruns, 1973, Rothstein and Lisuzzo, 2003), increase lateral bud development (Timmer et al., 1977), and increase tree growth (Hawkins et al., 1998) in several *Abies* species. Applying excess N can result in luxury consumption. Luxury consumption is continued N uptake by the tree with no increase in growth. Increased N fertilization in *A. balsamea* resulted in increased needle dry weight. Fertilizing beyond a foliar N content of 2.3%, however, resulted in luxury consumption (Timmer and Stone, 1978).

Conifers keep their needles for several growing season. Some nutrients are mobile and can move from older tissue to younger tissues while other nutrients cannot. Younger branches tend to produce more photosynthate than older branches (Larsen and Mekic, 1990; Jach and Ceulemans, 2000). As a result, trees alter nutrient levels and needle quantities in older needles to maintain the productivity of younger shoots. Hinesley and Wright (1989) found that in *A. fraseri* N, P, and K concentrations were higher in younger branches than branches 4- to 5-years-old. *Abies amabilis* are slow growing trees and tend to keep their needles for a several growing seasons. Due to the increased importance of its older needles, little N and P are exported to newly emerging shoots in the spring (Hawkins et al., 1998). However, any exported nutrients were partially replaced later in the growing season. Lignin concentrations increase as trees age. Hinesley and Wright (1989) also found that Ca levels increase in older *A. fraseri* branches as lignin levels increase and productivity declines.

Chlorophyll and Nitrogen

Chlorophyll and nitrogen are two important components of the photosynthetic process. Trees vary their concentrations in response to contrasting light environments. Nitrogen concentrations increased in *A. amabilis* (Brooks et al, 1994; Stenberg et al., 1998) and *A. alba* (Robakowski et al., 2003) as irradiance increased. However in *A. balsamea*, the N concentrations in sun and shade shoots were not different. Richardson (2004) suggests this contradiction could be due to sun shoots undergoing photoinhibition where additional N would not increase photosynthetic output or due to shade shoots capturing light more efficiently than sun shoots. According to Grassi and Bagnaresi (2001), shade-tolerant plants commonly have a higher chlorophyll/mass ratio than plants acclimated to high light environments, but chlorophyll/area is unaffected by the light environment. Increased irradiance resulted in lower chlorophyll/mass in *A. alba* (Grassi and Bagnaresi, 2001; Robakowski et al., 2003). In *A. amabils* (Brooks et al., 1994) chlorophyll/area increased with increasing irradiance while it was unchanged in *A. alba* (Grassi and Bagnaresi, 2001; Robakowski et al., 2003).

As irradiance decreased, chlorophyll b concentrations increased more than chlorophyll a and resulted in a decline in the chlorophyll a/b ratio in *A. alba* (Brooks et al., 1994). Since both N and chlorophyll are important components of photosynthesis, their increasing concentrations in sun foliage results in higher photosynthetic output in sun shoots than shade shoots (Brooks et al., 1994; Carter and Smith, 1985; Robakowski et al., 2003).

Lime

Lime ($CaCO_3$) application is a broadly accepted practice to raise soil pH levels. Using lime to raising soil pH levels reduces aluminum toxicity, improves nutrient

availability, returns Ca lost by tree harvest to the soil (Jablanczy, 1971). In *A. balsamea*, lime application improves tree quality (Jablanczy, 1971). However, other studies question the effectiveness of lime applications. Lime applications did not improve growth in the 2 years following application in *A. balsamea* on sites with a pH of 3.7 (Timmer et al., 1977). Rothstein and Lisuzzo (2003) found high foliar Ca levels in *A. fraseri* imply nutrient imbalances.

Light

In the landscape, trees are often planted on exposed sites only to become shaded over time. *Abies* species have adapted to acclimate differently to varying light environments. In Japan, under moderate light, *A. veitchii* has a higher growth rate than *A. mariesii*. Under low light however, *A. mariesii* regenerates more successfully than *A. veitchii* (Kohyama, 1984). Changing shoot dynamics can increase the light harvested by the needles while reducing maintenance costs. Trees can alter their morphological and chemical characteristics to maximize their light harvest in response to changing light environments. Tolerance and adaptation to a wide range of light conditions would be beneficial in future tree introductions.

Biomass Allocation

Changes in light environment can lead to changes in biomass allocation. Tree crowns are more conical in high light environments and more globose in low light environments (King, 1997, Kohyama, 1980). Under low light conditions, both *A. amabilis* and *A. lasiocarpa* displayed reduced height growth and increased caliper growth and resulted in increased lateral shoot growth and increased light interception (Klinka et al., 1992). King (1997) however, found that light increases branch biomass allocation

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while limiting stem biomass allocation under low light conditions. In *A. balsamea* biomass allocation to foliage, branches, stems, and total shoot remained constant at 13, 25, 45, and 100% light intensities, while total biomass accumulation was highest at 45% light. In the same study, root mass increased with increasing light intensity in 4-year-old *A. balsamea* seedlings (Logan, 1969). Life-spans for needles on shade shoots were longer than life-spans for needles on sun shoots thus compensating for increased branch biomass costs (Kohyama, 1980, Mori and Takeda, 2004). Mori and Takeda (2004) found little difference in shoot length between sun and shade trees in *A. veitchii* while sun shoots were longer than shade shoots in *A. mariesii*.

Photoinhibition

Many conifers, including *Abies*, maintain their needles year-round and have limited photosynthetic capacity during winter months when warm temperatures occur (Schaberg et al., 1998). Snow cover is an important insulator of plant material during cold winter months. However, snow has a high albedo and increases the amount of reflected light. Increased reflection raised light intensity levels and resulted in needle photodamage when exposed to low temperatures (Yamazaki, et al., 2003).

Light Response

Light response curves are used to distinguish changes in photosynthetic response at different light intensities and include the following: dark respiration, the light compensation point, apparent quantum efficiency, light saturation point, and maximum photosynthetic gain (A_{max}) (Figure 1). Dark respiration is the amount of respiration when no light is present. The light compensation point is the light intensity where dark respiration equals the gain due to photosynthesis and results in a net carbon gain of zero.

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Apparent quantum efficiency is the change in photosynthesis for change in incident photosynthetically active radiation (PAR). With increasing light intensities, shoots eventually reach A_{max} at the light saturation point. At varying light levels, trees alter their photosynthetic response to maximize their photosynthetic gain. In *A. alba*, dark respiration, quantum efficiency, and the light compensation point increased with increased irradiance (Grassi and Bagnaresi, 2001). Brooks et al. (1994) found A_{max} was higher in sun shoots than shade shoots in *A. amabilis*.

Needle Morphology and Shoot Architecture

Light plays an important role by influencing the needle morphology of conifers. In *A. amabilis*, needle weight, needle thickness, and leaf mass/area increased with increased irradiance (Brooks et al., 1994; Sprugel et al., 1996). Leaf area and needle weight increase with higher light levels in *A. amabils* and *A. lasiocarpa* (Klinka et al., 1992).

Conifers will change their shoot architecture to limit self-shading in shade conditions, while producing shoots to maximize light harvest in high irradiance conditions. Shoots growing in full light tend to have more needles than shoots grown in the shade. Smith and Carter (1988) found that in *A. lasiocarpa* needle density (needles·cm^{-1}) was 50% higher in sun shoots than shade shoots. Needles of *A. lasiocarpa* growing at high light exposure were more vertically oriented, while trees growing at reduced light exposure were more horizontally oriented to maximize light harvest and reduce self-shading (Germino and Smith, 1999). In *A. amabilis*, leaf mass/shoot silhouette area increases linearly as canopy openness increases suggesting trees in full sun spread incoming light over more area than trees growing in shade (Sprugel et al.,

1996). Shade shoots undergo less self-shading than sun shoots and therefore increase

their light-capturing efficiency (Stenberg, et al., 1998). Increased fertilization resulted in

improved growth leading to increased self-shading of older needles in *A. grandis*, which

eliminated a positive carbon balance in those shoots and caused needle abscission

(Balster and Marshall, 2000). By altering shoot architecture, sun and shade shoots can

maximize their area for light harvest while limiting maintenance costs.

Light Acclimation

As trees age, sun shoots can eventually become shade shoots and trees can

acclimate in response to this change in irradiance. After applying shade to sun shoots, *A.*

amabilis reduced its photosynthetic production at light saturation, chlorophyll-to-nitrogen

ratio, and chlorophyll a:b ratio to levels similar to shoots that were naturally shaded,

while chlorophyll content increased (Brooks et al., 1994). Even though chlorophyll a:b

ratio declined, both chlorophyll a and chlorophyll b levels increased with decreased

irradiance. Needle thickness was unchanged as shoots became shaded but needle weight

decreased suggesting internal changes and needle abscission.

Summary

Abies use in the landscape and by the Christmas tree industry has been rather

limited; however, different species and provenances have adapted to varying site

conditions (Table 2). Several adaptive characteristics warrant further investigation to

identify potential species and hybrids worthy of consideration for use in Michigan.

Considerable variation exists among species and provenances in their level of cold

hardiness and time to budbreak. Shoot adaptations result in needle temperatures above

that of the surrounding air to improve their photosynthetic output in some species.

Species and provenances may respond differently to drought and vary in drought tolerance. Foliar nutrient levels are largely dependent on soil pH; however, different species show tolerance to varying soil pH levels. Varying light environments elicit different responses exhibited by biomass allocation, shoot architecture, light response characteristics, and photosynthetic elements.

Table 1. Fourty-six species in 10 *Abies* sections as defined by Farjon (1990).

Section	Species
Abies	*alba* Mill.
	cephalonica Loud.
	cilicica (Ant. et Kotschy) Carrière
	nebrodensis (Lojac.) Mattei
	nordmanniana (Stev.) Spach.
Amabilis	*amabilis* (Dougl.) Forbes
	mariesii Mast.
Balsamea	*balsamea* (Linn.) Mill.
	fraseri (Pursh.) Poir.
	kawakamii (Hay.) Ito
	koreana Wils.
	lasiocarpa (Hook.) Nutt.
	nephrolepis (Tratv.) Maxim.
	sachalinensis (Fr. Schm.) Mast.
	sibirica Ledeb.
	veitchii Lindl.
Bracteata	*racteata* D. Don ex Poiteau
Momi	*beshanzuensis* Wu
	chensiensis Van Tiegh.
	firma Sieb. et Zucc.
	holophylla Maxim.
	homolepis Sieb. et Zucc.
	pindrow (Lamb.) Royle
	recurvata Mast.
	ziyuanensis Fu et Mo

Figure 1 Cont'd.

Section	Species
Grandis	*concolor* (Gord. et Glend.) Lindl.
	durangensis Mart.
	grandis (Dougl.) Forbes
	guatemalensis Rehd.
Nobilis	*magnifica* A. Murr.
	procera Rehd.
Oiamel	*hickeli* Flous et Gauss
	religiosa (H.B.K.) Schlect. et Cham.
	vejari Mart.
Piceaster	*numidica* De Lann.
	pinsapo Boiss.
Pseudopicea	*densa* Griff.
	fabric (Masters) Craib
	fanjingshanensis Huang, Tu et Fang
	fargesii French.
	forrestii C. Coltm. Rogers
	spectabilis (D.Don) Spach
	chengii Rushforth
	delavayi Van Tiegh.
	squamata Masters
	yuanbaoshanensis Lü et Fu

Table 2. List of species tolerant or intolerant of extreme winter temperatures, drought, and soil pH.

Cold Hardiness	
<u>Less Cold Hardy (Temps ≥ -20 °C)</u>	
A. spectabilis	Sakai, 1982
<u>Medium Cold Hardy (Temps -21 to -39 °C)</u>	
A. firma	Sakai, 1982
A. homolepis	Sakai, 1982
A. veitchii	Sakai, 1982
A. procera	Sakai, 1982
A. sachalinensis	Sakai, 1982
<u>More Cold Hardy (Temps ≤ -40 °C)</u>	
A. koreana	Sakai, 1982
A. balsamea	Sakai, 1982
A. sibirica	Sakai, 1982
A. lasiocarpa	Gordon-Kamm, 1980
Drought tolerance	
<u>Poor Water Use Efficiency</u>	
A. alba - Eastern European provenances	Guehl and Assenanc, 1987
A. lasiocarpa	Piñol and Sala, 2000
Soil Chemistry	
<u>Tolerant of high pH soils</u>	
A. balsamea	Bakuzis and Hansen, 1965
A. veitchii	Cregg et al., 2004
A. lasiocarpa	Cregg et al., 2004
<u>Intolerant of high pH soils</u>	
A. alba	Hiltbrunner and Flückiger, 1996
A. borisii regis	Cregg et al., 2004
A. sibirica	Cregg et al., 2004
A. sachalinensis	Cregg et al., 2004
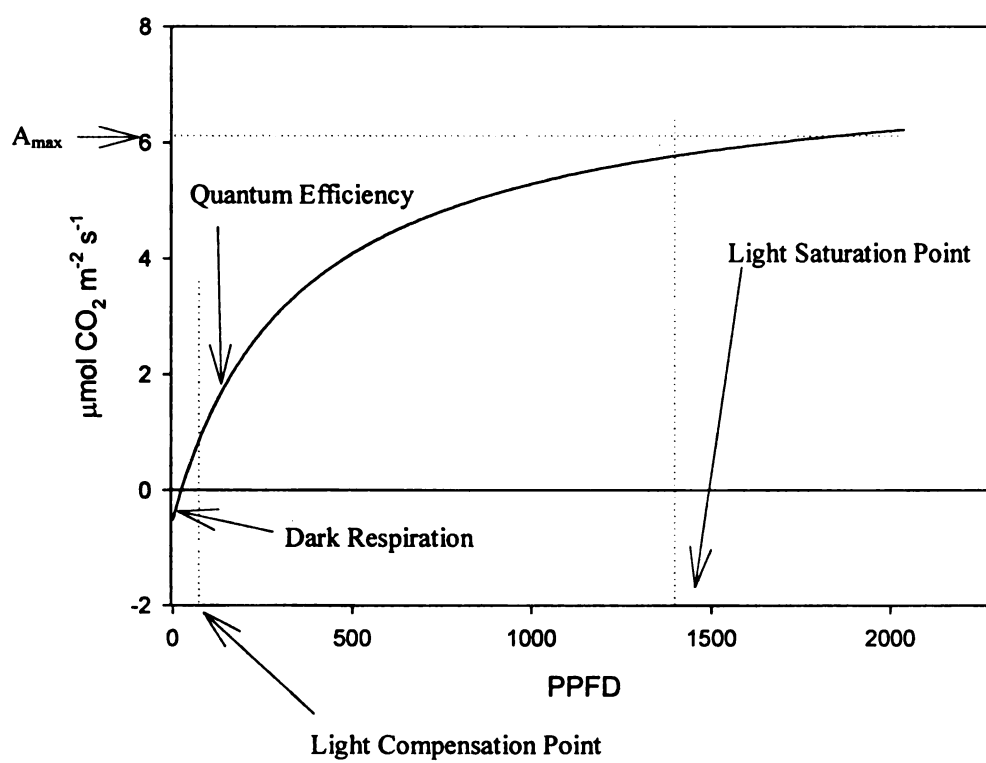
Figure 1. Sample light response curve for *Abies koreana* x *balsamea*.
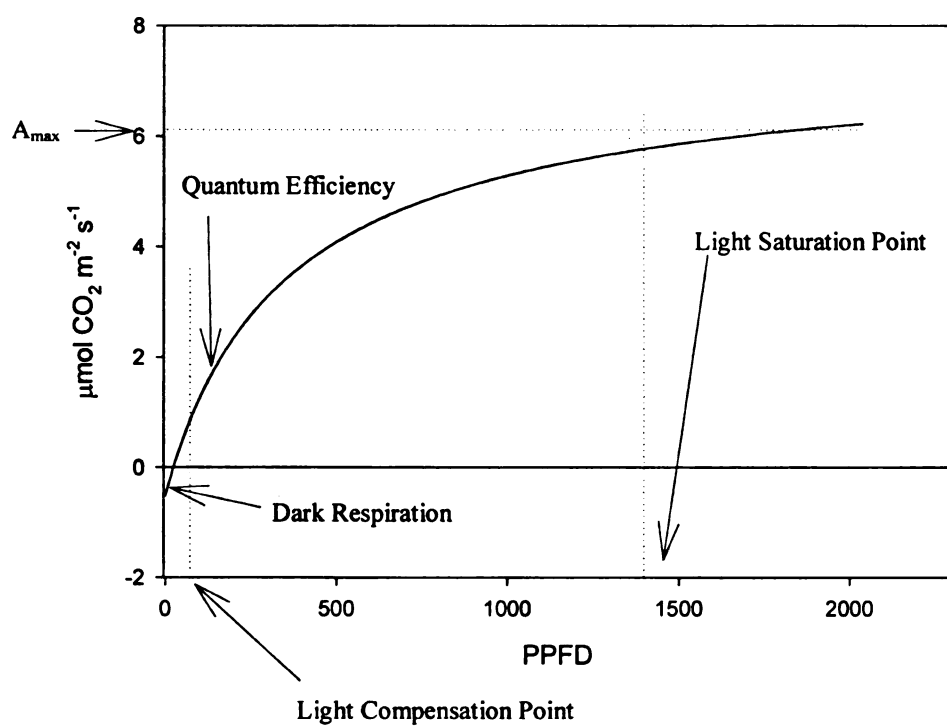
Figure 1. Sample light response curve for *Abies koreana* x *balsamea*.

Literature Cited

Alexander, R.R., R.C. Shearer, and W.D. Shepperd. 1990. Subalpine fir. In: Russell M. Burns and Barbara H. Honkala (tech coords.). Silvics of North American: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

Aussenac, G. 2002. Ecology and ecophysiology of circum-Mediterranean firs in the context of climate change. Ann. For Sci. 59: 823-832.

Bakuzis, E.V. and H.L. Hansen. 1965. Balsam fir-a monographic review. University of Minnesota Press, Minneapolis. 445p.

Balster, N.J. and J.D. Marshall. 2000. Decreased needle longevity of fertilized Douglas-fir and grand fir in the northern Rockies. Tree Physiol. 20: 1191-1197.
3394087 - 304

Berry, J. and O. Björkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31: 491-543.

Bigras, F.J., A. Ryyppö, A. Lindström, and E. Stattin. 2001. Cold acclimation and deacclimation of shoots and roots of conifer seedlings, p. 57-88. In: F.J. Bigras and S.J. Colombo (eds.). Conifer cold hardiness. Kluwer Academic Publishers, Norwell, Mass.

Brooks, J.R., T.M. Hinckley, and D.G. Sprugel. 1994. Acclimation responses of mature *Abies amabilis* sun foliage to shading. Oecologia. 100: 316-324.

Brown, J.H. 2000. Survival and growth of trees of a Canaan Valley, West Virginia, seed source in relation to varying soil/site conditions. Ohio State Univ. Ext. Spec. Circ. 175-00.

Bruns, P.E. 1973. Cultural practices, fertilizing and foliar anaylsis of balsam fir Christmas trees. N.H. Agric. Exp. Stn. Bull. 501, Durham, NH.

Bryan, J.A., J.R. Seiler, and R.D. Wright. 1989. Influence of growth medium pH on the growth of container-grown fraser fir seedlings. J. Environ. Hort. 7(2) 62-64.

Campbell, R.K. and A.I. Sugano. 1979. Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. Bot. Gaz. 140(2): 223-231.

Carter, G.A. and W.K. Smith. 1985. Influence of shoot structure on light interception and photosynthesis in conifers. Plant. Physiol. 79: 1038-1043.

Coleman, M.D., T.M. Hinckley, G. McNaughton, and B.A. Smit. 1992. Root cold hardiness and native distribution of subalpine conifers. Can. J. For. Res. 22: 932-938.

Cregg, B.M., M.W. Duck, C.M., Rios, D.B. Rowe, and M.R. Koelling. 2004. Chlorophyll fluorescence and needle chlorophyll concentration of fir (*Abies* sp.) seedlings in response to pH. Hort Sci. 39(5): 1121-1125.

Critchfield, W.B. 1988. Hybridization of the California firs. For. Sci. 34(1): 139-51.

Dickson, R.L., G.B. Sweet, N.D. Mitchell. 2000. Predicticing *Pinus radiata* female strobilus production for seed orchard site selection in New Zealand. For. Ecol. Mgt. 133: 197-215.

Eiga, S. and A. Sakai. 1984. Altitudinal variation in freezing resistance of Saghalien fir (*Abies sachalinensis*). Can. J. Bot. 62: 156-160.

Eiga, S. and A. Sakai. 1987. Regional variation in cold hardiness of Sakhalin fir (*Abies sachalinensis* Mast.) in Hokkaido, Japan. In Li (ed.). Plant cold hardiness. Liss, New York, N.Y.

Farjon, A. 1990. Pinaceae drawings and descriptions of the genera *Abies, Cedrus, Psudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix,* and *Picea*. Champaign, IL.

Foiles, M.W., R.T. Graham, and D.F. Olson, Jr. 1990. Grand fir. In: Russell M. Burns and Barbara H. Honkala (tech coords.). Silvics of North American: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

Franklin, J.F. 1990. Noble fir. In: Russell M. Burns and Barbara H. Honkala (tech coords.). Silvics of North American: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

Fryer, J.H. and F.T. Ledig. 1972. Microevolution of the photosynthetic temperature optimum in relation to the elevational complex gradient. Can. J. Bot. 50: 1231-1235.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. And Plant Mol. Biol. 40: 503-537.

Gordon-Kamm, W.J. 1980. Freezing tolerance of several conifers in a western Washington forest community. Western Washington Univ. Bellingham, M.S. Thesis.

Germino, M.J. and W.K. Smith. 1999. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. Plant Cell Environ. 22: 407-415.

Grassi, G. and U. Bagnaresi. 2001. Foliar morphological and physiological plasticity in *Picea abies* and *Abies alba* saplings along a natural light gradient. Tree Physiol. 21: 959-967.

Guehl, J.M. and G. Aussenac. 1987. Photosynthesis decrease and stomatal control of gas exchange in *Abies alba* Mill. in response to vapor pressure difference. Plant Physiol. 83: 316-322.

Guehl, J.M, G. Aussenac, J. Bouachrine, R. Zimmerman, J.M. Pennes, A. Ferhi, and P. Grieu. 1991. Sensitivity of leaf gas exchange to atmospheric drought, soil drought, and water-use efficiency in some Mediterranean Abies species. Can. J. For. Res. 21: 1507-1515.

Guehl, J.M., J. Bouachrine, R. Zimmermann, and E. Dreyer. 1989. Responses of photosynthesis and stomatal conductance to atmospheric humidity in some Mediterranean *Abies* species. Ann. Sci. For. 46(S): 401s-405s.

Hallgren, S.W. and J.A. Helms. 1988. Control of height growth components in seedlings of California red and whit fir by seed sources and water stress. Can. J. For. Res. 18: 521-529.

Hansen, J.K. and J.B. Larsen, 2004. European silver fir (*Abies alba* Mill.) provenances for Calabria southern Italy: 15-year results from Danish provenance field trials. Eur. J. For. Res. 123: 127-138.

Hawkins, B.J., G. Henry, and S.B.R. Kiiskila. 1998. Biomass and nutrient allocation in Douglas-fir and amabilis fir seedlings: influence of growth rate and nutrition. Tree. Physiol. 18: 803-810.

Hiltbrunner, E. and W. Flückiger. 1996. Manganese deficiency of silver fir trees (*Abies alba*) at a reforested site in the Jura mountains, Switzerland: aspects of cause and effect. Tree Physiol. 16:963-975.

Hinesley, L.E. and R.D. Wright. 1989. Biomass and nutrient accumulation in fraser-fir Christmas trees. HortScience 24(2): 280-282

Howe, G.T., S.N. Aitken, D.B. Neale, K.D., Jermstad, N.C. Wheeler, and T.H.H. Chen. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can. J. Bot. 81: 1247-1266.

Isoda, K., S. Shiraishi, S. Watanabe, and K. Kitamura. 2000. Molecular evidence of natural hybridization between *Abies veitchii* and *A. homolepis* (Pinaceae) revealed by chloroplast, mitochondrial and nuclear DNA markers. Mol. Ecol. 9: 1965-1973.

Jablanczy, A. 1971. Use of lime for tree cultivation. Am. Christmas Tree J. 15: 21-23.

Jach, M.E. and R. Ceulemans. 2000. Effects of season, needle age and elevated atmospheric CO_2 on photosynthesis in Scots pine (Pinus sylvestris). Tree Physiol. 20: 145-157.

Jacobs, B.F., C.R. Werth, and S.I. Guttman. 1984. Genetic relationships in *Abies* of eastern United States: an electrophoretic Study. Can. J. of Bot. 62: 609-616.

Johnson, D.M., M.J. Germino, and W.K. Smith. 2004. Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* seedlings below and above the alpine timberline. Tree Physiol. 24: 377-386.

King, D.A. 1997. Branch growth and biomass allocation in *Abies amabilis* saplings in contrasting light environments. Tree Physiol. 17: 251-258.

Klaehn, F.U. and J.A. Winieski. 1962. Interspecific hybridization in the genus Abies. Silvae Genet. 11: 130-140.

Klinka, K., Q. Wang, G.J. Kayahara, R.E. Carter, and B.A. Blackwell. 1992. Light-growth response relationships in Pacific silver fir (*Abies amabilis*) and subalpine fir (*Abies lasiocarpa*). Can. J. Bot. 70: 1919-1930.

Kohyama, T. 1980. Growth pattern of *Abies mariesii* saplings under conditions of open-growth and suppression. Bot. Mag. Tokyo. 93: 13-24.

Kohyama, T. 1984. Regeneration and coexistence of two *Abies* species dominating subalpine forests in central Japan. Oecologia. 62:156-161.

Laacke, R.J. 1990a. California red fir. In: Russell M. Burns and Barbara H. Honkala (tech coords.). Silvics of North American: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

Laacke, R.J. 1990b. White fir. In: Russell M. Burns and Barbara H. Honkala (tech coords.). Silvics of North American: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

Larsen, J.B. and F. Mekic. 1991. The geographic variation in European silver fir (Abies alba Mill.). Silvae Genet. 40(5/6): 188-198.

Lavigne, M.B., C.H.A. Little, and J.E. Major. 2001. Increasing the sink:source balance enhances photosynthetic rate of 1-year-old balsam fir foliage by increasing allocation of mineral nutrients. Tree Physiol. 21: 417-426.

Liu, T.S. 1971. A monograph of the genus *Abies*. Taipei, Taiwan.

Logan, K.T. 1969. Growth of tree seedlings as affected by light instensity IV. black spruce, white spruce, balsam fir, and eastern white cedar. Can. For. Serv. Publ. No. 1256.

Lucas, R.E. and J.F. Davis. 1961. Relationships between pH values of organic soils and availabilities of 12 Plant Nutrients. Soil Sci. 92:177-182.

Martin, T.A., T.M. Hinckley, F.C. Meinzer, and D.G. Sprugel. 1999. Boundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. Tree Physiol. 19: 435-443.

Masle, J. and G.D. Farquhar. 1988. Effects of soil strength on the relation of water-use efficiency and growth to carbon isotope discrimination in wheat seedlings. Plant Physiol. 86: 32-38.

McCullough, D.G., S.A. Katovich, D.L. Mahr, D.D. Neumann, C.S. Sadof, M.J. Raupp. 1999. Biological control of insect pests in forested ecosystems: a manual for foresters, Christmas tree growers and landscapers. Mich. State Ext. Bull. E-2679.

McCullough, D.G., S.A. Katovich, M.E. Ostry, and J. Cummings-Carlson. 1998. Christmas tree pest manual. 2nd ed. Mich. State Ext. Bull. E-2676.

Moore, J.A., P.G. Mika, T.M. Shaw, and M.I. Garrison-Johnston. 2004. Foliar nutrient characteristics of four conifer species in the interior northwest United States. Western J. Appl. For. 19(1): 13-24.

Mori, A. and H. Takeda. 2004. Functional relationships between crown morphology and within-crown characteristics of understory saplings of three codominant conifers in a subalpine forest in central Japan. Tree Physiol. 24: 661-670.

Myers Jr., O. and F.H. Bormann. 1963. Phenotypic variation in *Abies balsamea* in response to altitudinal and geographic gradients. Ecology. 44(3): 429-436.

Nicholls, T.H., and M.A. Palmer. 1985. Christmas tree winter injury in the lake states. Amer. Christmas tree J. 29: 21-25.

Owston, P.W. and T.T. Kozlowski. 1981. Growth and cold hardiness of container grown Douglas-fir, noble fir, and Sitka spruce seedlings in simulated greenhouse regimes. Can. J. For. Res. 11:465-474.

Parducci, L., A.E. Szmidt, A. Madaghiele, M. Anzidei, and G. G. Vendramin. 2001. Genetic variation at chloroplast microsatellites (cpSSRs) in *Abies nebrodensis* (Lojac.) Mattei and three neighboring *Abies* Species. Theor. Appl. Genet. 102:733-740.

Piñol, J and A. Sala. 2000. Ecological implications of xylem cavitation for several Pinaceae in the Pacific Northern USA. Funct. Ecol. 14: 538-545.

Perry, T.O. and W.C. Wu. 1960. Genetic variation in the winter chilling requirement for date of dormancy break for *Acer rubrum*. Ecology. 41(4): 790-794.

Peterson, D.W., D.L. Peterson, and G.J. Ettl. 2002. Growth responses of subalpine fir to climatic variability in the Pacific Northwest. Can. J. For. Res. 32(9): 1503-1517.

Richardson, A.D. 2004. Foliar chemistry of balsam fir and red spruce in relation to elevataion and the canopy light gradient in the mountains of the northeastern United States. Plant and Soil. 260: 291-299.

Ritchie, G.A. 2003. Root physiology and phenology: the key to transplanting success. In: Riley L.E., R.K. Dumroese, and T.D. Landis (tech. coords.). National proceedings: Forest and conservation nursery associations -2002. Ogden, UT: USDA For. Serv., Rocky Mountain research station. Proceedings RMRS-P-28: 98-104.

Robakowski, P., P. Montpied, and E. Dreyer. 2003. Plasticity of morphological and physiological traits in response to different levels of irradiance in seedlings of silver fir (*Abies alba* Mill). Trees. 17: 431-441.

Robakowski, P., P. Montpied, and E. Dreyer. 2002. Temperature response of photosynthesis of silver fir (*Abies alba* Mill.) seedlings. Ann. For. Sci. 59: 163-170.

Rothstein, R.E. and N. Lisuzzo. 2003. Optimal nutrition of fraser fir Christmas trees in Michigan, p. 19-21. In: D. Brown-Rytlewski and J. O'Donnell (eds.). Nursery, Landscape and Christmas Tree Research Projects and Educational Programs. Michigan State Univ. Extension, East Lansing, MI.

Rushforth, K.D. 1987. Conifers. Christopher Helm. London.

Sakata, M. and K. Suzuki. 2000. Evaluating possible causes for the decline of Japanese fir (Abies firma) forests based on δ^{13}C records of annual growth rings. Environ. Sci. Technol. 34: 373-376.

Sakai, A. 1970. Mechanism of desiccation damage of conifers wintering in soil-frozen areas. Ecology. 51(4): 657-664.

Sakai, A. 1982. Extraorgan freezing of primordial shoots of winter buds of Conifers, p. 199-209. In P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress mechanisms and crop implications v. 2. Academic Press, New York, NY.

Sala, A., E. V. Carey, R.e. Kaene, and R.M. Callaway. 2001. Water use by whitebark pine and subalpine fir: potential consequences of fire exclusion in the northern Rocky Mountains. Tree Physiol. 21: 717-725.

Schaberg, P.G., J.B. Shane, P.F. Cali, J.R. Donnelly, and G.R. Strimbeck. 1998. Photosynthetic capacity of red spruce during winter. Tree Physiol. 18: 271-276.

Scholz, F. and B.R. Stephan. 1982. Growth and reaction to drought of 43 Abies grandis provenances in a greenhouse study. Silvae Genet. 31(1): 27-35.

Smith, W.K. and G.A. Carter. 1988. Shoot structure effects on needle temperatures and photosynthesis in conifers. Amer. J. Bot. 75(4): 496-500.

Smith, W.K. 1980. Importance of aerodynamic resistance to water use efficiency in three conifers under field conditions. Plant Physiol. 65: 132-135.

Sprugel, D.G., J.R. Brooks, and T.M. Hinckley. 1996. Effects of light on shoot geometry and needle morphology in Abies amabilis. Tree Physiol. 16: 91-98.

Stenberg, P., H. Smolander, D. Sprugel, and S. Smolander. 1998. Shoot structure, light interception, and distribution of nitrogen in an *Abies amabilis* canopy. Tree Physiol. 18: 759-767.

Strimbeck, G.R., Schaberg, P.G., DeHayes, D.H., Shane, J.B., and G.J. Hawley. 1995. Midwinter dehardening of montaine red spruce during a natural thaw. Can. J. For. Res. 25: 2040-2044.

Sutinen, M.L., R. Arora, M. Wisniewski, E. Ashworth, R. Strimbeck, and J. Palta. 2001. Mechanisms of frost survival and freeze-damage in nature, p. 89-120. In: F.J. Bigras and S.J. Colombo (eds.). Conifer cold hardiness. Kluwer Academic Publishers, Norwell, Mass.

Timmer, V.R., E.L. Stone, and D.G. Embree. 1977. Growth response of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Can. J. For. Res. 7: 441-446.

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

United States Department of Agriculture. 2001. Nursery crops 2000 summary. 13 Feb 2005. < http://usda.mannlib.cornell.edu/reports/nassr/other/nursery/nurser01.pdf>

van der Kamp, B.J. and J. Worrall. 1990. An unusual case of winter bud damage in British Columbia interior conifers. Can. J. For. Res. 20: 1640-1647.

Webster, R., A. Rigling, and L. Walthert. 1996. An analysis of crown conditions of *Picea, Fagus* and Abies in relation to environment in Switzerland. Forestry 69: 347-355.

Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science. 169: 1269-1278.

Worrall, J. 1983. Temperature – Bud-burst relationships in amabilis and subalpine fir provenance tests replicated at different elevations. Silvae Genet. 32:203-9.

Xiaohong, L., Q. Dahe, S. Xuemei, C. Tuo, and R. Jiawen. 2003. Climatic significance of stable carbon isotope in tree rings of Abies spectabibis in southeastern Tibet. Chinese Sci. Bul. 48(18): 2000-2004.

Xie, C.Y. and C.C. Ying. 1993. Geographic variation of grand fir (*Abies grandis*) in the Pacific coast region: 10-year results from a provenance trial. Can. J. For. Res. 23: 1065-1072.

Yamazaki, J., A. Ohashi, U. Hashimoto, E. Negishi, S. Kumagai, T. Kubo, T. Oikawa, E. Maruta, and Y. Kamimura. 2003. Effect of high light and low temperature during harsh winter on needle photodamage of *Abies mariesii* growing at the forest limit on Mt. Norikura in Central Japan. Plant Sci. 165: 257-264.

CHAPTER ONE

BUDBREAK AND WINTER INJURY IN EXOTIC FIRS

For submission to HortScience

BUDBREAK AND WINTER INJURY IN EXOTIC FIRS

Additional index words. Cold Hardiness, chlorophyll fluorescence, frost damage

Abstract

Fir (*Abies* spp.) trees are occasionally used as landscape trees but are more commonly grown as Christmas trees. Recently, the Michigan State University Department of Forestry and the Michigan Christmas tree industry initiated a field test of exotic firs for potential use as Christmas trees. In the present study we expanded the evaluation of these exotic fir species to include their suitability as landscape trees and characterize their tolerance to cold damage. In spring 2004 and 2005, trees were surveyed weekly for budbreak and late spring frost damage. Freeze tests were conducted on four species growing at the Horticulture Teaching and Research Center to determine cold hardiness levels during winter. Species differed significantly in their days to budbreak at all locations. Trees that had already broken bud were more prone to late spring frost damage than trees yet to break bud. Chlorophyll fluorescence, bud damage, and needle damage differed among species at -44 °C. Bud, foliar, and cambium damage was correlated with chlorophyll fluorescence following freeze tests. Budbreak and cold hardiness were correlated with species breaking bud earlier displaying greater mid-winter cold hardiness than species breaking bud later. Selection criteria for future *Abies* introductions to the upper Midwest should include identifying species with late budbreak.

Introduction

The genus *Abies* Mill. (true firs) consists of 46 species (Farjon, 1990) found only in the northern hemisphere at higher latitudes or at higher elevations in lower latitudes. They generally prefer sites with cool temperatures, adequate moisture, well-drained soil, and low pH soils. Expanded use of *Abies* in landscapes has been limited by their intolerance of many site conditions. However, *Abies* may be more tolerant of environmental conditions than originally thought as species differ in their response to soil pH (Cregg et al., 2004), drought (Guehl et al., 1991), light (Kohyama, 1984), and freezing temperatures (Sakai, 1982). Collectively, *Abies* cover large elevational and latitudinal ranges (Liu, 1971). Langlet (1963) suggests large species distribution ranges increase the likelihood of genetic adaptation to diverse external factors.

Several *Abies* species are native to North America, but their use in the landscape, Christmas tree, and forestry industries has been limited to a few species. *Abies concolor* is common to the landscape in cooler regions. For Christmas tree production, *A. fraseri* is commonly grown in the eastern United States, while *A. procera* and *A. nordmanniana* are commonly grown in the Pacific Northwest. Forestry use has been limited to *A. amabilis, A. grandis,* and *A. procera* in the Pacific Northwest.

Recent efforts to increase conifer diversity have sparked increased interest in planting exotic conifers. Much of this interest has centered on exotic firs and their hybrids. Throughout the Midwest, planting of conifers has been typically limited to a few types of trees. Trees like Douglas-fir [*Pseudotsuga menziesii* (Mirbel.) Franco], Norway spruce [*Picea abies* (L.) Karst.], Colorado blue spruce [*Picea pungens* Englem.], Scotch

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pine (*Pinus sylvestris* L.), Austrian pine (*Pinus nigra* Arnold), and eastern white pine [*Pinus strobus* L.] are frequently used to the point of overplanting. The resulting lack of diversity has led to increased disease problems and insect pressures (McCullough et al., 1999; McCullough et al., 1998).

For future evergreen conifer introductions in the upper Midwest, tolerance of freezing temperatures is a necessary characteristic. Trees with adequate cold hardiness are required as average winter temperatures range from –20 to –42 °C in the upper Midwest (USDA Plant Hardiness Zone Map, 1990). Species also need to break bud in late spring to reduce damage from late spring frosts.

In conifers, cold hardiness levels increase in the late fall, reach a maximum in midwinter, and decrease as temperatures rise in late winter (Ritchie, 2003). In *A. lasiocarpa* (Gordon-Kamm, 1980; van der Kamp and Worrall, 1990), *A. procera* (Owston and Kozlowski, 1981), and *A. amabilis* (van der Kamp and Worrall, 1990) cold hardiness was reduced following periods of warm temperatures. Maximum cold hardiness also varies among species. For example, *A. spectabilis* is cold hardy to -25 °C while *A. balsamea* and *A. sibirica* are cold hardy to -70 °C (Sakai, 1982). Considerable intra-specific variation has also been documented for provenances of various species of *Abies* including *A. grandis* (Xie and Ying, 1993) and *A. sachalinensis* (Eiga and Sakai, 1984; Eiga and Sakai, 1984). Provenance variation in cold hardiness may be related to latitude, elevation, and winter snowfall.

The direct effects of freezing temperatures result in the failure of cell biological functions (Binder and Fielder, 1996; Yordanov, 1992) and cause secondary limitation to the photoharvesting system (Adams and Perkins, 1993). Chlorophyll fluorescence is

used to measure the efficiency by which photosystem II captures light and is often expressed as the ratio of variable fluorescence to maximum fluorescence (FvFm) (Bjorkman and Demming, 1987). Chlorophyll fluorescence is useful in comparing plant stress (Cregg et al., 2004; Maxwell and Johnson, 2000; Toivonen and Vidaver, 1988; Ritchie and Landis, 2005) and quantifying cold hardiness (Binder and Fielder, 1996; Adams and Perkins; 1993).

Budbreak is under strict genetic control (Worrall, 1983) and is an adaptive response to climate conditions at the population's origin (Campbell and Sugano, 1979). First a chilling requirement and then a growing degree day (GDD) requirement must be satisfied for budbreak to occur (Howe et al., 2003; Campbell and Sugano, 1979). Northern provenances have a longer chilling requirement than southern provenances (Perry and Wu, 1960). Provenances from colder regions have a reduced GDD requirement for budbreak than provenances from warmer regions (Campbell and Sugano, 1979). For example, *Picea mariana* (Mill.) B. S. P. provenances from northern Canada broke bud 7 days earlier than provenances from the northern United States when grown at the same location (Morgenstern, 1978).

Species that break bud earlier in the spring are more susceptible to late spring frosts than those breaking bud later in the spring (Hansen and Larsen, 2004; Leege, 2002). Timing of budbreak differs among provenances for *A. alba* (Hansen and Larsen, 2004), *A. amabilis* (Worrall, 1983), *A. grandis* (Scholz and Stephan, 1982), and *A. lasiocarpa* (Hansen et al., 2004; Worrall, 1983). High elevation provenances have reduced threshold temperatures and thus break bud earlier than lower elevation provenances, suggesting adaptation to a shorter growing season (Worrall, 1983).

The goal of this project is to identify *Abies* species tolerant of the Michigan climate, promote increased plant diversity, and thus reduce disease problems and insect pressures. Objectives in this paper are to 1) compare the date of budbreak between species, 2) identify differences in winter injury, and 3) test the hypothesis that midwinter cold hardiness is inversely related to budbreak.

Materials and Methods

Site Locations

Over 1100 trees representing 38 species, hybrids, provenances, and varieties of *Abies* were part of a true fir species and provenance trial initiated at the Kellogg Research Forest (KRF), Augusta, Michigan, in early 1991. In the fall of 2002 and spring of 2003, 246 trees representing 21 species and hybrids (Table 1) were transplanted to three locations in Michigan: Clarksville Horticulture Experiment Station, Clarksville, MI (CHES); Horticulture Teaching and Research Center, East Lansing, MI (HTRC); and Northwest Michigan Horticulture Research Station, Traverse City, MI (NWMHRS) (Figure 1). These three locations along with the KRF represent different climate regions (Table 2) and soil environments (Table 3) in Michigan. Trees were dug and ball and burlapped as 60 to 76 cm root balls in accordance with American nursery standards (American Nursery and Landscape Association, 2004)

In the fall of 2003, 57 additional trees were transplanted from the KRF to replace 37 trees that died due to transplant stress during the previous year and add three new species and varieties to the study. At least four trees of each species or hybrid were planted at each location with the exception of the HTRC where one to four more trees of

each species were planted when available. Trees were planted in a complete randomized design at each location. Trees were planted at approximately 4.5 m intervals.

Fertilization

In spring 2004, sites were fertilized with 21-0-0 ammonium sulfate at a rate of approximately 133 g per tree, to lower soil pH by 0.5 and insure that nitrogen was not limiting. Fertilizer was applied at CHES on 29 April, HTRC on 22 April, and NWHRS on 11 May. On 7 July 2004, 46-0-0 urea was applied at CHES at a rate of 91 g per tree. Trees at KRF were fertilized every fall with 21-0-0 at 85 g per tree. Trees exceeding 0.9 m received 28 g of fertilizer for each additional 0.3 m in height.

Budbreak

Beginning 16 March, 2004, each of the three outlying sites and the KRF were surveyed for budbreak, considered to have occurred once one bud broke its bud scale. Trees were inspected weekly until all trees at each location had broken bud. For each inspection date, growing degree days (GDD) were calculated using a base temperature of 10 °C (Dickson et al., 2000) and the numerical integration method using the Michigan Automated Weather Network (MAWN) web-site (http://www.agweather.geo.msu.edu/mawn/).

Frost damage

Temperatures reached -2.2 and -2.4 °C on 3 and 4 May 2004 at the KRF after trees had begun to break bud. Trees were visually rated on 5 May 2004 using the following 0-4 scale: 0= no shoots damaged, 1= 1-25%, 2= 26-50%, 3= 51-75%, and 4= 76-100% of shoots damaged. Shoots were considered damaged if they were brown in color or were dropping. All trees in each species block (n=7-42) were inspected for frost

damage, in addition to the four individuals previously selected at random for the budbreak study.

Cold Hardiness

Four species were chosen to measure cold hardiness and represent trees in four different bud break groups: *A. balsamea* var. *phanerolepis*; *A. chensiensi*; *A. nephrolepis*; and *A. veitchii*. Shoots from the current year's growth were collected from three trees of each species from HTRC. Samples were collected on 22 Nov 2004, 13 Dec 2004, 24 Jan 2005, and 7 Mar 2005 with freeze tests beginning 1-3 days later. Twelve samples for each temperature (4 spp x 3 rep) treatment were laid on moist cheese cloth, covered with aluminum foil, and rolled into bundles. A thermocouple was inserted into the stem of one sample in each bundle to measure stem temperature. Bundles were then placed into a freezer (ScienTemp, Adrian, MI) and stored at 2 °C until the test began. Temperatures were lowered at 3 °C·hr^{-1} and a bundle was removed at each targeted temperature until completion of the run. A control bundle was kept in a walk-in cooler at 2 °C where remaining bundles were allowed to thaw following removal from the freezer.

In the 22 Nov 2004 test, a bundle was removed at the following temperatures; 2, -6, -9, -12, -15, -18, -21, -24, -27, -30, -33 and -36 °C. For both the 13 Dec 2004 and 24 Jan 2005 tests, a bundle was removed at the following temperatures: 2, -18, -21, -24, -27, -30, -33, -36, -39, -42, and -44 °C. In the 7 Mar 2005 test, a bundle was collected at the following temperatures: 2, -6, -9, -12, -15, -18, -21, -24, -27, -30, -33, -36, -39, -42, -44, and -78 °C. Bundles were placed in a walk-in cooler and allowed to thaw at 2 °C for 2-3 days and then placed in a high humidity chamber at room temperature (25 °C) for 4-5 days. Then samples were visually rated for needle damage, bud damage, and cambium

damage using the following 0-2 scale: 0= no damage; 1= partial browning of the tissue; 2=dead tissue.

Chlorophyll fluorescence (F_v/F_m) was measured using two needles from every sample in each temperature treatment using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, England). Samples were clipped and dark-acclimated for 15 min before readings were taken. An index of injury percentage was calculated for each species comparing the F_v/F_m at each temperature to the F_v/F_m of the control.

Statistical Analysis

Species effects on budbreak and cold hardiness damage were determined using PROC MIXED (SAS Inc., Cary, NC). When significant differences were indicated, means were separated using Tukey's Studentized test (Sexton, 1998). Species and year differences for required growing degree day to budbreak were determined using PROC GLM (SAS Inc.) and means were separated using the Tukey's Studentized test. Damage ratings were analyzed using non-parametric measures and means were separated by comparing two species using Kruskal-Wallis. Correlation between tissue damage, F_v/F_m, and the mean date of budbreak were identified using PROC CORR (SAS Inc.).

Results

Budbreak

The date of budbreak varied with planting location ($p<0.0001$), species (<0.0001), and year ($p<0.03$) (Table 4). Budbreak began in mid to late April in both years, lasting between 29-49 days in 2004 and 36-49 days in 2005. Trees at southern sites began and finished breaking bud earlier than northern sites. All trees had broken bud by 17 June

2004 and 9 June 2005. In both years, *A. nephrolepis*, *A. bifolia*, *A. holophylla*, and *A. lasiocarpa* were among the first to break bud at all four locations, while *A. veitchii* and *A. homolepis* were two of the last species to break bud at all four locations.

The GDD required for budbreak differed among species ($p \leq 0.0001$), locations ($p \leq 0.0001$), and years ($p \leq 0.04$). Trees at the southern locations accumulated GDD faster (Figure 2) and required more GDD for budbreak than at northern locations (Table 5). GDD accumulation was initially slower in 2005 but by early June GDD accumulation was nearly equal to 2004. Fewer GDD were required at NWHRS for budbreak than at the other locations.

Budbreak was ranked among species at each location and a strong location: location correlation existed (Table 6) suggesting budbreak among species was generally related at each location. Location × species interaction for both days to budbreak and GDD was significant ($p \leq 0.0001$), indicating the rank order of some species changed among locations. For example, *A. koreana* was in the last group to break bud at CHES, HTRC, and NWHRS while being one of the first species to break bud at KRF. In *A. koreana* x *veitchii* and *A. fraseri* x *homolepis*, budbreak was not closely related to the parent species, while budbreak for *A. koreana* x *balsamea* was similar to its parents'.

Mean days to budbreak at the HTRC were correlated ($R^2 = 0.38$, $p=0.033$) with average F_v/F_m at -44°C (Figure 3). Trees breaking bud earlier had higher F_v/F_m values than trees breaking bud later. *Abies veitchii* had the lowest F_v/F_m of the species included in the cold hardiness study.

Late Frost damage

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Late frost damage following the May 2004 freeze was related to the date of budbreak. Trees breaking bud early displayed more damage from late spring frosts than those breaking bud later. At KRF, *A. nephrolepis*, *A. bifolia*, *A. holophylla*, and *A. lasiocarpa* displayed a high percent of frost damage in a large number of trees (Figure 4). In contrast, *A. procera*, *A. koreana*, *A. chensiensis*, *A. nordmanniana* ssp. *equi-trojani*, *A. fraseri* x *homolepis* displayed no frost damage. At the time of frost, the following species had not completed budbreak: *A. fraseri* x *homolepis*, *A. koreana*, *A. koreana* x *balsamea*, *A. homolepis*, *A. procera*, and *A. veitchii*. In both *A. homolepis* and *A. veitchii* late frost damage occurred to some trees not included in the budbreak survey but surveyed for late frost damage.

Cold Hardiness

Cold hardiness varied among species and by test date. Chlorophyll fluorescence values differed at different temperatures ($p \leq 0.001$). F_v/F_m values decreased as temperatures were lowered during controlled freeze tests in *A. chensiensis* and *A. veitchii* but remained constant in *A. balsamea* var. *phanerolepis* and *A. nephrolepis* as temperatures reached -44 °C (Table 7). Species differed for tests during December 2004 ($p \leq 0.002$) and January 2005 ($p \leq 0.001$), but not March ($p = 0.10$). Needle damage differed among species at -44 °C in all tests ($p \leq 0.05$) (Table 8). Needle damage was greatest in *A. chensiensis* during all tests. Damage to stem tissue and buds was not significant among species during any tests. Damage to needles, stem tissue, and buds were highly correlated ($p < 0.001$) with declining in F_v/F_m values (Table 9).

Discussion

In each species, the date of budbreak was similar in both years; however, GDD at the time of budbreak differed in both years for each species. Also, rank correlations between locations suggest budbreak is under strong genetic control which is supported by previous studies (Worral, 1983). Meeting a chilling requirement and accumulating a set number of GDD are necessary for trees to break bud (Howe et al., 2003). In both years, budbreak first occurred at southern locations in mid April and began at NWHRS in late April. The difference between the first and last species to break bud ranged between 29- and 49-days depending on location suggesting late bud breaking species such as *A. koreana* and *A. veitchii* require more GDD to break bud than species with earlier budbreak. Within species, some variation in budbreak occurred at different locations and was most prevalent in hybrids; although several species were influenced by unknown location factors.

Abies balsamea is native to Michigan and can serve as a point of reference for the species included in this study. At all three transplanted locations, the following species broke bud following *A. balsamea*: *A. procera*; *A. fraseri* x *homolepis*; *A. fraseri*; *A. chensiensis*; *A. nordmanniana* ssp. *equi-trojani*; *A. homolepis*; *A. koreana*; and *A. veitchii*. In *A. balsamea*, a Michigan native, late spring frost damage is a problem (Lantagne and Koelling, 2004). Trees breaking bud after A. balsamea showed no evidence of late frost damage with the exception of *A. homolepis* and *A. veitchii*. Damage in these two species was limited to trees located on top of a hill which received more thermal time than those on the slope or at the base of the hill.

The mean number of GDD required to break bud was different ($p \leq 0.0001$) at each location. At the NWHRS, trees required fewer GDD to break bud than more southern

locations, implying that another factor in addition to thermal time may be influencing budbreak. There is some evidence that photoperiod has some influence on budbreak (Partanen et al., 1998); although its effect is debated (Worral, 1983). In 2004, daylength for trees at the NWHRS were nearly 1-hour longer when the last tree broke bud than at KRF, which perhaps can explain the difference in the GDD required for budbreak. In *P. menziesii* var. *menziesii* (Mirb.) Franc. populations from regions with similar winter temperatures, trees from regions with the largest moisture deficit broke bud earlier than the average (Campbell and Sugano, 1979). This suggests that trees from regions frequented by summer drought break bud early in the spring to complete stem elongation and set bud before summer drought conditions begin (Kaya et al., 1994). Both *A. koreana* and *A. veitchii*, two of the last species to break bud in this study, are native to regions with increased summer precipitation (Farjon, 1990) so perhaps their late budbreak is an adaptive response to a mild climate and adequate summer precipitation.

Within species, provenances can vary greatly in time of budbreak (Hansen and Larsen, 2004; Scholz and Stephen, 1982; Worral, 1983) and cold hardiness levels (Dolnicki and Kraj, 1998; Eiga and Sakai, 1984; Eiga and Sakai, 1987, Xie and Ying, 1993). One of the limitations of the current study is that provenance information for each species is unknown. Moreover, it is unknown if the parent trees of the hybrids were from the same provenance as the straight species included in this study. For example, the balsam parent of the Korean x Balsam hybrid is not necessarily from the same seed source as the straight balsam species included in this study, which could explain some of the inconsistencies in the budbreak between the parents and their hybrids. Also, variation within a species

is not accurately represented because each species is represented by a single provenance.

Chlorophyll fluorescence was a good indicator of cold injury during controlled freeze tests as F_v/F_m values declined with increasing temperatures. These results paralleled increasing needle, stem, and bud damage, which is consistent with previous studies (Adams and Perkins, 1993; Binder and Fielder, 1996). Cold hardiness between different plant organs differs in the temperature at which damage occurs (Coleman et al., 1992; Sakai, 1982). The temperature where damage occurred was different for buds, stems, and needles. However, damage variables and F_v/F_m values were strongly correlated suggesting that while the temperatures that damage different organs may vary, relative cold hardiness is related.

Many studies show differences in cold hardiness among species and provenances (Sakai, 1982; Xie and Ying, 1993; Eiga and Sakai, 1987; Eiga and Sakai, 1984). As expected, trees included in the cold hardiness study also varied in the temperature at which they displayed damage to freezing temperatures. One limitation of the current study was the inability of the freezer to be lowered beyond -44 °C. From a practical stand point, this is near to the lowest annual temperatures in the coldest regions of the upper Midwest. In most years, species showing no signs of damage should be able to survive most winters if given the necessary time to acclimate.

The degree of cold hardiness in trees reaches a maximum during mid-winter and with a gradual acclimation and de-acclimation period before and after respectively (Ritchie, 2003). Bud damage was the greatest in the March test. Some GDD accumulation had begun by then, internal development processes related to budbreak may

have begun, and warmer temperatures likely reduced cold hardiness levels leading to increased freeze damage. Needle damage ratings were the lowest in January. Typically January temperatures were the lowest so more conditioning lead to greater cold hardiness. Increased damage in December and March was likely due to incomplete acclimation and the start of de-acclimation leading to less cold hardiness. Stem damage ratings were not different suggesting cambium tissue was adequately insulated at -44 °C, the lowest temperature possible in our controlled freeze test. F_v/F_m declined progressively between each test suggesting that repairs to cold damage did not begin until growth began again in the spring. F_v/F_m at -44 °C differed significantly among species in December and January, but not March. In the March test, species breaking bud had the highest F_v/F_m values suggesting they may have already begun recovery from cold damage that occurred during the winter.

Other factors being equal, trees from colder regions are cold hardy at lower temperatures (Sakai, 1982) and break bud earlier in a common site, due to a reduced chilling and heat accumulation requirement, than trees from warmer regions. Worral (1983) suggests this may be an adaptation allowing trees to complete their growth before fall frosts in cold regions with short growing seasons. In the present study, date of budbreak and F_v/F_m readings at -44 °C were strongly correlated for the individual trees included in the cold hardiness experiment. Trees with maximum cold hardiness levels were among the first species to break bud in the spring while species with reduced cold hardiness were among the last, suggesting trade-offs between mid-winter cold hardiness and the timing of budbreak in the spring. Interestingly, *A. chensiensis* had the lowest

F_v/F_m while being the third of the four species in the study to break bud, but its subsequent cause is unknown.

In summary, species varied in their tolerance of freezing temperatures and in the date they broke bud. Strong correlations existed between the temperatures different plant tissues showed visual signs of damage. Trees that were among the first to break bud in the spring withstood colder winter temperatures than trees breaking bud later. Species breaking bud early in the spring were more likely to be damaged by late spring frosts. Budbreak should continue as an important selection criterion for conifer species introduced to the landscape and Christmas tree industries in the upper Midwest. Species such as *A. homolepis*, *A. koreana*, and *A. veitchii* were among the last species to break bud at all locations and should be considered for future introduction. Additional studies should focus on larger provenance tests could be conducted to select provenances species such as *A. bifolia*, *A. lasiocarpa*, and *A. nephrolepis* which break bud later, yet display desirable ornamental characteristics.

Table 1. List of *Abies* species planted at four locations in Michigan.

Common Name	Scientific Name	Geographic Origin	Elevation (m)	Latitude (°N)
Ernst fir	*A. chensiensis* Van Tiegh.	Asia	2500 - 3800	25 - 33
Korean fir	*A. koreana* Wils.	Asia	1000 - 2000	33 - 36
Needle fir	*A. holophylla* Maxim.	Asia	0 - 1400	33 - 49
Nikko fir	*A. homolepis* Sieb. et Zucc.	Asia	600 - 2200	33 - 38
Siberian White fir	*A. nephrolepis* (Trautv.) Maxim.	Asia	500 - 2000	35 - 55
Veitch fir	*A. veitchii* Lindl.	Asia	1200 - 3000	33 - 38
Balsam fir	*A. balsamea* (Linn.) Mill.	N. America	0 - 1500	38 - 59
Cannan fir	*A. balsamea var phanerolepis* (Fern.) Liu	N. America	0 - 1500	38 - 59
Corkbark fir	*A. bifolia* (Merr.) Lemm.	N. America	0 - 1700	37 - 39
Fraser fir	*A. fraseri* (Pursh.) Poir.	N. America	1100 - 2100	35 - 37
Noble fir	*A. procera* Rehd.	N. America	100 - 2700	41 - 48
Sub-Alpine fir	*A. lasiocarpa* (Hook.) Nutt.	N. America	0 - 3500	32 - 64
Nordman fir	*A. nordmanniana* (Steven) Spach.	Mediterranean	1000 - 2200	40 - 44
Turkish fir	*A nordmanniana ssp. equi-trojani* (Aschers. et Sint. Ex Boiss.) Coode et Cullen	Mediterranean	1000 - 2200	40 - 44
Fraser x Nikko Hybrid	*A. fraseri x homolepis*	N. America/Asia	-	-
Korean x Balsam Hybrid	*A. koreana x balsamea*	N. America/Asia	-	-
Korean x Veitch Hybrid	*A. koreana x veitchii*	N. America/Asia	-	-

Table 2. Thirty-year climate summary and USDA plant hardiness zones for four *Abies* planting sites in Michigan.

Location	Average January Low (°C)[1]	Average July High (°C)[1]	Annual Precipitation (cm)[1]	Annual Snowfall (cm)[1]	Growing Season (days)[1]	USDA Hardiness Zone[2]
HTRC	-11	28	78.5	99	150	5A
NWHRS	-10	27	85.1	244	135	5B
CHES	-10	28	90.7	145	147	5B
KRF	-9	28	89.4	135	149	5B

1 Illinois Dept. of Nat. Res., 2005. 2 USDA Plant Hardiness Map, 1990.

Table 3. Soil properties of four *Abies* planting sites in Michigan.

Location	Sand (%)[1]	Silt (%)[1]	Clay (%)[1]	Soil Type[1]	2003 pH	2004 pH
HTRC	83.1	8.7	9.3	Loamy Sand	6.25	5.72
NWHRS	83.3	7.6	9.1	Loamy Sand	7.11	6.66
CHES	61.3	23.5	15.1	Sandy Loam	6.76	4.62
KRF	72.4	17.1	10.5	Sandy Loam	4.63	4.12

[1] Analyzed at the MSU Soil and Plant Nutrition Laboratory, East Lansing, MI

Table 4. Budbreak date of 17 *Abies* species grown at four locations in Michigan in 2004 and 2005.

Species	2004				2005			
	CHES	HTRC	KRF	NWHRS	CHES	HTRC	KRF	NWHRS
A. nephrolepis	April 20 a	April 24 abc	April 22 a	May 4 a	April 20 a	April 20 a	April 20 a	April 28 a
A. bifolia	April 22 a	April 23 ab	April 22 a	May 4 a	April 19 a	April 20 a	April 20 a	May 1 ab
A. holopylla	April 23 a	April 21 a	April 22 a	May 6 ab	April 20 a	April 20 a	April 20 a	May 3 ab
A. lasiocarpa	April 29 ab	April 22 ab	April 22 a	May 6ab	April 30 abcd	April 26 ab	April 20 a	April 29 a
A. balsamea	May 2 abc	May 1 abc	NR	May 10 ab	April 24 ab	May 2 abc	April 26 abc	May 10 abc
A. koreana x *veitchii*	May 3 abc	May 3 bcde	April 26 ab	May 17 abcde	May 2 abcd	May 5 bcd	April 22 ab	May 18 abc
A. bal. var. *phanerolepis*	May 5 abc	May 3 bcde	NR	May 15 abcde	April 28 abcd	May 5 bcd	April 27 abc	May 15 abc
A. nordmanniana	May 5 abc	May 5 cdef	NR	NR	April 27 abc	May 9 bcde	May 4 abcd	NR
A. koreana x *balsamea*	May 5 abc	May 26 i	May 3 abc	June 4 e	May 6 abcd	May 26 fg	May 2 abc	May 25 bc
A. fraseri x *homolepis*	May 12 bc	May 7 defg	May 5 abc	May 25 bcde	May 9 abcd	May 9 bcde	May 8 bcde	May 18 abc
A. procera	May 12 bc	May 12 defgh	May 7 bc	May 13 abcd	May 9 abcd	May 18 defg	May 10 cde	May 30 c
A. nord. ssp. *equi-trojani*	May 12 bc	May 14 efgh	May 5 abc	May 27 cde	May 1 abcd	May 12 cdef	May 8 bcde	May 22 abc
A. chensiensis	May 14 bc	May 12 defgh	May 3 abc	May 13 abcd	May 10 bcd	May 10 bcde	May 10 cde	May 17 abc
A. homolepis	May 16 c	May 18 ghi	May 12 c	May 20 abcde	May 18 d	May 24 efg	May 20 de	May 25 bc
A. fraseri	May 17 c	May 16 fghi	NR	May 29 cde	May 10 abcd	May 15 cdefg	May 11 cde	May 20 abc
A. koreana	May 19 c	May 22 hi	May 2 abc	May 27 cde	May 17 cd	May 25 fg	May 10 cde	May 20 abc
A. veitchii	May 19 c	May 22 hi	May 14 c	June 3 de	May 17 cd	May 28 g	May 24 e	June 1 c

Means within columns followed by the same letter were not statistically different, α=0.05, Tukey. No records were available for trees listed as NR.

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Table 5. Mean growing degree days required before budbreak in 17 *Abies* species grown at four locations in Michigan in 2004 and 2005.

Species	2004				2005			
	CHES	HTRC	KRF	NWHRS	CHES	HTRC	KRF	NWHRS
A. nephrolepis	114 a	128 a	150 a	81 a	153 a	148 a	176 a	102 a
A. bifolia	119 ab	124 a	150 a	95 ab	153 a	148 a	176 a	102 a
A. holophylla	122 ab	117 a	150 a	83 a	153 a	148 a	176 a	106 a
A. lasiocarpa	170 abc	123 a	150 a	110 ab	163 ab	175 ab	176 a	113 a
A. nordmanniana	182 abcd	209 abc	NR	NR	159 a	204 ab	227 ab	NR
A. bal. var phanerolepis	193 abcd	179 ab	NR	157 abc	162 ab	180 ab	201 a	162 abc
A. koreana x veitchii	198 abcde	179 ab	163 a	169 abc	197 abc	180 ab	177 a	181 abc
A. koreana x balsamea	223 abcdef	447 f	220 ab	286 c	218 abc	310 de	224 a	242 abc
A. balsamea	226 abcdef	167 a	NR	114 ab	158 a	193 ab	181 a	142 ab
A. nord. ssp. equi-trojani	269 bcdef	310 cde	221 ab	214 abc	180 abc	231 abcd	231 ab	206 abc
A. fraseri x homolepis	270 bcdef	223 abc	246 ab	201 abc	210 abc	208 abc	231 ab	198 abc
A. procera	272 bcdef	285 bcd	247 ab	146 abc	223 abc	252 bcd	251 abc	284 bc
A. chensiensis	292 cdef	285 bcd	208 ab	146 abc	237 abc	219 abcd	251 abc	168 abc
A. homolepis	317 cdef	355 def	325 b	184 abc	276 c	306 cde	324 bc	219 abc
A. fraseri	331 def	325 cde	NR	220 abc	220 abc	248 bcd	270 abc	189 abc
A. koreana	347 ef	403 ef	233 ab	249 bc	264 bc	310 de	258 abc	194 abc
A. veitchii	364 f	396 def	342 b	299 c	266 c	351 e	348 c	324 c

Means within columns followed by the same letter were not statistically different, $\alpha= 0.05$. No records available for trees listed as NR.

Table 6. Pearson correlation coefficients for budbreak of 17 *Abies* species at four locations in Michigan.

	KRF	NWHRS	HTRC
CHRS	0.61***	0.53***	0.71***
KRF		0.58***	0.60***
NWHRS			0.71***

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

Table 7. Mean F_v/F_m value of four *Abies* species following controlled freeze tests to -44 °C.

	F_v/F_m		
Species	December*	January*	March
A. bal. var. *phanerolepis*	0.627 a	0.657 a	0.462 ns
A. nephrolepis	0.611 a	0.654 a	0.705 ns
A. veitchii	0.375 a	0.492 a	0.291 ns
A. chensiensis	0.022 b	0.071 b	0.304 ns

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively and indicates significant species effects for controlled freeze test that month.

Means within a column followed by the same letter were not significantly different, $\alpha = 0.05$, Tukey.

Table 8. Mean needle damage ratings of four *Abies* species following controlled freeze tests to -44 °C.

| Species | Needle Damage | | |
	December*	January*	March*
A. bal. var. *phanerolepis*	1.0 a	0.0 a	0.3 ab
A. nephrolepis	1.0 a	0.0 a	0.0 a
A. veitchii	1.7 a	0.7 a	1.7 bc
A. chensiensis	2.0 b	2.0 b	2.0 c

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively and indicates significant species effects for controlled freeze test that month.

Means within a column followed by the same letter were not significantly different, $\alpha = 0.05$, Kruskal-Wallis.

Table 9. Pearson's correlation coefficient for winter damage in four *Abies* species growing in Michigan in March 2005 following controlled freeze test.

	Needle Damage	Stem Damage	Bud Damage
F_v/F_m	-0.60***	-0.69***	-0.46***
Needle Damage		0.77***	0.65***
Stem Damage			0.68***

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively
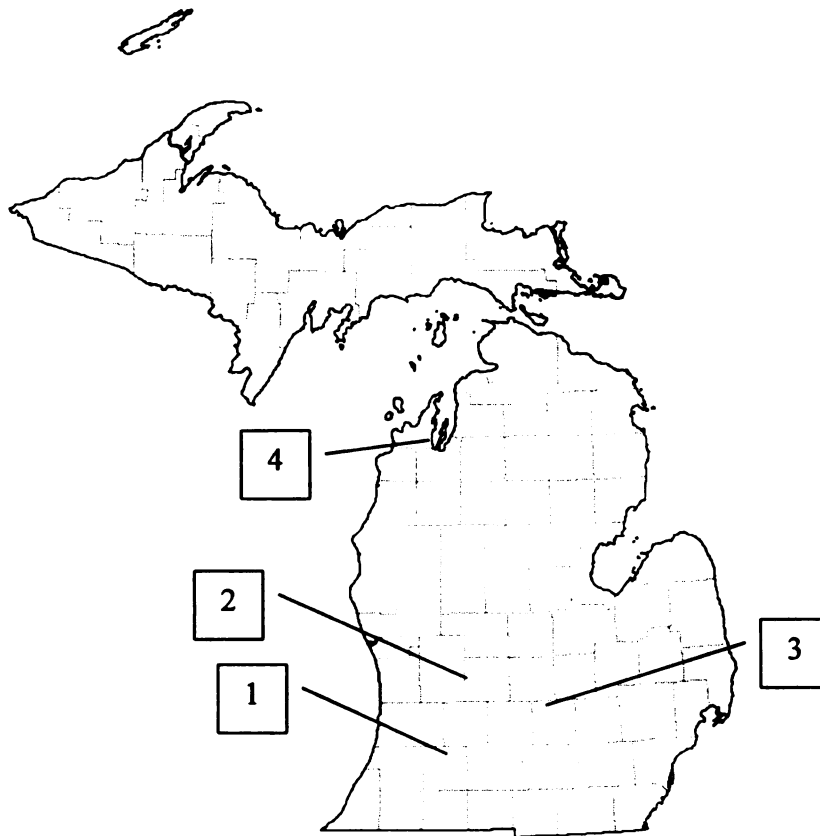
Figure 1. Location of four *Abies* trials in Michigan. 1) Kellogg Research Forest (KRF), 2) Clarksville Horticulture Experiment Station (CHES), 3) Horticulture Teaching and Research Center (HTRC), 4) Northwest Michigan Horticultural Research Station (NWHRS).
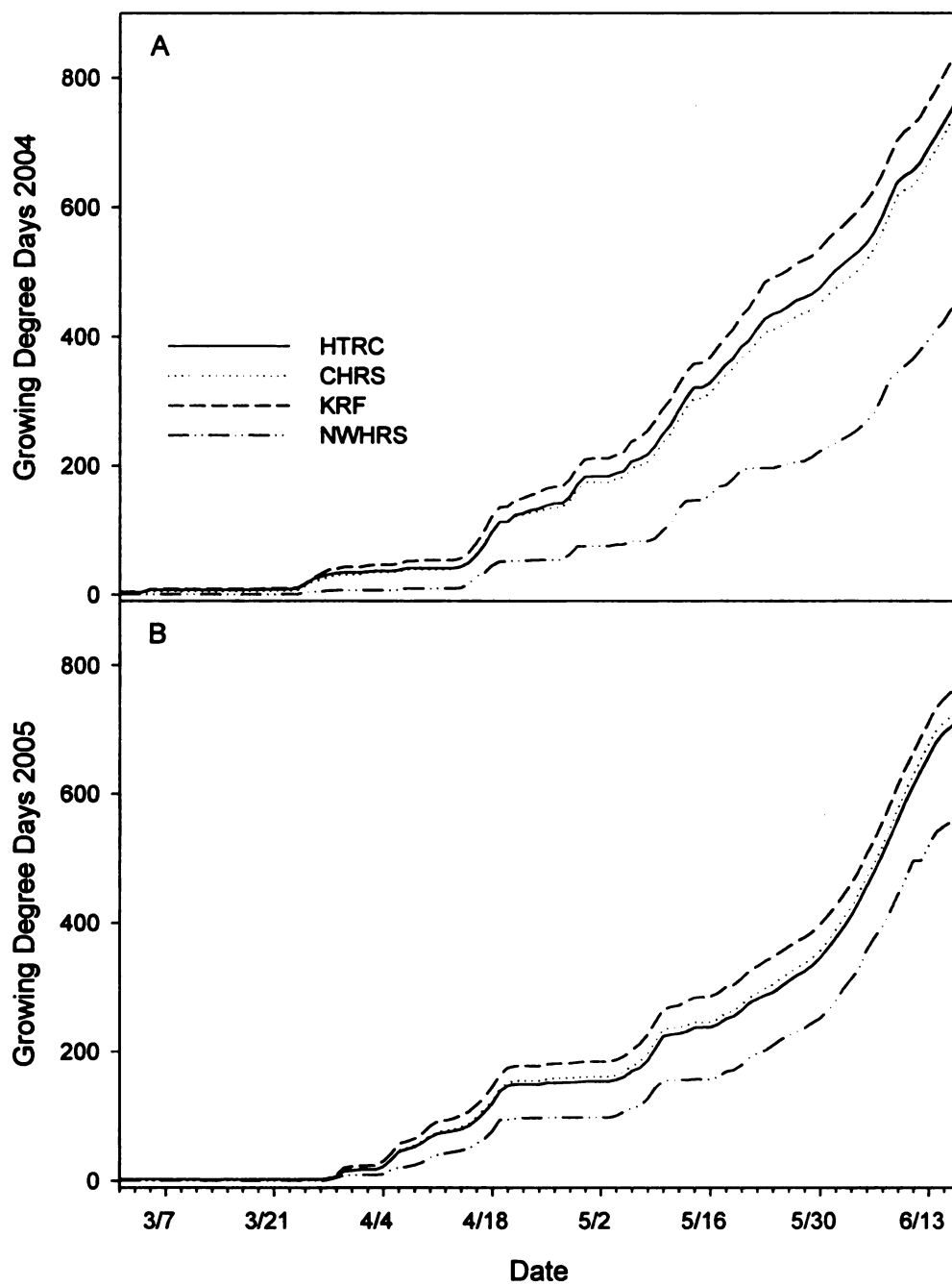
Figure 2. Growing degree day accumulation since January 1 in A) 2004 and B) 2005 at four *Abies* test plots in Michigan.
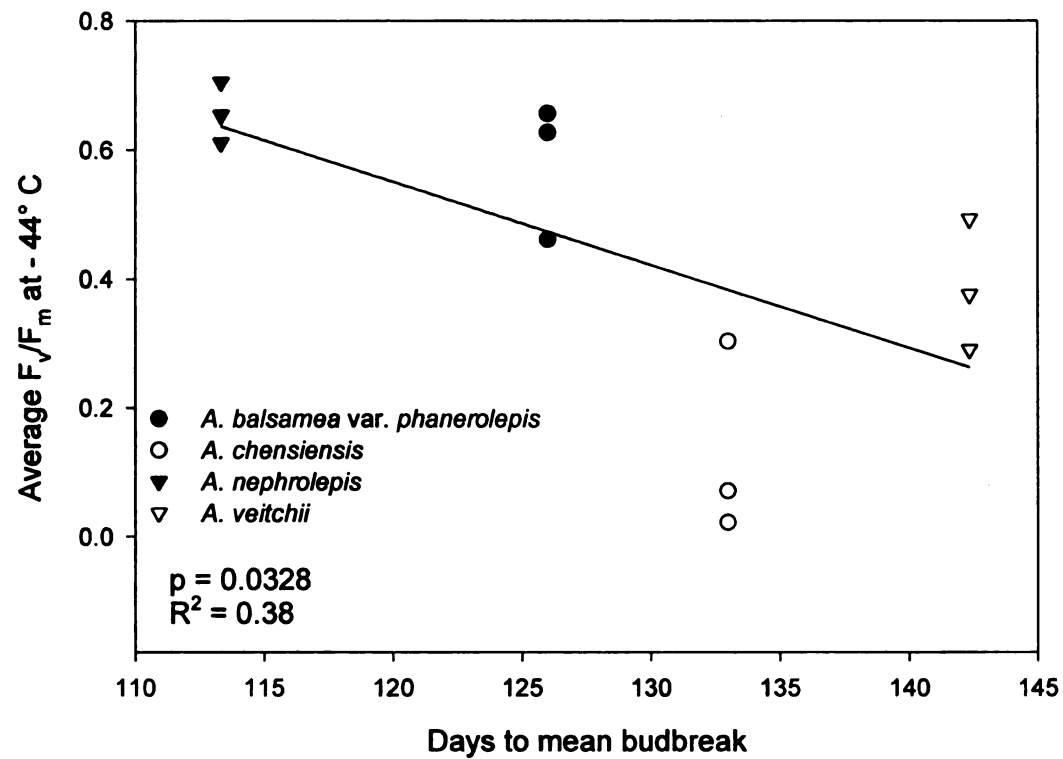
Figure 3. Comparison of average F_v/F_m at -44 °C and mean day to budbreak in four *Abies* species at HTRC in December 2004 and January and March 2005.
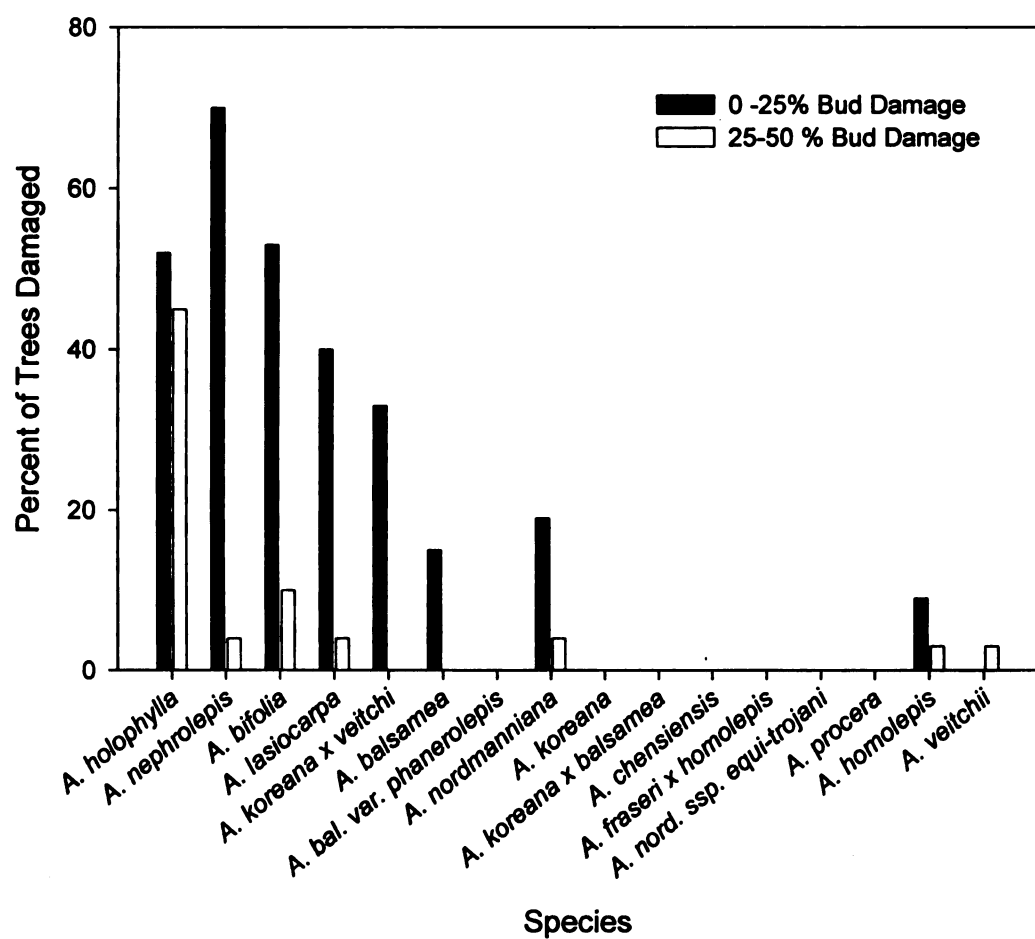
Figure 4. Frost damage to recently emerged shoots at the Kellogg Research Forest, 5 May 2004.

Literature Cited

Adams, G.T. and T.D. Perkins. 1993. Assessing cold tolerance in *Picea* using chlorophyll fluorescence. Environ Exp. Bot. 33(3): 377-382.

American Standards for Nursery Stock. 2004. Amer. Nurs. Land. Asso. Washington, DC.

Binder, W.D. and P. Fielder. 1996. Chlorophyll fluorescence as an indicator of frost hardiness in white spruce seedlings from different latitudes. New For. 11: 233-253.

Björkman, O. and B. Demming. 1987. Photo yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta. 170: 489-504.

Campbell, R.K. and A.I. Sugano. 1979. Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. Bot. Gaz. 140(2): 223-231.

Coleman, M.D., T.M. Hinckley, G. McNaughton, and B.A. Smit. 1992. Root cold hardiness and native distribution of subalpine conifers. Can. J. For. Res. 22: 932-938.

Cregg, B.M., M.W. Duck, C.M., Rios, D.B. Rowe, and M.R. Koelling. 2004. Chlorophyll fluorescence and needle chlorophyll concentration of fir (*Abies* sp.) seedlings in response to pH. Hort Sci. 39(5): 1121-1125.

Dickson, R.L., G.B. Sweet, and N.D. Mitchell. 2000. Predicting Pinus radiata female strobilus production for seed orchard site selection in New Zealand. For. Ecol. Mgt. 133: 197-215.

Dolnicki, A. and W. Kraj. 1998. Dynamics of frost resistance in various provenances of *Abies grandis* Lindl. Acta Societatis Botanicorum Poloniae. 67(1): 51-58.

Eiga, S. and A. Sakai. 1984. Altitudinal variation in freezing resistance of Saghalien fir (*Abies sachalinensis*). Can. J. Bot. 62: 156-160.

Eiga, S. and A. Sakai. 1987. Regional variation in cold hardiness of Sakhalin fir (*Abies sachalinensis* Mast.) in Hokkaido, Japan. In Li (ed.). Plant cold hardiness. Liss, New York, N.Y.

Farjon, A. 1990. Pinaceae drawings and descriptions of the genera *Abies, Cedrus, Psudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix*, and *Picea*. Champaign, IL.

Gordon-Kamm, W.J. 1980. Freezing tolerance of several conifers in a western Washington forest community. Western Washington Univ. Bellingham, M.S. Thesis.

Guehl, J.M, G. Aussenac, J. Bouachrine, R. Zimmerman, J.M. Pennes, A. Ferhi, and P. Grieu. 1991. Sensitivity of leaf gas exchange to atmospheric drought, soil drought, and water-use efficiency in some Mediterranean Abies species. Can. J. For. Res. 21: 1507-1515.

Hadley, J.L. and W.K. Smith. 1987. Influence of krummholz mat microclimate on needle physiology and survival. Oecologia. 73: 82-90.

Hansatech Instruments Ltd. 1997. Operating instructions for Plant Efficiency Analyzer (PEA) advanced fluorescence analysis. Hansatech Instruments Ltd., Norfolk, U.K.

Hansen, J.K. and J. B. Larsen. 2004. European silver fir (Abies alba Mill.) provenances from Calabria, southern Italy: 15-year results from Danish provenance field trials. Eur. J. For. Res. 123: 127-138.

Hansen, O.K., U.B. Nielsen, Ø.M. Edvardsen, B. Skúlason, and J. Skage. 2004. Nordic provenance trials with *Abies lasiocarpa* and *Abies lasiocarpa* var. *arizonica*: Three-year results. Scand. J. For. Res. 19: 112-126.

Howe, G.T., S.N. Aitken, D.B. Neale, K.D., Jermstad, N.C. Wheeler, and T.H.H. Chen. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can. J. Bot. 81: 1247-1266.

Illinois Department of Natural Resources. 2005. Midwest Regional Climate Center. Champaign, IL. 20 June 2005. http://sisyphus.sws.uiuc.edu/index.html.

Kaya, Z., W.T. Adams, and R. K. Campbell. 1994. Adaptive significance of intermittent shoot growth in Douglas-fir seedlings. Tree Physiol. 14: 1277-1289.

Kohyama, T. 1984. Regeneration and coexistence of two *Abies* species dominating subalpine forests in central Japan. Oecologia. 62:156-161.

Langlet, O. 1963. Patterns and terms of intra-specific ecological variability. Nature. 200: 347-348.

Lantagne, D. and M. Koelling. 2004. Tree planting in Michigan. MSU Extension Publication. http://forestry.msu.edu/extension/ExtDocs/treeplnt.htm

Leege, T. 2002. An evaluation of exotic true firs in the inland northwest. Amer. Christmas Tree J. 46: 49-51.

Liu, T.S. 1971. A monograph of the genus *Abies*. Taipei, Taiwan.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51: 659-668.

McCullough, D.G., S.A. Katovich, D.L. Mahr, D.D. Neumann, C.S. Sadof, M.J. Raupp. 1999. Biological control of insect pests in forested ecosystems: a manual for foresters, Christmas tree growers and landscapers. Mich. State Ext. Bull. E-2679.

McCullough, D.G., S.A. Katovich, M.E. Ostry, and J. Cummings-Carlson. 1998. Christmas tree pest manual. 2nd ed. Mich. State Ext. Bull. E-2676.

Morgenstern, E.K. 1978. Range-wide genetic variation of black spruce. Can. J. For. Res. 8: 463-473.

Owston, P.W. and T.T. Kozlowski. 1981. Growth and cold hardiness of container grown Douglas-fir, noble fir, and Sitka spruce seedlings in simulated greenhouse regimes. Can. J. For. Res. 11:465-474.

Partanen, J., V. Koski, and H. Hänninen. 1998. Effects of photoperiod and temperature on the timing of bud burst in Norway spruce (*Picea abies*). Tree Physiol. 18: 811-816.

Perry, T.O. and W.C. Wu. 1960. Genetic variation in the winter chilling requirement for date of dormancy break for *Acer rubrum*. Ecology. 41(4): 790-794.

Ritchie, G.A. 2003. Root physiology and phenology: the key to transplanting success. In: Riley L.E., R.K. Dumroese, and T.D. Landis (tech. coords.). National proceedings: Forest and conservation nursery associations -2002. Ogden, UT: USDA For. Serv., Rocky Mountain research station. Proceedings RMRS-P-28: 98-104.

Ritchie, G. and T.D. Landis. 2005. Seedling quality tests: chlorophyll fluorescence. For. Nurs. Notes. Publ. R6-CP-TP-11-04. Winter: 12-16 .

Sakai, A. 1982. Extraorgan freezing of primordial shoots of winter buds of Conifers, p. 199-209. In P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress mechanisms and crop implications v. 2. Academic Press, New York, NY.

Scholz, F. and B.R. Stephan. 1982. Growth and reaction to drought of 43 Abies grandis provenances in a greenhouse study. Silvae Genet. 31(1): 27-35.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, pp1243-1246. Nashville, TN, March 22-25.

Toivonen, P. and W. Vidaver. 1988. Variable chlorophyll a fluorescence and CO_2 uptake in water-stressed white spruce seedlings. Plant Physiol. 86: 744-748.

USDA Plant Hardiness Zone Map. 1990. USDA Misc. Publ. 1475. Washington D.C.

van der Kamp, B.J. and J. Worrall. 1990. An unusual case of winter bud damage in British Columbia interior conifers. Can. J. For. Res. 20: 1640-1647.

Worrall, J. 1983. Temperature – bud-burst relationships in amabilis and subalpine fir provenance tests replicated at different elevations. Silvae Genet. 32(5-6): 203-209.

Xie, C.Y. and C.C. Ying. 1993. Geographic variation of grand fir (*Abies grandis*) in the Pacific coast region: 10-year results from a provenance trial. Can. J. For. Res. 23: 1065-1072.

Yordanov, I. 1992. Response of photosynthetic apparatus to temperature stress and molecular mechanisms of its adaptation. Photosynthetica. 26(4): 517-531.

CHAPTER TWO

CHLOROPHYLL FLUORESCENCE, PHOTOSYNTHESIS, GROWTH, AND FOLIAR

NUTRIENT CONCENTRATION OF *ABIES* IN RESPONSE TO SOIL pH

CHLOROPHYLL FLUORESCENCE, PHOTOSYNTHESIS, GROWTH, AND FOLIAR NUTRIENT CONCENTRATION OF *ABIES* IN RESPONSE TO SOIL pH.

Additional index words. *Abies*, foliar nutrition, growth, photosynthesis, F_v/F_m, soil chemistry

Abstract

We measured foliar nutrition, maximum photosynthesis (A_{max}), variable chlorophyll fluorescence (F_v/F_m) and leader growth in 17 *Abies* Mill. species, hybrids, and varieties grown under field conditions at four locations in Michigan. Sites represented soil pH ranging from 3.5 to 7.6. Increased soil pH significantly reduced foliar nutrient concentrations of N, P, K, S, B, Zn, Mn, Fe, and Cu. F_v/F_m decreased with increasing pH and was correlated with foliar N, P, K, and Cu. A_{max} declined as soil pH increased, although, the response varied by species ($p \leq 0.05$). Both *A. lasiocarpa* and *A. homolepis* were more tolerant of higher soil pH soils than *A. balsamea* var. *phanerolepis*. Foliar N, P, and K were negatively correlated with soil pH, explaining photosynthetic limitations in high pH soils. Leader growth was more closely related to foliar N, P, and K concentrations than A_{max}. Tolerance of high pH soil is an important selection criterion for future *Abies* introductions to the landscape and Christmas tree industry in the upper Midwest.

Introduction

Adequate foliar nutrition is required for most plant physiological processes to function properly. Soil pH may influence the availability of several plant nutrients such as K, P, Fe, Mn, B, Zn (Lucas and Davis, 1961). Cregg et al. (2004) observed decreased foliar chlorophyll concentrations and foliar nutrient concentrations in response to

elevated soil pH levels in several *Abies* species. However, some species were more tolerant of higher pH soil. Throughout the upper Midwest and Great Lakes region, soil pH levels are quite variable. Coniferous forests tend to be acidic while grassland regions are more alkaline. Therefore, tolerance of *Abies* to varying soil pH and nutrient availability is necessary for future plant introductions in the upper Midwest.

Chlorophyll fluorescence is a rapid and non-destructive method to measure the efficiency by which photosystem II captures light and is often expressed as the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) (Bjorkman and Demming, 1987). The effects of various environmental plant stresses on plant health have been identified using chlorophyll fluorescence (Toivonen and Vidaver, 1988; Adams and Perkins, 1993; Sun et al., 2001). In *Abies*, photosynthetic efficiency declined due to the development of nutrient deficiencies caused as soil pH increased, making chlorophyll fluorescence an important means to identify nutrient stress (Cregg et al., 2004).

The genus *Abies* Mill. (true firs) contains 46 species (Farjon, 1990) and covers large elevational and latitudinal ranges (Liu, 1971), which increase the probability of genetic adaptation to diverse external factors (Langlet, 1963). In *A. fraseri*, seedling growth is highest at soil pH of 4.2 to 4.5 (Bryan et al., 1989), while *A. balsamea* grows best on sites with soil pH of 6.5 and 7.0 (Bakuzis and Hansen, 1965). Cregg et al. (2004) showed *A. lasiocarpa* and *A. veitchii* were more tolerant of soils with higher soil pH than *A. sachalinensis* Mast., *A. sibirica* Ledeb., or *A. borisii regis* Mattf.

Soil that is more acidic or more alkaline than preferred ranges is problematic as well. Extreme alkaline conditions resulted in lower N and Mn concentrations in several conifers (Mandre et al., 1999). Increasing soil pH led to needle chlorosis, nutrient

deficiencies, and tree decline in *A. alba* Mill. (Hiltbrunner and Flückiger, 1996). Needle

chlorosis developed in *A. nordmanniana* grown in high pH soils (Khalil et al., 1989).

Conversely, extremely low soil pH led to increased Al toxicity, interfered with Mg

uptake, and reduced specific needle mass in *A. firma* (Nakatani et al., 2004; Igawa et al.,

1997).

Nutrient deficiencies affect different plant physiological processes. Magnesium

deficiencies led to needle chlorosis, reduced biomass accumulation, and decreased

photosynthetic output (Sun and Payn, 1999; Laing et al., 2000; Sun et al., 2001). Excess

K uptake inhibited Mg translocation from roots to foliage (Sun and Payn, 1999).

Decreased needle growth, bud frequency, photosynthetic output, and stomatal

conductance resulted from N deficiencies (Chandler and Dale, 1995; Tan and Hogan,

1995; Hinsley et al., 2000; Timmer et al., 1977). Phosphorous deficiencies reduced

carboxylation of Rubisco and electron transport resulting in decreased A_{max} in *Pinus*

pinaster Ait. (Loustau et al., 1999).

The goal of this project is to identify *Abies* species tolerant of different edaphic

conditions in the upper Midwest, to promote increased plant diversity, and thus reduce

disease problems and insect pressures. The objective of this paper is to 1) identify the

influence of soil pH on important macro- and micro-nutrients, 2) investigate how soil pH

and nutrient deficiencies impede physiological processes, and 3) understand how nutrient

deficiencies and reduced physiological processes affect plant growth.

Materials and Methods

Site Locations

Over 1100 trees representing 38 species, hybrids, provenances, and varieties of *Abies* were part of a true fir species and provenance trial initiated at the Kellogg Research Forest (KRF), Augusta, Michigan, in early 1991. In the fall of 2002 and spring of 2003, 246 trees representing 21 species and hybrids (Table 1) were transplanted to three locations in Michigan: Clarksville Horticulture Experiment Station, Clarksville, MI (CHES); Horticulture Teaching and Research Center, East Lansing, MI (HTRC); and Northwest Michigan Horticulture Research Station, Traverse City, MI (NWMHRS) (Figure 1). These three locations along with the KRF represent different climate regions (Table 2) and soil environments (Table 3). Trees were dug and ball and burlapped as 60 to 76 cm root balls in accordance with American nursery standards (American Nursery and Landscape Association, 2004)

In the fall of 2003, 57 additional trees were transplanted from the KRF to replace 37 trees that died due to transplant stress during the previous year and add three new species and varieties to the study. At least four trees of each species or hybrid were planted at each location with the exception of the HTRC where one to four more trees of each species were planted when available. Trees were planted in a complete randomized design at each location. Trees were planted at approximately 4.5 m intervals.

Fertilization

In spring 2004, sites were fertilized with 21-0-0 ammonium sulfate at a rate of approximately 133 g per tree, to lower soil pH by 0.5 and insure that N was not limiting. Fertilizer was applied at CHES on 29 April, HTRC on 22 April, and NWHRS on 11 May. On 7 July 2004, 46-0-0 urea was applied at CHES at a rate of 91 g per tree. Trees at KRF

were fertilized every fall with 21-0-0 at 85 g per tree. Trees exceeding 0.9 m received 28 g of fertilizer for each additional 0.3 m in height.

Soil pH Test

Four evenly spaced soil samples (15 cm depth) were collected approximately 30 cm from the stem of each tree at the three transplant sites and KRF. Samples for each tree were combined and stored in a polyethylene bag. In 2003, samples were taken from 13 *Abies* species at the CHES on 21 Oct, HTRC on 10 Oct, KRF on 7 Oct, and NWHRS on 16 Oct. In 2004, after fertilization, samples were taken from 17 *Abies* species at the following locations: CHES on 7 Oct; HTRC on 5 Oct; KRF on 10 Oct; NWHRS on 8 and 9 Oct.

Soil pH was measured using an Orion Soil pH Meter, Model 410 (Orion Research Incorporated, Boston, MA). An equal volume of soil and reverse osmosis water were mixed. Soil pH readings were taken after 15 minutes. In 2003, four samples from each location were chosen at random and a soil texture analysis was conducted at the Michigan State University Soil and Plant Nutrition Laboratory (East Lansing, MI) (Table 3).

Soil Nutrition Samples

In 2004, four soil samples from each site were chosen at random from excess soil not used in soil pH tests for a full soil nutrient and cation exchange capacity analysis.

Chlorophyll Fluorescence

Chlorophyll fluorescence (F_v/F_m) was measured on three needles from every tree at all four locations using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, England). Readings were taken on sunny days when temperatures were above 16 °C. Needles were dark-acclimated for 15 min

using the manufacturer's plastic/foam clips before measurements were recorded. In 2003, readings were taken from 13 *Abies* species at CHES on 13 Oct, HTRC on 8 Oct, KRF on 7 Oct, and NWHRS on 11 Oct. In 2004, 17 *Abies* species were measured at CHES on 7 Oct, HTRC on 6 Oct, KRF on 10 Oct, and NWHRS on 9 Oct.

Foliar Nutrient Samples

Foliar nutrient samples were taken from current year needles selected at random from each tree at all four locations. In 2003, samples were collected from 13 *Abies* species at CHES on 13 Oct, HTRC on 8 Oct, KRF on 7 Oct, and NWHRS on 11 Oct. In 2004, samples were collected from 17 *Abies* species at CHES on 7 Oct, HTRC on 5, 11, and 12 Oct, KRF on 10 Oct, and NWHRS on 8 Oct. Samples were stored in paper bags and oven-dried at 60 °C for four days. Needles were stripped from branches, ground in a coffee grinder, and sifted through a #40 sieve. In both years, samples were sent to Waters Agricultural Laboratories, Inc. (Camilla, GA) for a full foliar nutrient analysis.

Gas Exchange

Gas exchange was measured on current years growth using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) fitted with a conifer chamber (LI-6400-05, Li-Cor). The maximum photosynthetic rate (A_{max}) was measured from sun-exposed shoots in the upper one-third of the tree canopy on sunny days with PPFD greater than 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Block temperature of the conifer chamber was maintained near 25 °C. The flow of air into the chamber was 500 $\mu mol \cdot s^{-1}$. The reference CO_2 concentration was slightly above ambient at 400 $\mu mol\ CO_2 \cdot mol^{-1}$.

Gas exchange was measured at CHES on 29 June, 6 August, and 30 August 2004, HTRC on 23 June, 28 July, and 2 September 2004, KRF on 1 July, 1 August, and 10

September 2004, and NWHRS on 15 July, 5 August, and 4 September 2004.

Immediately after gas exchange measurements, shoots used for gas exchange

measurements were removed. Samples were transported in a cooler with ice and then

transferred to a cooler at 2.5 °C. Needle area was determined in two ways: 1) projected

shoot area and 2) projected needle area. To determine projected shoot area, samples were

passed through a leaf area meter (LI-3000, Li-Cor). Projected needle area was measured

by removing all the needles on each shoot and scanning them at 200 dpi using an Epson

Perfection 2450 scanner (Seiko Epson Corp., Long Beach, CA). Images were analyzed

using WinSeedle (Regent Instruments, Inc., Quebec, Canada) to determine projected

needle area. Maximum photosynthetic rates are expressed in terms of projected shoot

area (PSA_{max}) and projected needle area (PNA_{max}).

Growth

The topmost shoot of each tree was considered the leader. Current year leader

heights were measured at CHES, HTRC, and NWHRS following the completion of

growth in September 2004. Trees at KRF were sheared while they were still growing, so

no shoot growth measurements were taken on those trees.

Statistical Analysis

PROC MIXED (SAS Inc., Cary, NC) was used for analysis of variance for

location, species, year effects, interactions, and means were separated using Tukey's

Studentized test. PROC CORR (SAS) was used to identify Pearson's correlation

coefficients between leader growth, photosynthesis, soil pH, F_v/F_m, and the following

foliar elements: N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, and Cu. Species differences in

response to pH were tested by Analysis of Covariance using PROC GLM.

Results

Mean soil pH levels differed significantly among location ($p \leq 0.001$) and years ($p \leq 0.001$) but not species ($p \leq 0.46$). Soil pH levels decreased in 2004 following ammonium sulfate application. In both years, mean soil pH was highest at NWHRS and lowest at KRF. Photosynthetic efficiency (F_v/F_m) declined across sites in 2004 ($p \leq 0.02$). Mean F_v/F_m values were highest at NWHRS and lowest at HTRC in both years.

Foliar nutrient concentrations of P, K, Zn, Cu, B, and S concentrations were higher ($p \leq 0.05$) in 2004 than 2003 and nutrient concentrations were generally higher in 2004 than 2003, except for Mn. In 2003, 95% confidence intervals indicated P was lower than suggested optimal ranges (Rothstein and Lisuzzo, 2003), while K, Mg, and Fe concentrations were higher than optimal. In 2004, 95% confidence intervals were higher than optimal ranges for K, Mg, Ca, S, and Fe.

Soil concentrations of 11 essential mineral plant nutrients varied among locations (Table 4). KRF had lower Ca and Mg than the three horticultural stations where the sites had a history of crop production and presumed lime application. Species × location effects existed for all variables ($p \leq 0.05$). Species differed for foliar N, P, K, Mg, Ca, S, Fe, Zn, B, Cu, leader growth, F_v/F_m, and PNA_{max}, but not foliar Mn ($p = 0.12$) (Table 5). Foliar N was lower at HTRC than at other sites and *A. balsamea* var. *phanerolepis* had higher P and Ca at KRF than at other locations (Figure 3). Generally, nutrient concentrations were highest in *A. nephrolepis*.

Soil pH was negatively correlated with PSA_{max}, N, P, K, S, B, Zn, and Cu in both 2003 and 2004 ($p \leq 0.05$). In addition, soil pH was negatively correlated with Mn, (Pearson's r=-0.19, p=0.0015) Fe, (Pearson's r =-0.17, p=0.003) and PNA_{max} (Pearson's

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r=-0.40, p≤0.0001) in 2004 (Table 6). With the exception of Mg, all nutrients correlated with pH were also correlated with PSA_{max} when data for 2003 and 2004 were combined. Similarly, all nutrients correlated with pH were also correlated with PSA_{max}, with the exceptions of Ca in 2003 and Mn in 2004.

Net photosynthesis (PNA_{max}) varied by species and location (p≤0.0001) (Table 7) and was positively correlated with N, P, K, Mg, Ca, S, B, Zn, Fe, Cu, and leader growth. However, a species × location interaction was significant (p=0.046) In addition, PNA_{max} declined with increasing soil pH (Figure 2). Analysis of covariance indicated a significant interaction between species and pH (p=0.003). At the upper quartile of soil pH (pH=6.38), PNA_{max} was significantly higher in *A. lasiocarpa* than *A. balsamea* var. *phanerolepis* but at the lower quartile of soil pH (pH=4.28) species were not significantly different. Increased soil pH reduced available N, P, and K, which resulted in reduced photosynthesis (Figure 4).

Photosynthetic efficiency (F_v/F_m) differed significantly by species, year, and location (p≤0.05). However, in 2004 a species × location interaction was significant (p=0.037). Photosynthetic efficiency (F_v/F_m) was correlated with foliar N, Ca, S, and Zn in 2003 and foliar N, P, K, Mn, Cu, leader growth, and PSA_{max} were in 2004. Soil pH and F_v/F_m were not correlated in 2003 (p≤0.77) or 2004 (p≤0.10). Increased concentrations of foliar N ($R^2 = 0.39$, p≤0.0001) and K ($R^2 = 0.22$, p≤0.0001) resulted in increased F_v/F_m values when all species were combined (Figure 3). F_v/F_m values were highest in *A. fraseri*, *A. fraseri* x *homolepis*, and *A. koreana* x *veitchii* and lowest in *A. chensiensis*. In both *A. koreana* x *balsamea* and *A. koreana* x *veitchii*, F_v/F_m values were closely related to their *A. koreana* parent.

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Leader growth differed by species and location in 2004 (p≤0.0001) and was positively correlated with foliar N, P, K, Ca, S, Fe, Cu, PNA_{max}, and F_v/F_m, and negatively correlated with soil pH and foliar Mn (p≤0.05). However, a species × location interaction was significant (p=0.026). While foliar N (Pearson's r = 0.46, p≤0.0001), P (Pearson's r = 0.35, p≤0.0001), and K (Pearson's r = 0.56, p≤0.0001) were closely related to increased leader growth (Figure 5). Analysis of covariance indicated significant interaction between species and foliar K (p=0.046). At the upper quartile of foliar K (K=1.04), leader growth was significantly higher in *A. koreana* x *balsamea* and *A. koreana* x *veitchii* than *A. nordmanniana* ssp. *equi-trojani* but at the lower quartile of foliar K (K=0.59) *A. koreana x balsamea* and *A. nordmanniana* ssp *equi-trojani* are not different (Figure 5).

Discussion

Soil pH levels declined at NWHRS, HTRC, and CHES in response to ammonium sulfate application in 2004. Soil nutrient and plant foliar nutrient concentrations differed suggesting an additional factor influenced nutrient availability in the trees. In this case, increased soil pH reduced most foliar nutrient concentrations but its influence was strongest for foliar N, P, K, and Cu concentrations. Foliar nutrient concentrations have been negatively correlated with soil pH in previous studies (Cregg et al., 2004; Lucas and Davis, 1961).

Mean foliar nutrient concentrations were in the range of adequate nutritional guidelines proposed for *A. fraseri* (FRA) by Rothstein and Lisuzzo (2003). However, foliar K, Mg, and Fe concentrations were above optimal guidelines. In our study, Mg was not correlated with soil pH, which contrasts with previous findings (Cregg et al.,

2004; Lucas and Davis, 1961). Before being transplanted, trees were grown at KRF in low pH soils and supplied with annual fertilization. As a result, some antecedent effects, such as high foliar Mg concentrations, may have existed in the two years following transplanting. Immobile nutrient concentrations such as Mn and Fe declined in 2004 and were not correlated with soil pH in 2003 but were in 2004, further supporting this explanation.

Little relation between soil pH and F_v/F_m was evident either year, which contradicts previous findings in a greenhouse study (Cregg et al., 2004). In the present study, trees were grown under field conditions and influenced by many factors such as drought stress, nutrient availability, soil type, and temperature among others. Adequate foliar nutrient concentrations and antecedent effects may have impeded the interaction between soil pH and F_v/F_m.

Increased foliar N coincided with F_v/F_m in all species until N was no longer limiting. When foliar N was greater than 2%, F_v/F_m increased little. Rothstein (2005) found initial N application resulted in increased F_v/F_m. In the same study though, additional N, did not increase F_v/F_m but did cause nutrient imbalances and deficiencies. In the present study, foliar P and K were correlated with F_v/F_m in 2004 following ammonium sulfate application. Following N application, foliar P and B concentrations increased in *A. procera* (Fletcher et al., 1998). In *A. balsamea*, foliar Ca, Mg, Na, Fe, and Zn concentrations increased following N fertilization, however, foliar P, K, and Mn concentrations declined (Czapowskyj et al., 1980; Timmer and Stone, 1978).

Photosynthetic rates generally declined with increasing soil pH, although, some species were more tolerant of increasing soil pH than others. Declines in PNA_{max} were

due to the influence of soil pH on foliar N, P, and K, although other elements were influential to a lesser extent. Surprisingly, *A. balsamea* var. *phanerolepis* was the least tolerant species to increasing soil pH. Previously, *A. balsamea* var. *phanerolepis* was typically considered tolerant of higher soil pH (Brown, 2000; Frank, 1990). In the fall of 2003, *A. balsamea* var. *phanerolepis* was transplanted and establishment effects may have reduced its photosynthetic output in 2004. *A. lasiocarpa* maintained a higher PNA_{max} rate at elevated soil pH, while *A. homolepis* was more insensitive to increased soil pH. Photosynthetic efficiency was maintained in *A. lasiocarpa* while declining in other *Abies* species as soil pH increased (Cregg et al., 2004). Field observations indicate *A. lasiocarpa* is tolerant of many soil conditions and a wide range of soil pH levels (Alexander et al., 1990).

Leader growth differed among species. In *A. koreana* x *balsamea* and *A. koreana* x *veitchii*, leader growth increased more with foliar K than in *A. nordmanniana* ssp. *equi-trojani*. Hybrid species had the greatest leader growth and is evidence of hybrid vigor, which has been reported in other *Abies* hybrids (Klaehn, and Winieski, 1962). Leader growth was strongly correlated with N, P, and K. Also, leader growth was more strongly correlated with several nutrients than with PNA_{max}.

Leader growth is but one growth parameter and biomass is allocated to shoot, stem, root, and reproductive growth. Trees growing under nutrient deficiencies, allocate more biomass to roots than shoots (Kaakinen et al., 2004). Both root and shoot growth increased in *A. amabilis* (Dougl.) Forbes when N and P were applied at optimal levels than when N or P was limiting (Hawkins et al., 1998). The leaf area index (LAI) and biomass accumulation per LAI increased following either fertilizer, irrigation, or both in

Pinus taeda L. (Albuagh et al, 1998; Albaugh et al., 2004). Schoettle (1994) suggests increased foliar biomass per shoot compensates for decreased photosynthesis in *P. contorta* (Dougl.) Loud. We found that when multiple foliar nutrient concentrations were optimum, tree health improved and leader growth increased.

In summary, soil pH influenced nutrient availability. Photosynthetic efficiency as indicated by F_v/F_m was not related to soil pH, although, site factors and antecedent nutrient effects influenced these results. Photosynthetic response differed among species to increasing soil pH suggesting photosynthesis of *A. balsamea* and *A. balsamea* var. *phanerolepis* is especially sensitive to soil pH, while *A. nephrolepis* and *A. lasiocarpa* are less sensitive to soil pH. Negative relationships between foliar N, P, and K and soil pH explained photosynthetic limitations in high pH soils. In many nutrients, concentrations were influenced by soil pH suggesting declined photosynthetic production was a result of multiple nutrient limitations. Hybrids crosses had the greatest leader growth and showed signs of hybrid vigor. Leader growth was influenced more by foliar nutrient concentrations than by photosynthetic output.

Table 1. List of *Abies* species planted at four locations in Michigan.

Common Name	Scientific Name	Geographic Origin	Elevation (m)	Latitude (N)
Ernst fir	*A. chensiensis* Van Tiegh.	Asia	2500 - 3800	25 - 33
Korean fir	*A. koreana* Wils.	Asia	1000 - 2000	33 - 36
Needle fir	*A. holophylla* Maxim.	Asia	0 - 1400	33 - 49
Nikko fir	*A. homolepis* Sieb. et Zucc.	Asia	600 - 2200	33 - 38
Siberian white fir	*A. nephrolepis* (Trautv.) Maxim.	Asia	500 - 2000	35 - 55
Veitch fir	*A. veitchii* Lindl	Asia	1200 - 3000	33 - 38
Balsam fir	*A. balsamea* (Linn.) Mill.	N. America	0 - 1500	38 - 59
Cannan fir	*A. balsamea* var. *phanerolepis* (Fern.) Liu	N. America	0 - 1500	38 - 59
Corkbark fir	*A. bifolia* (Merr.) Lemm.	N. America	0 - 1700	37 - 39
Fraser fir	*A. fraseri* (Pursh.) Poir.	N. America	1100 - 2100	35 - 37
Noble fir	*A. procera* Rehd.	N. America	100 - 2700	41 - 48
Sub-Alpine fir	*A. lasiocarpa* (Hook.) Nutt.	N. America	0 - 3500	32 - 64
Nordman fir	*A. nordmanniana* (Steven) Spach.	Mediterranean	1000 - 2200	40 - 44
Turkish fir	*A nordmanniana* ssp. *equi-trojani* (Aschers. et Sint. ex Boiss.) Coode et Cullen	Mediterranean	1000 - 2200	40 - 44
Fraser x Nikko Hybrid	*A. fraseri x homolepis*	N. American/Asia	NA	NA
Korean x Balsam Hybrid	*A. koreana x balsamea*	N. American/Asia	NA	NA
Korean x Veitch Hybrid	*A. koreana x veitchii*	N. American/Asia	NA	NA

NA indicates data was not available (Liu, 1971).

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Table 2. Thirty-year climate summary and USDA plant hardiness zones for four *Abies* planting sites in Michigan.

Location	Average January Low (°C)[1]	Average July High (°C)[1]	Annual Precipitation (cm)[1]	Annual Snowfall (cm)[1]	Growing Season (days)[1]	USDA Hardiness Zone[2]
HTRC	-11	28	78.5	99	150	5A
NWHRS	-10	27	85.1	244	135	5B
CHES	-10	28	90.7	145	147	5B
KRF	-9	28	89.4	135	149	5B

1 Illinois Dept. of Nat. Res., 2005. 2 USDA Plant Hardiness Map, 1990.

Table 3. Soil properties of four *Abies* planting sites in Michigan

Location	Sand (%)	Silt (%)	Clay (%)	Soil Type	2003 pH	2004 pH
HTRC	83.1	8.7	9.3	Loamy Sand	6.25	5.72
NWHRS	83.3	7.6	9.1	Loamy Sand	7.11	6.66
CHES	61.3	23.5	15.1	Sandy Loam	6.76	4.62
KRF	72.4	17.1	10.5	Sandy Loam	4.63	4.12

Table 4. Soil nutrient concentrations and cation exchange capacity (CEC) of four *Abies* test plots in Michigan.

Location	P	K*	Mg	Ca*	S	B	Zn*	Mn*	Fe**	Cu	CEC
					kg·ha⁻¹						
CHES	66.5	166.8 a	172.4	992.4 bc	31.7	0.2	3.0 b	80.4 a	49.8 a	3.7	6.5
KRF	82.7	136.6 ab	68.5	388.1 c	23.7	0.2	3.3 b	76.3 a	70.5 a	0.4	5.7
NWHRS	45.6	95.0 b	418.3	3579.6 a	8.6	0.9	6.5 ab	24.5 b	15.1 b	0.4	12.5
HTRC	82.8	89.3 b	303.0	2985.0 ab	51.6	1.0	9.8 a	44.6 ab	24.5 b	1.2	11.1

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

Means within a column followed by the same letter were not significantly different, $\alpha = 0.05$, Duncan.

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Table 5. Mean foliar nutrient levels in 17 *Abies* species growing at four locations in Michigan in 2004.

Species	g·kg⁻¹						Mn	mg·kg⁻¹			
	N****ˣ	P****ˣ	K****ˣ	Mg****ˣ	Ca****ˣ	S****ˣ		Fe****ˣ	Zn****ˣ	B****ˣ	Cu***ˣ
A. balsamea	20.5	1.9	6.9	1.4	5.2	1.7	548.4	76.3	45.3	22.6	4.1
A. bal. var. phanerolepis	21.1	2.1	7.7	1.2	4.9	1.9	611.5	72.8	46.2	19.8	4.1
A. bifolia	20.5	2.3	7.8	2.6	8.0	2.1	411.9	64.4	49.8	35.7	3.3
A. chensiensis	21.3	1.7	9.0	1.6	7.5	1.6	480.3	55.9	40.6	19.3	4.6
A. fraseri	17.7	1.9	7.5	1.4	4.5	1.7	450.2	69.2	32.2	26.9	4.0
A. fraseri x homolepis	21.2	2.2	9.6	1.4	5.8	1.9	405.6	66.9	44.9	23.1	3.7
A. koreana	20.5	2.0	8.3	1.7	5.4	2.1	585.9	55.7	21.8	30.3	2.9
A. koreana x balsamea	21.3	2.3	8.2	1.5	5.3	2.0	525.4	64.6	31.1	28.3	3.4
A. koreana x veitchii	21.6	2.3	9.0	1.6	6.9	1.9	420.6	73.3	43.2	26.3	4.0
A. holophylla	21.5	1.9	6.6	1.4	9.3	1.7	452.3	68.7	46.5	24.2	3.8
A. homolepis	19.1	1.7	6.8	1.4	5.5	1.6	509.1	55.2	38.9	22.2	3.0
A. procera	18.8	1.8	8.3	1.6	4.8	1.8	697.8	57.1	54.3	26.7	3.9
A. nordmanniana	18.5	1.8	10.2	1.3	6.1	1.3	514.3	57.0	36.8	24.0	4.3
A. nephrolepis	22.6	2.5	10.6	1.8	9.4	2.2	448.3	84.7	68.4	35.2	3.9
A. lasiocarpa	19.9	2.1	8.9	1.5	5.9	1.9	527.6	63.8	56.5	38.4	3.7
A. nord. ssp. equi-trojani	20.8	1.9	9.1	1.4	5.6	1.6	523.4	70.2	38.3	21.7	4.5
A. veitchii	18.4	1.7	7.5	1.4	5.0	1.5	550.3	55.1	34.0	21.7	3.2

*, **, *** Significant at $p \leq 0.05$, 0.01, or 0.0001, respectively

ˣ Species were significant at $p \leq 0.0001$, however, location × species effect was significant at $p \leq 0.05$.

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Table 6. Pearson's correlation coefficient for leader height, soil pH, photosynthesis, chlorophyll fluorescence and 11 foliar nutrient elements of 17 *Abies* species at four locations in Michigan sampled in October 2004.

	Leader	PSA$_{max}$	PNA$_{max}$	F$_v$/F$_m$	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
pH	-0.19**	-0.15**	-0.40***	-0.10	-0.40***	-0.44***	-0.51***	0.10	-0.11	-0.12*	-0.28***	-0.30***	-0.19**	-0.17**	-0.41***
Leader		0.04	0.18**	0.35***	0.48***	0.35***	0.56***	-0.07	0.23**	0.14*	-0.10	0.09	-0.17*	0.27***	0.27***
PSA$_{max}$			0.65***	0.20**	0.15**	0.12*	0.21**	0.11	0.10	0.18**	0.16**	0.20**	-0.02	0.15**	0.18**
PNA$_{max}$				0.04	0.33***	0.39***	0.51***	0.16**	0.42***	0.20**	0.36***	0.45***	0.05	0.21**	0.35***
F$_v$/F$_m$					0.54***	0.21***	0.36***	-0.07	0.03	0.06	-0.10	0.06	-0.15*	0.04	0.39***
N						0.52***	0.66***	0.04	0.35***	0.35***	0.04	0.34***	-0.07	0.36***	0.67***
P							0.60***	0.11	0.34***	0.34***	0.46***	0.45***	-0.01	0.22**	0.48***
K								-0.12*	0.26***	0.21**	0.28***	0.27***	-0.02	0.17**	0.58***
Mg									0.46***	0.49***	0.27***	0.34***	-0.15**	0.22***	-0.05
Ca										0.32***	0.33***	0.50***	-0.19**	0.24***	0.26***
S											0.32***	0.37***	-0.08	0.44***	0.15*
B												0.41***	0.02	0.10	0.12
Zn													-0.03	0.43***	0.44***
Mn														-0.06	-0.005
Fe															0.28***

*, **, *** Significant at p≤0.05, 0.01, or 0.0001, respectively

Figure 7. Mean leader growth, chlorophyll fluorescence (F_v/F_m), and net photosynthesis (PNA_{max}) in 17 *Abies* species grown at four locations in Michigan.

Species	Leader Growth***[x]	F_v/F_m*[x]	PNA_{max}***[x]
	cm		μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$
A. balsamea	15.5	0.663	6.2
A. bal. var. *phanerolepis*	20.1	0.698	5.8
A. bifolia	11.0	0.655	9.0
A. chensiensis	17.1	0.628	7.6
A. fraseri	12.0	0.714	6.4
A. fraseri x *homolepis*	37.8	0.711	7.2
A. koreana	22.3	0.700	5.9
A. koreana x *balsamea*	35.9	0.699	6.5
A. koreana x *veitchii*	38.5	0.709	7.1
A. holophylla	16.8	0.667	7.3
A. homolepis	25.2	0.638	6.1
A. procera	10.6	0.713	8.2
A. nordmanniana	21.7	0.670	8.0
A. nephrolepis	30.1	0.668	7.4
A. lasiocarpa	11.9	0.680	8.6
A. nord. ssp. *equi-trojani*	13.0	0.675	7.5
A. veitchii	30.4	0.644	5.6

*, **, *** Significant at $p \leq 0.05$, 0.01, or 0.0001, respectively

[x] Species were significant at $p \leq 0.05$, however, location x species effect was significant at $p \leq 0.05$.

Table 8. Mean foliar nutrient of four *Abies* test plots in Michigan in 2004.

Location	g·kg⁻¹						mg·kg⁻¹				
	N***	P***	K***	Mg***	Ca***	S***	Mn***	Fe***	Zn***	B***	Cu***
CHES	25.7 a	2.13 b	10.23 b	1.66 ab	6.87 a	2.04 a	520.6 a	89.0 a	51.9 a	24.4 b	4.5 a
HTRC	15.3 d	1.78 c	5.93 d	1.73 a	6.07 bc	1.84 b	540.8 a	66.6 b	43.5 b	29.7 a	2.8 c
KRF	22.0 b	2.51 a	10.99 a	1.22 c	6.57 ab	1.60 c	567.1 a	49.3 d	44.7 b	32.7 a	4.5 a
NWHRS	20.4 c	1.73 c	6.87 c	1.56 ab	5.62 c	1.69 bc	376.8 b	55.1 c	30.7 c	17.0 c	3.5 b

*, **, *** Significant at p≤0.05, 0.01, or 0.0001, respectively

Means within the same column followed by the same letter are not statistically different. α = 0.05, Tukey.

Table 9. Mean leader growth, chlorophyll fluorescence (F_v/F_m), and photosynthetic values (PNA_{max}) of four *Abies* test plots in Michigan in 2004.

Location	cm		µmol CO₂·m⁻²·s⁻¹
	Leader***	F_v/F_m***	PNA_{max}***
CHES	30.91 a	0.720 b	8.42 a
HTRC	14.70 c	0.584 c	6.67 b
KRF	NA	0.696 b	8.02 a
NWHRS	22.43 b	0.749 a	5.22 c

*, **, *** Significant at p≤0.05, 0.01, or 0.0001, respectively

Means within the same column followed by the same letter are not statistically different. α = 0.05, Tukey.
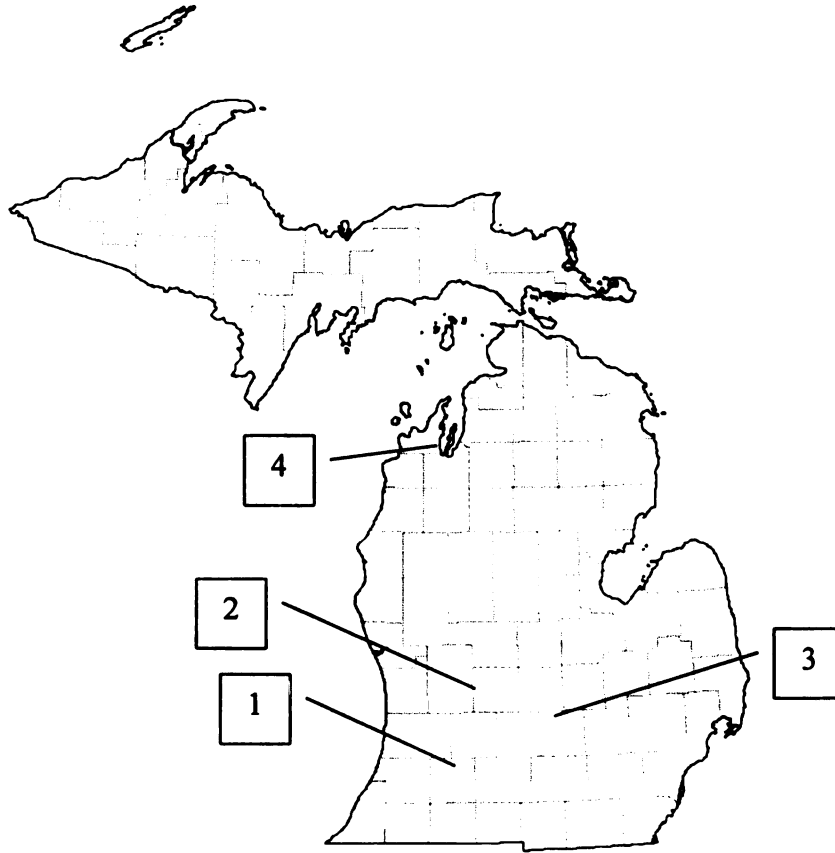
Figure 1. Location of four *Abies* test plots in Michigan. 1) Kellogg Research Forest (KRF), 2) Clarksville Horticulture Experiment Station (CHES), 3) Horticulture Teaching and Research Center (HTRC), 4) Northwest Michigan Horticultural Research Station (NWHRS)
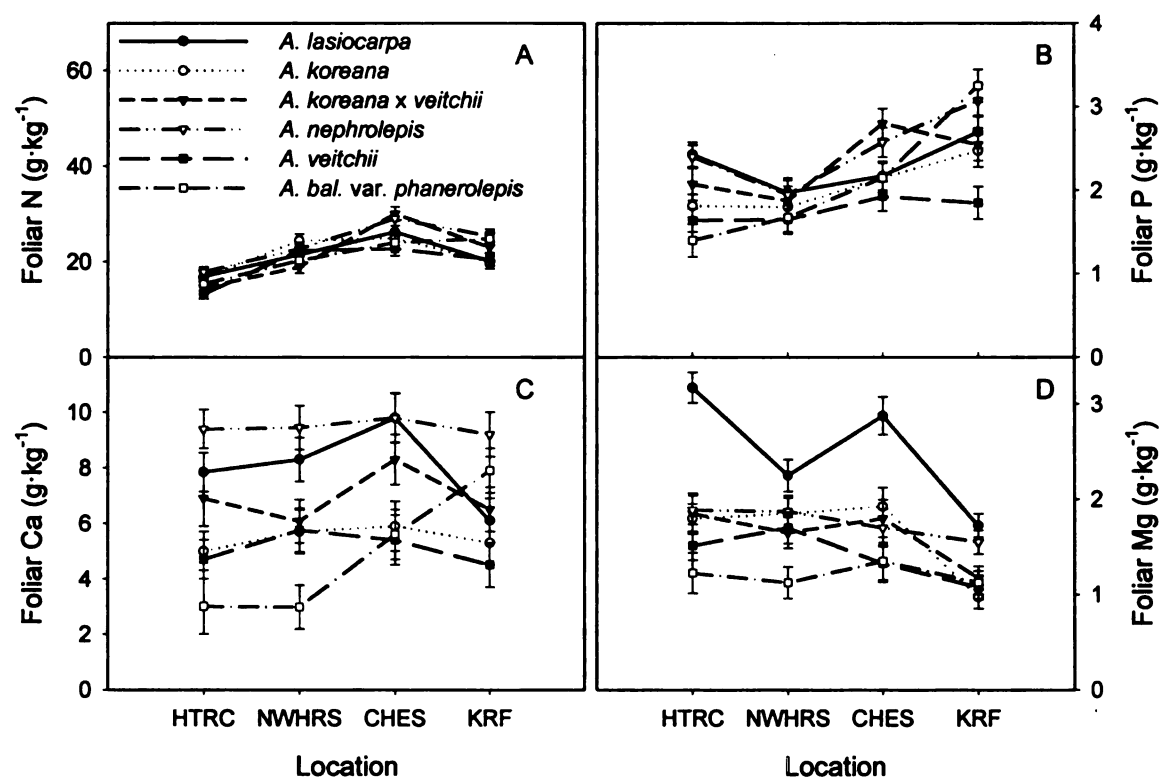
Figure 3. Relationship between average foliar A) N, B) P, C) Ca, and D) Mg in six *Abies* species grown at four locations in Michigan in 2004.
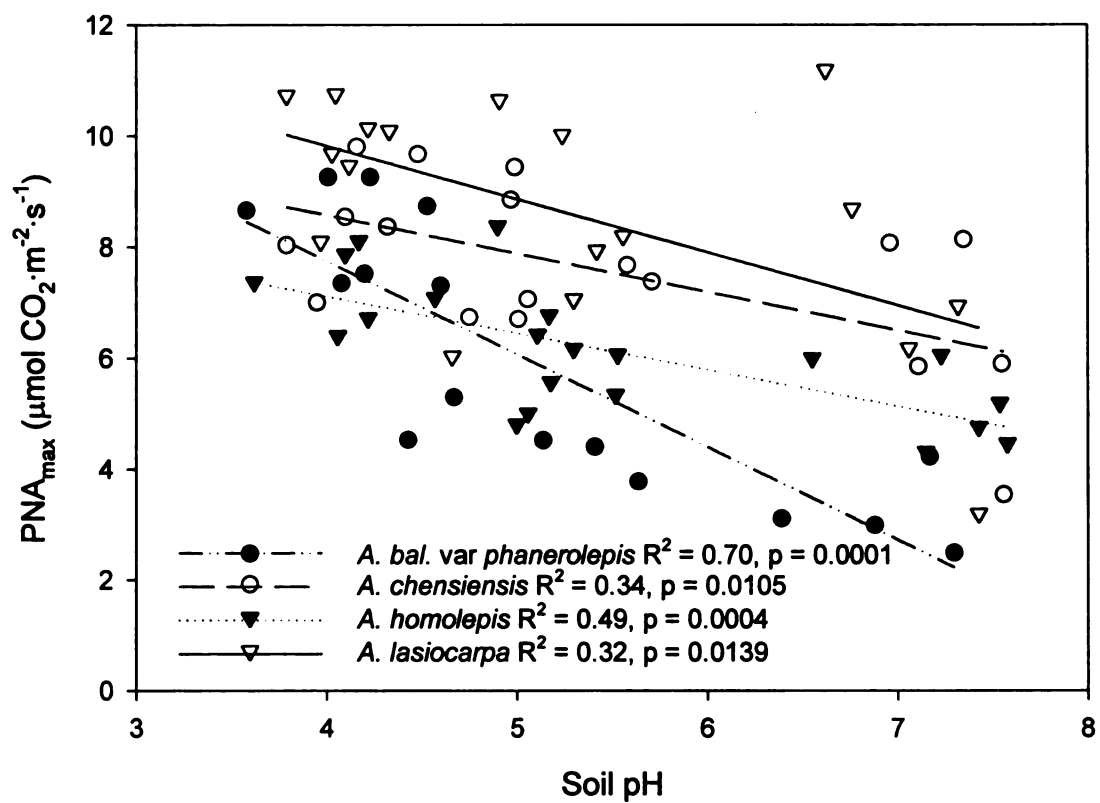
Figure 3. Relationship between PNA_{max} and soil pH in four *Abies* species grown at four locations in Michigan in 2004.
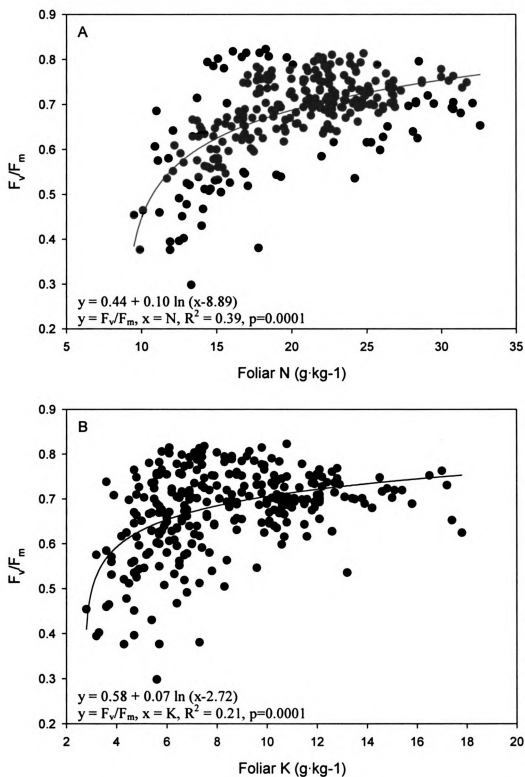
Figure 4. Relationships between photosynthetic efficiency (F_v/F_m) and A) N and B) K in 17 *Abies* species grown at four locations in Michigan in 2004.
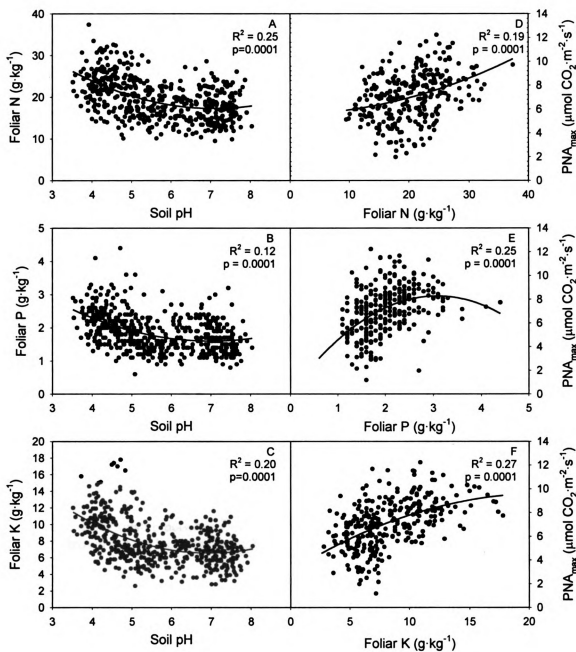
Figure 5. Relationships between A) N, C) P, E) K, and soil pH and PNA_{max} and B) N, D) P, and F) K in 17 *Abies* species grown at four locations in Michigan in 2004.
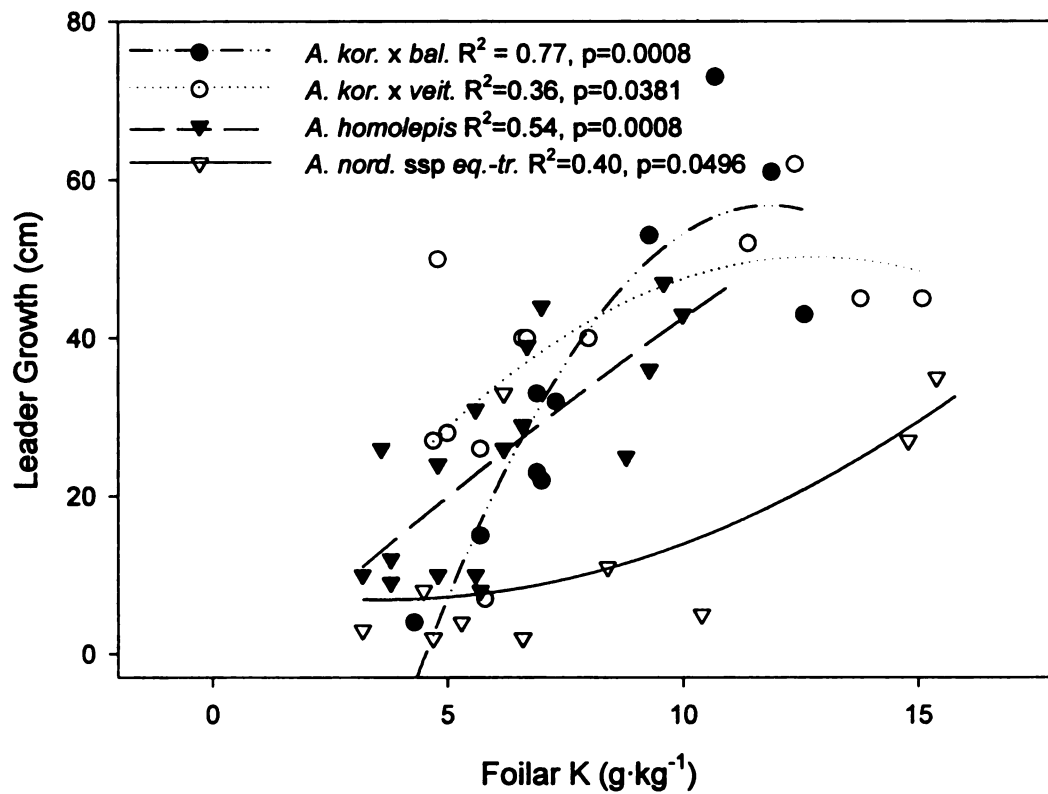
Figure 6. Relationship between leader growth and foliar K concentration in four *Abies* species grown at four locations in Michigan in 2004.

Literature Cited

Adams, G.T. and T.D. Perkins. 1993. Assessing cold tolerance in *Picea* using chlorophyll fluorescence. Environ. Exp. Bot. 33(3): 377-382.

Albaugh, T.J., H.L. Allen, P.M. Dougherty, and K.H. Johnsen. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. For. Ecol. Mgt. 192: 3-19.

Albaugh, T.J., H.L. Allen, P.M. Dougherty, L.W. Kress, and J.S. King. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. For. Sci. 44(2): 317-328.

Alexander, R.R., R.C. Shearer, and W.D. Shepperd. 1990. Subalpine fir. In: R.M. Burns and B.H. Honkala (tech. cords.). Silvics of North America: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

American Standards for Nursery Stock. 2004. Amer. Nurs. Land. Asso. Washington, DC.

Bakuzis, E.V. and H.L. Hansen. 1965. Balsam fir-a monographic review. University of Minnesota Press, Minneapolis. 445p.

Björkman, O. and B. Demming. 1987. Photo yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta. 170: 489-504.

Brown, J.H. 2000. Survival and growth of trees of a Canaan Valley, West Virginia, seed source in relation to varying soil/site conditions. Ohio State Univ. Ext. Spec. Circ. 175-00.

Bryan, J.A., J.R. Seiler, and R.D. Wright. 1989. Influence of growth medium pH on the growth of container-grown fraser fir seedlings. J. Environ. Hort. 7(2) 62-64.

Chandler, J.W. and J.E. Dale. 1995. Nitrogen deficiency and fertilization effects on needle growth and photosynthesis in Sitka spruce (*Picea sitchensis*). Tree Physiol. 15: 813-817.

Cregg, B.M., M.W. Duck, C.M., Rios, D.B. Rowe, and M.R. Koelling. 2004. Chlorophyll fluorescence and needle chlorophyll concentration of fir (*Abies* sp.) seedlings in response to pH. Hort Sci. 39(5): 1121-1125.

Czapowskyj M.M., L.O. Safford, and R.D. Briggs. 1980. Young nutrient status of young red spruce and balsam fir in a fertilized stand. USDA For. Ser. Res. Paper NE-467.

Farjon, A. 1990. Pinaceae drawings and descriptions of the genera *Abies, Cedrus, Psudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix,* and *Picea*. Champaign, IL.

Fletcher, R., C. Landgren, S. Webster, and M. Bondi. 1998. Fertilizing noble fir. Christmas Tree Lookout. 31(1): 21-13, 26-28.

Frank, R.M. 1990. Balsam fir. In: R.M. Burns and B.H. Honkala (tech. cords.). Silvics of North America: 1. Conifers. USDA For. Serv., Agr. Hdbk. 654.

Hansatech Instruments Ltd. 1997. Operating instructions for Plant Efficiency Analyzer (PEA) advanced fluorescence analysis. Hansatech Instruments Ltd., Norfolk, U.K.

Hawkins, B.J., G. Henry, and S.B.R. Kiiskila. 1998. Biomass and nutrient allocation in Douglas-fir and amabilis fir seedlings: influence of growth rate and nutrition. Tree. Physiol. 18: 803-810.

Hiltbrunner, E. and W. Flückiger. 1996. Manganese deficiency of silver fir trees (*Abies alba*) at a reforested site in the Jura mountains, Switzerland: aspects of cause and effect. Tree Physiol. 16:963-975.

Hinesly, L.E., L.K. Snelling, C.R. Campbell, D.K. Roten, and J. Hartzog. 2000. Nitrogen increases fresh weight and retail value of fraser fir Christmas trees. HortScience. 35(3): 860-862.

Hinesley, L.E. and R.D. Wright. 1989. Biomass and nutrient accumulation in Fraser-fir Christmas trees. HortScience. 24(2): 280-282.

Igawa, M., H. Kameda, F. Maruyama, H. Okochi, and I. Otsuka. 1997. Effect of simulated acid fog on needles of fir seedlings. Environ. Exp. Bot. 38: 155-163.

Illinois Department of Natural Resources. 2005. Midwest Regional Climate Center. Champaign, IL. 20 June 2005. http://sisyphus.sws.uiuc.edu/index.html.

Kaakinen, S., A. Jolkkonen, S. Iivonen, and E. Vapaavuori. 2004. Growth, allocation and tissue chemistry of *Picea abies* seedlings affected by nutrient supply during the second growing season. Tree Physiol. 24: 707-719.

Klaehn, F.U. and J.A. Winieski. 1962. Interspecific hybridization in the genus Abies. Silvae Genet. 11: 130-140.

Khalil, N., C. Leyval, M. Bonneau, and B. Guillet. 1989. Influence du type de nutrition azotée sur le déclenchement de la chlorose du sapin de Nordmann (*Abies nordmanniana,* Spach, 1842) (Summary in English). Ann. Sci. For. 46: 325-343.

Laing, W., D. Greer, O. Sun, P. Beets, A. Lowe, and T. Payn. 2000. Physiological impacts of Mg deficiency in *Pinus radiata*: growth and photosynthesis. New Phytol. 146: 47-57.

Langlet, O. 1963. Patterns and terms of intra-specific ecological variability. Nature. 200: 347-348.

Liu, T.S. 1971. A monograph of the genus *Abies*. Taipei, Taiwan.

Loustau, D., M. Ben Brahim, J.P. Gaudillère, and E. Dreyer. 1999. Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. Tree Physiol. 19: 707-715.

Lucas, R.E. and J.F. Davis. 1961. Relationships between pH values of organic soils and availabilities of 12 Plant Nutrients. Soil Sci. 92:177-182.

Mandre, M., J. Klõšeiko, K. Ots, and L. Tuulmets. 1999. Changes in phytomass and nutrient partitioning in young conifers in extreme alkaline growth conditions. Environ. Poll. 105: 209-220.

Nakatani, N., A. Kume, T. Kobayashi, T. Hirakawa, and H. Sakugawa. 2004. Needle morphology related to chemical contents in the needles of Japanese Fir (Abies firma) trees subjected to acidic depositions at Mt. Oyama, Eastern Japan. Water Air Soil Poll. 152: 97-110.

Rothstein, D.E. 2005. Nitrogen management in a fraser fir (Abies fraser [Pursh] Poir.) Christmas tree plantation: effects of fertilization on tree performance and nitrogen leaching. For. Sci. 51(2): 175-184.

Rothstein, D.E. and N. Lisuzzo. 2003. Optimal nutrition of fraser fir Christmas trees in Michigan, p. 19-21. In: D. Brown-Rytlewski and J. O'Donnell (eds.). Nursery, Landscape and Christmas Tree Research Projects and Educational Programs. Mich. State Univ. Ext., East Lansing, MI.

Schoettle, A. 1994. Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. Tree Physiol. 14: 1055-1068.

Sun, O.J., G.J.H.P. Gielen, R. Sands, C.T. Smith, and A.J. Thorn. 2001. Growth, Mg nutrition and photosynthetic activity in *Pins radiata*: evidence that NaCl addition counteracts the impact of low Mg supply. Trees: Structure and Function. 15: 335-340.

Sun, O.J. and T.W. Payn. 1999. Magnesium nutrition and photosynthesis in *Pinus radiata*: clonal variation and influence of potassium. Tree Physiol. 19: 535-540.

Tan, W. and G.D. Hogan. 1995. Limitations to net photosynthesis as affected by nitrogen status in jack pine (*Pinus banksiana* Lamb.) seedlings. J. Exp. Bot. 46: 407-413.

Timmer, V.R., E.L. Stone, and D.G. Embree. 1977. Growth response of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Can. J. For. Res. 7: 441-446.

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

Toivonen, P. and W. Vidaver. 1988. Variable chlorophyll a fluorescence and CO_2 uptake in water-stressed white spruce seedlings. Plant Physiol. 86: 744-748.

USDA Plant Hardiness Zone Map. 1990. USDA Misc. Publ. 1475. Washington D.C.

CHAPTER THREE

NEEDLE MORPHOLOGY, SHOOT ARCHITECTURE, AND NET PHOTOSYNTHETIC RESPONSE IN *ABIES* SPECIES.

List of Abbreviations

Abbreviation	Name	Unit of Measurement
A_{max}	Net Photosynthesis	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
g_w	Needle Conductance	$mol\ H_2O \cdot m^{-2} \cdot s^{-1}$
WUE	Water Use Efficiency	$mol\ CO_2 \cdot mol\ H_2O^{-1}$
Ci	Intercellular CO2	$\mu mol\ CO_2$
Δ	Carbon Isotope Discrimination	per mil
PSA	Projected Shoot Area	cm^2
PNA	Projected Needle Area	cm^2
TNA	Total Needle Area	cm^2
PSA_{max}	Net Photosynthesis - Projected Shoot Area	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
PNA_{max}	Net Photosynthesis - Projected Needle Area	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
TNA_{max}	Net Photosynthesis - Total Needle Area	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
CE	Carboxylation Efficiency	$\mu mol\ m^{-2} \cdot s^{-1}$
R	Respiration	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
Q	Incoming Radiation	$mol \cdot s^{-1}$
ϕ	Quantum Efficiency	$\mu mol\ CO_2 \cdot \mu mol^{-1}$
Rd	Dark Respiration	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$
k	Convexity Coefficient	Unitless
STAR	Shoot Silhouette Area : Total Needle Area	Unitless
PNA/TNA	Projected Needle Area : Total Needle Area	Unitless
PSA/PNA	Projected Shoot Area : Projected Needle Area	Unitless
$\delta^{13}C$	Stable Isotope Ratio	Unitless

NEEDLE MORPHOLOGY, SHOOT ARCHITECTURE, AND NET PHOTOSYNTHETIC RESPONSE IN *ABIES* SPECIES.

Addition index words: Net photosynthesis, carbon isotope discrimination, needle morphology, shoot architecture, water use efficiency, photosynthetic light response curves, A/C$_i$ curves, *Abies*.

Abstract

We measured gas exchange, carbon isotope discrimination (Δ), needle morphology, shoot architecture, photosynthetic light response curves, and A/C$_i$ curves in 17 Abies species and hybrids grown at four locations in Michigan. Net photosynthesis (PSA$_{max}$, PNA$_{max}$, and TNA$_{max}$) varied among species and taxonomic subsections. Carbon isotope discrimination (Δ) differed among species and subsections, was negatively correlated with instantaneous water use efficiency, and was positively correlated with PSA$_{max}$, PNA$_{max}$, and TNA$_{max}$. Needle morphology and shoot architecture differed among species and subsections and was correlated with Δ. Photosynthetic light response and A/C$_i$ curves indicate main species differences were in net photosynthesis and not apparent quantum efficiency, apparent carboxylation efficiency, or dark respiration. Foliar P and K were strongly correlated with increased PSA$_{max}$, PNA$_{max}$, and TNA$_{max}$ from photosynthetic light response and A/C$_i$ curves.

Introduction

Plant production is dependent on the energy produced during photosynthesis (Körner, 1991). Site conditions and morphological and physiological characteristics impact net photosynthesis (A$_{max}$) rates in conifers. Improved nutrition increased A$_{max}$ rates and improved Rubisco capacity in *Picea mariana* Mill. B.S.P. (Paquin et al., 2000). Atmospheric and soil drought conditions reduce A$_{max}$ although species respond

differently (Cregg, 1994; Silim et al., 2001; Cregg and Zhang, 2001). Increased light intensity affects dark respiration, quantum efficiency, and the light compensation point in *A. alba* Mill. (Grassi and Bagnaresi, 2001). In sun shoots, A_{max} rates were higher than shade shoots in *A. amabilis* Dougl. ex Forb. (Brooks et al., 1994).

Photosynthetic gas exchange characteristics vary among conifer species. Net photosynthesis is closely related to stomatal conductance (g_w) (Teskey et al., 1986; Cregg, 1994; Monson and Grant, 1989). Genotypes from dry climates frequently have fewer stomata than genotypes from climates with more precipitation (Knauf and Bilan, 1974: Cregg et al., 2000). Stomatal conductance decreases as water vapor pressure deficits increase (Guehl et al., 1991). Stomatal conductance and transpiration rate are closely related when compared to xylem potentials, but increased vapor pressure deficits can cause transpiration to increase while stomatal conductance decreases (Teskey et al., 1986). The relationship between A_{max} and g_w is frequently used to describe the water use efficiency (WUE) between genotypes or environmental treatments (Silim et al., 2001; Cregg and Zhang, 2001).

Stomata conductance influences the internal CO_2 (C_i) levels inside the leaf (Farquahr and Sharkey, 1982). Air contains three carbon isotopes, of which ^{12}C and ^{13}C represent 98.9% and 1.1% of atmospheric carbon respectively. C_3 plants discriminate against ^{13}C, however, lower C_i levels reduce the plants ability to discriminate against isotopes (Farquhar et al., 1989). Increased WUE is frequently correlated with reduced carbon isotope discrimination (Δ) and used to quantify drought adaptation (Farquhar et al., 1989; Masle and Farquhar, 1988; Farquhar and Richards, 1984).

Periods of decreased g_w from drought or pollutants are often correlated with decreased Δ (Guehl et al., 1991; Sakata and Suzuki, 2000). In *Pinus sylvestris* L., genotypes from drier regions had less discrimination than genotypes from wetter regions (Cregg and Zhang, 2001). Reduced Δ resulted in decreased A_{max} and dry matter accumulation in two *Picea* species (Silim et al., 2001).

Conifers respond to drought conditions in several ways. Shoot internode number and length were reduced under drought conditions in *A. magnifica* A. Murr. and *A. concolor* (Gord. and Glend.) Lindl. (Hallgren and Helms, 1988). In Switzerland, *A. alba* shed needles in response to drought conditions (Webster et al., 1996). To avoid drought conditions species such as *A. cephalonica* Loud. have optimized stomatal regulation of gas exchange while species like *A. bornmulleriana* Mattf. avoid internal water stress (Guehl et al., 1991).

Needle morphology influences many physiological processes in conifers. Increased needle packing or needles per unit of shoot area can increase boundary layer effects and shoot temperatures (Martin et al., 1999 and Smith and Carter, 1988). More commonly, the relationship between needle morphology and light harvesting is investigated. Sun exposed shoots in *A. lasiocarpa* were more vertically oriented than shade shoots (Germino and Smith, 1999). In *A. mariesii* Mast. and *A. veitchii*, needle packing (needles·cm^{-1} of shoot) was less dense in shoots from shade seedlings than from sun seedlings (Mori and Takeda, 2004). This suggests acclimation to reduce self-shading occurs in sun shoots and to maximize light capture in shade shoots. Needle morphology characteristics in *Pinus monticola* (Dougl.) D. Don seedlings from the same genetic background varied when grown at different locations suggesting environmental factors

such as soil pH, precipitation, and temperature can affect needle morphology (Woo et al., 2002). Needle growth was reduced in trees growing on nutrient-poor sites (Ishii et al., 2003).

Needle area of single needle conifers is frequently expressed as projected needle area (PNA), shoot silhouette area (SSA) or total needle area (TNA). Photosynthetic rates can vary by species depending upon the method used to express needle area (Ishii et al., 2003). PNA tends to over-estimate light interception by needles while SSA accounts for self-shading with-in shoots (Smith et al., 1991).

Ratios such as STAR (the SSA to TNA ratio) and TNA/PNA are used to investigate shoot architecture (Stenberg et al., 1999: Ishii et al., 2003) and vary among *Abies* species (Figure 1). Ratios such as TNA/PNA are related to cross-sectional needle measurements such as needle thickness (Sellin, 2000). Shade shoots have increased STAR values (Stenberg, 1996). However, STAR values had little impact on photosynthetic rates in *A. balsamea* (Bernier et al., 2001).

Photosynthetic response to varying light intensities and CO_2 concentrations provides insight to physiological limitations and adaptations in conifers. Light saturation was lower in *A. balsamea* shade seedlings, while dark respiration, quantum efficiency, and the light compensation point increased with light intensity in *A. alba* Mill. (Landhäusser and Lieffers, 2001; Grassi and Bagnaresi, 2001). In *A. amabilis*, A_{max} was higher in sun shoots than shade shoots (Brooks et al., 1994). A/C_i curves provide good ways to study stomatal limitations to photosynthesis (Farquahr and Sharkey, 1982). For example, in *Picea abies*, A/C_i curves show apparent carboxylation efficiency (CE) and

light respiration were higher in sun shoots than in shade shoots (Sprtová and Marek, 1999).

The genus *Abies* Mill. (true firs) contains 46 species (Farjon, 1990). However, research has been generally limited to a few species. Recent efforts to increase conifer diversity in the upper Midwest have created interest in additional *Abies* species. The following paper contains two parts. In experiment one we characterize gas exchange, needle morphology, shoot architecture, and carbon isotope discrimination in 17 *Abies* species and hybrids. In experiment two we characterize photosynthetic light response curves and A/Ci curves for a subset of species used in experiment one.

Materials and Methods

Site Locations

Over 1100 trees representing 38 species, hybrids, provenances, and varieties of *Abies* were part of a true fir species and provenance trial initiated at the Kellogg Research Forest (KRF), Augusta, Michigan, in early 1991. In the fall of 2002 and spring of 2003, 246 trees representing 21 species and hybrids and seven taxonomic sections or subsections (Table 1) were transplanted to three locations in Michigan: Clarksville Horticulture Experiment Station, Clarksville, MI (CHES); Horticulture Teaching and Research Center, East Lansing, MI (HTRC); and Northwest Michigan Horticulture Research Station, Traverse City, MI (NWMHRS) (Figure 2). These three locations along with the KRF represent different climate regions (Table 2) and soil environments (Table 3). Trees were dug and ball and burlapped as 60 to 76 cm root balls in accordance with American nursery standards (American Nursery and Landscape Association, 2004)

In the fall of 2003, 57 additional trees were transplanted from the KRF to replace 37 trees that died due to transplant stress during the previous year and add three new species and varieties to the study. At least four trees of each species or hybrid were planted at each location with the exception of the HTRC where one to four more trees of each species were planted when available. Trees were planted in a complete randomized design at each location. Trees were planted at approximately 4.5 m intervals.

Fertilization

In spring 2004, sites were fertilized with 21-0-0 ammonium sulfate at a rate of approximately 133 g per tree, to lower soil pH by 0.5 and insure that nitrogen was not limiting. Fertilizer was applied at CHES on 29 April, HTRC on 22 April, and NWHRS on 11 May. On 7 July 2004, 46-0-0 urea was applied at CHES at a rate of 91 g per tree. Trees at KRF were fertilized every fall with 21-0-0 at 85 g per tree. Trees exceeding 0.9 m received 28 g of fertilizer for each additional 0.3 m in height.

<u>Experiment One</u>

Needle Morphology and Shoot Architecture

Three needles were collected at random from sun-exposed shoots in the upper one-third of the tree canopy twice during the growing season. Shoots were sampled at CHES on 29 June and 30 August 2004, HTRC on 23 June and 31 August 2004, KRF on 1 July and 1 September 2004, and NWHRS on 15 July and 3 September 2004. Needle length was measured using a digital caliper (Mitutoyo Corp., Aurora, IL). Free-hand cross-sections were cut from the center of each needle and photographed using a digital camera (Micropublisher 3.3, QImaging, Burnaby, BC) and software (Qcapture Suite, QImaging) under a lab microscope at 30x magnification and saved as a TIF image.

Images were hand traced using a pen tablet (Graphire3, Wacom Technology, Vancouver, WA) and cross-sectional area, needle width, thickness, perimeter, and shape factor (Figure 3) were measured with image analysis software (SigmaScan Pro 5.0, SPSS Inc., Chicago). Shape factor is a determination of the roundness of a needle cross-section where a value of 1 is a circle and 0 is a flat line. The shape factor was calculated as:

$$\text{Shape factor} = \frac{4\pi \times area}{perimeter^2} \text{ (Sigma Scan Pro 5.0).}$$

Shoot architecture was examined for all trees. The ratio of shoot silhouette area to total needle area (STAR) was calculated as PSA/TNA. The ratio of TNA to PNA was also calculated.

Gas Exchange

Gas exchange was measured on current year growth using a portable IRGA (LI-6400, Li-Cor, Lincoln, NE) fitted with a conifer chamber (LI-6400-05, Li-Cor). Sun-exposed shoots were measured in the upper one-third of the tree canopy on sunny days with PPFD greater than 1500 μmol m^{-2} s^{-1}. Block temperature of the conifer chamber was maintained near 25 °C. The flow of air into the chamber was 500 μmol s^{-1}. The reference CO_2 concentration was slightly above ambient at 400 μmol CO_2 mol^{-1}.

Shoots were sampled at CHES on 29 July 2003 and 29 June, 6 August, and 30 August 2004, HTRC on 25 July 2003 and 23 June, 28 July, and 2 September 2004, KRF on 1 July, 1 August, and 10 September 2004, and NWHRS on 30 July 2003 and 15 July, 5 August, and 4 September 2004. Shoots were harvested immediately after gas exchange measurements and transported in a cooler with ice and then stored at 2.5 °C until needle area was measured. Needle area was determined in three ways: 1) projected shoot area (PSA), 2) projected needle area (PNA), and 3) total needle area (TNA). To determine

PSA, samples were passed through a leaf area meter (LI-3000, Li-Cor). For determining PNA, all the needles on each shoot were removed and scanned at 200 dpi using an Epson Perfection 2450 scanner (Seiko Epson Corp., Long Beach, CA). Images were analyzed using WinSeedle (Regent Instruments, Inc., Quebec, Canada) to determine PNA. TNA was determined by multiplying the projected needle area by the average perimeter:needle width ratio of three needle cross-sections calculated using SigmaScan (SPSS Inc.). Maximum photosynthetic rates are expressed in terms of projected shoot area (PSA_{max}), projected needle area (PNA_{max}), and total needle area (TNA_{max}) (Figure 4).

Soil Moisture

Volumetric soil water content was measured using a portable TDR unit (Trase I, Soil Moisture Equipment Corp., Santa Barbara, CA) on the days gas exchange was measured. Due to an equipment problem, volumetric soil moisture was not measured during gas exchange measurements in late August/ early September 2004.

Water Use efficiency

Intrinsic water use efficiency (WUE) was calculated from gas exchange data. WUE was expressed as the ratio of photosynthesis (A_{max}) to needle conductance (g). Ratios were calculated from gas exchange measurements collected on the dates listed in the previous section.

Carbon Isotope Discrimination

Carbon isotope discrimination was measured in shoots from current year growth from select species at all four locations. Species were selected to represent a range of shoot morphologies, geographic ranges, and all species hybrids. Species sampled include: *A. balsamea, A. lasiocarpa, A. fraseri, A. fraseri* x *homolepis, A. koreana, A.*

koreana x *balsamea*, *A. koreana* x *veitchii*, *A. holophylla*, *A. nephrolepis*, and *A. veitchii*.

Shoots were harvested on the following days: CHES on 30 August 2004, HTRC on 2 September 2004, KRF on 1 September 2004, and NWHRS on 4 September 2004. Samples were oven-dried at 60 °C for four days. Needles were stripped from branches, ground in a coffee grinder, and sifted through a #40 sieve. Between 2-3 mg of each sample were weighed into 5x9 mm tin capsules and placed in a plastic, 96-well sample tray. Relative abundance of ^{13}C and ^{12}C was determined with a gas phase isotope ratio mass spectrometer at the Center for Stable Isotope Biogeochemistry, University of California at Berkeley. The stable carbon isotope ratio ($\delta^{13}C$) was expressed as the $\dfrac{^{13}C}{^{12}C}$ ratio relative to PeeDee Belemnite (limestone) (Craig, 1957). The resulting $\delta^{13}C$ values were used to estimate isotope discrimination (Δ) as

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}$$

where δ_p is the isotopic composition of the plant material and δ_a is that of the air (assumed to be -8‰, Farquhar et al., 1989).

Experiment Two

Photosynthetic Light Response and A/C$_i$ Curves

Photosynthetic light response and A/C$_i$ curves were measured in five *Abies* species and one hybrid: *A. balsamea*, *A. bifolia*, *A. holophylla*, *A. koreana*, *A. koreana* x *balsamea*, and *A. nephrolepis*. A/C$_i$ curves were measured on the following days: CHES on 20, 21, and 22 September 2004, HTRC on 7, 8, and 9 September 2004, KRF on 18, 19, and 21 September 2004, and NWHRS on 4 and 5 September 2004. Light response was measured on the following days: CHES on 20, 21, and 22 September 2004, HTRC

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on 13, 14, and 15 September 2004, KRF on 17, 18, and 19 September 2004, and NWHRS

on 11 and 12 September 2004. Trees were blocked by species to reduce time of day

effects. Shoots were selected at random from sun-exposed shoots in the upper one-third

of the tree canopy to be measured. Shoots were illuminated with two halogen light bulbs

(Philips Halotone 50MR16/SP10, Phillips Electronics, New York, NY) on each side of

the conifer chamber. Lights were powered by a 12V deep cycle marine battery. While

measuring A/C_i curves, lights were repositioned to maintain PPFD near 2000 µmol CO_2

$\cdot m^{-2} \cdot s^{-1}$ and monitored while measuring each tree.

Gas exchange was measured at increasing CO_2 values of 0, 50, 125, 250, 400,

600, 900, and 1200 µmol $CO_2 \cdot mol^{-1}$ by manipulating the CO_2 concentration flowing into

the conifer chamber. Needle area was expressed as projected needle area and response

curves were fitted to the follow equation

$$A = \frac{(CE * C_i * A_{max})}{(CE * C_i + A_{max})} - R$$

using Photosyn Assistant software (Dundee Scientific, Dundee, Scotland, UK) where A is

needle photosynthesis, CE is the apparent carboxylation efficiency, C_i is intercellular

CO_2, R is respiration, and A_{max} is photosynthesis as the saturation of CO_2.

Light response was measured at decreasing light intensities. Incoming light was

filtered using a combination of mesh screens, black tinted plexiglass, and black plastic to

reach pre-determined light intensities of 2000, 1500, 1000, 800, 600, 400, 250, 150, 100,

50, 20, and 0 µmol·m^{-2}·s^{-1}. Needle area was expressed as project needle area and

response curves were fitted to the following equation

$$A = \frac{\phi Q + A_{max} - \sqrt{(\phi Q + A_{max})^2 - 4\phi Q k A_{max}}}{2k} - R_d$$

using Photosyn Assistant (Dundee Scientific) where Q is the light level, ϕ is the apparent quantum efficiency, A_{max} is light saturated maximum photosynthesis, k is the convexity, R_d is dark respiration, and A is shoot photosynthesis (Prioul and Chartier, 1977). After determining light response values, A_{max} was estimated for each tree by calculating the light response equation at light intensities of 2000 μmol m^{-2} s^{-1}.

Statistical Analysis

Species effects for needle morphology, shoot architecture, gas exchange, and carbon isotope discrimination were determined using PROC MIXED (SAS Inc., Cary, NC). When significant differences were indicated, means were separated using Tukey's Studentized test (Saxton, 1998). Correlation among needle morphology, gas exchange, shoot architecture ratios, and carbon isotope discrimination were identified using PROC CORR and linear regression was analyzed using PROC REG (SAS Inc.).

Results

Experiment One

Net Photosynthesis

When all species were combined, mean A_{max} differed among methods used to express needle area (p$\leq$0.0001). Rates were 10.97, 7.25, and 3.06 μmol CO_2·m^{-2}·s^{-1} for PSA$_{max}$, PNA$_{max}$, and TNA$_{max}$ respectively. Photosynthetic rates varied widely among species and locations. Volumetric soil moisture content was lowest at HTRC and NWHRS (Table 4). Photosynthesis expressed using projected shoot area (PSA$_{max}$) varied by species and location during all dates (p$\leq$0.0001). However, location × species interactions were significant (p$\leq$0.03) during measurements in late July and early September 2004. When PSA$_{max}$ was compared among taxonomic sections and sub-

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sections, location and subsection were significant (p≤0.01), while location × subsection effects were not (Table 5). In late July, PSA_{max} was highest in *A. balsamea* and *A. balsamea* var. *phanerolepis* at KRF, but among the lowest of all species at the other three locations. Net photosynthesis (PSA_{max}) was consistently low in *A. veitchii* across all locations and times. Net photosynthesis (PSA_{max}) was generally greatest in subsection *Laterales* and section *Nobilis* and lowest in subsections *Homolepides* and *Medianae* in 2004. In 2003, PSA_{max} was significantly (p≤0.0001) lower at HTRC than at CHES or HTRC. While PSA_{max} varied among locations during all measurements, location differences were less pronounced during later experiments.

Net photosynthesis expressed using projected needle area (PNA_{max}) varied by location and species during all experiments (p≤0.0001) (Table 6). However, species × location interaction was significant (p≤0.001) in late July 2004. Again, PNA_{max} was highest in *A. balsamea* and *A. balsamea* var. *phanerolepis* at KRF in late July, while they were among the lowest at all other locations. Net photosynthesis (PNA_{max}) was higher in *A. nordmanniana* ssp. *equi-trojani* and lower in *A. nordmanniana* compared to other species at KRF in June 2004 than found at other locations. When PNA_{max} was considered by taxonomic sections and sub-sections, PNA_{max} differed among location and subsection (p≤0.01), however, a location × subsection effect was significant (p≤0.05) in late August and early September 2004 (Table 7). Net photosynthesis (PNA_{max}) was generally greatest in the section *Nobilis* and lowest in subsection *Homolepides* in 2004. Throughout 2004, PNA_{max} increased progressively with additional measurements at all locations with the exception of the NWHRS in late July. The greatest increase occurred at CHES between late July and early September 2004.

Both PNA_{max} and TNA_{max} were strongly correlated (Pearson's r = 0.99, p≤0.0001) and species and location trends were similar throughout all experiments (Table 8). When TNA_{max} was considered by taxonomic sections and sub-sections, TNA_{max} differed among location and subsection (p≤0.01) in June 2004 but only differed among locations in late August and early September 2004 (Table 9). Net photosynthesis (TNAmax) was greatest in subsections *Laterales* and *Holophyllae* and lowest in subsections *Homolepides* and *Medianae*.

Water Use Efficiency and Carbon Isotope Discrimination

Water use efficiency (WUE) differed (p≤0.01) among locations during all measurements. However, WUE did not vary by species, except for early September 2004 (p≤0.01), or subsection. In June 2004, WUE was greatest at HTRC. In *A. procera*, WUE was lower among all species at HTRC than at other locations during late July and early September 2004. Carbon isotope discrimination (Δ) varied among locations (p≤0.0001), species (p≤0.01) (Table 10), and subsection (p≤0.05) (Table 11). However, location × species interaction (p≤0.01) was significant. Mean Δ was greatest at KRF and least at HTRC and NWHRS. At HTRC, Δ in *A. balsamea* was less compared to other species than among other locations. Generally Δ was greatest in the hybrid crosses, although some variation by location occurred. Both WUE and Δ were closely related (R^2 = 0.19, p≤0.0001) (Figure 5).

Needle Morphology

Needle cross-sectional area, width, thickness, perimeter, and shape factor varied among species and location (p≤0.01) during experiments in late June and early September 2004 (Table 12). However, species × location effects were significant for

needle thickness (p≤0.04) in September and shape factor (p≤0.01) during both June and September. Needle morphology parameters varied among taxonomic section and subsection classification (p≤0.001) in both late June and early September 2004 (Table 13). Cross-sectional area, needle width, and perimeter were generally the lowest at NWHRS. All needle morphology parameters increased during the growing season except needle width and perimeter. The increases were generally the greatest at CHES.

Shape factor, needle thickness, and cross-sectional area were correlated with PSA_{max}, PNA_{max}, and TNA_{max} (p≤0.0001) but not needle width. Increasing needle thickness was related to higher PSA_{max} (R^2 = 0.11, p≤0.0001), PNA_{max} (R^2 = 0.23, p≤0.0001), and TNA_{max} (R^2 = 0.21, p≤0.0001) (Figure 6). When species were combined, cross-sectional area, major diameter, minor diameter, and perimeter were strongly correlated. Shape factor was the only needle morphology parameter correlated with Δ (Pearson's r = -0.19, p≤0.02).

Shoot Architecture

Shoot architecture ratios (PSA/TNA, TNA/PNA, and PSA/PNA) differed among species and locations (p≤0.0001) (Table 14). However, species × location interactions were significant for PSA/TNA and TNA/PNA (p≤0.05) and for PNA/PSA (p≤0.0001) in early September. Shoot architecture ratios differed among taxonomic sections and subsections and locations (p≤0.05) in June and September 2004 (Table 15). Throughout the growing season PSA/TNA and TNA/PNA increased, while PNA/PSA remained constant. Both TNA/PNA and PSA/PNA were lowest at KRF. Carbon isotope discrimination (Δ) was negatively correlated with PNA/PSA (Pearson's r = -0.28, p≤0.001). However, Δ increased with PSA/TNA (R^2 = 0.10, p≤0.0001) (Figure 7).

Experiment Two

Photosynthetic Light Response

Location and species affected photosynthetic light response parameters. Dark respiration, PSA_{max}, and the LCP differed ($p \leq 0.01$) among species and location when PSA was used to express photosynthetic light response curves (Table 16). When TNA was used to express photosynthetic light response curves, TNA_{max} and LCP varied among species and location, while Rd and ϕ did not (Table 17) (Figure 8). When curves were expressed using projected needle area, Rd, PNA_{max}, and the LCP varied among locations ($p \leq 0.0001$) (Table 18).

When using all three methods of expressing needle area, Rd was greatest at NWHRS and lowest at KRF. However, LCP was lowest at KRF and highest at NWHRS. Midday xylem potential during light response measurements was lowest at the NWHRS (-1.3 MPa) and highest at KRF (-0.9 MPa). Apparent ϕ did not vary by location or species.

Light response measurements indicate foliar P was correlated with PSA_{max} (Pearson's r = 0.38, $p \leq 0.0001$), PNA_{max} (Pearson's r = 0.48, $p \leq 0.0001$), and TNA_{max} (Pearson's r = 0.43, $p \leq 0.0001$). Similar results existed for foliar K and net photosynthesis expressed using all three methods of expressing needle area. Carbon isotope discrimination (Δ) was correlated with PSA_{max} (Pearson's r = 0.52, $p \leq 0.0001$), PNA_{max} (Pearson's r = 0.58, $p \leq 0.0001$), and TNA_{max} (Pearson's r = 0.59, $p \leq 0.0001$). Dark respiration was negatively correlated with Mg and soil pH and positively correlated with Mn using all three methods of expressing needle area for photosynthetic light response curves.

When using PNA to generate curves, species differed in PNA_{max} ($p \leq 0.0001$) and light compensation point ($p \leq 0.05$). The LCP was lowest in *A. nephrolepis* and highest in *A. balsamea*. *Abies koreana* x *balsamea* had a lower LCP than either of its parents, however, it was more closely related to *A. koreana*. Dark respiration, PNA_{max}, and light saturation were the highest at KRF, however, the light compensation point was also the lowest. *Abies lasiocarpa* had the highest PNA_{max} and *A. balsamea* and *A. holophylla* had the lowest.

A/C$_i$ Curves

In A/C$_i$ experiments, apparent CE and A_{max} differed among species and location ($p \leq 0.05$) for all three methods of expressing needle area, while respiration differed among species only when using PSA (Figure 9). In A/C$_i$ curves expressed using projected shoot area, R, apparent CE, and PSA_{max} differed ($p \leq 0.05$) among species, although, a locations × species effect was significant for PSA_{max} (Table 19). When PNA was used to express curves, apparent CE and PNA_{max} differed ($p \leq 0.05$) among species, while respiration did not. Respiration was negatively correlated with apparent CE (Pearson's r = -0.52, $p \leq 0.0001$) (Table 20). Apparent CE was lowest in *A. holophylla* when all three methods of expressing needle are were used. When PNA was used to generate curves, apparent CE increased with PNA/PSA ($R^2 = 0.22$, $p \leq 0.0001$). In A/C$_i$ curves expressed using TNA, respiration, apparent CE, and TNA_{max} did not differ among species, although location effects were significant for apparent CE and TNAmax ($p \leq 0.01$) (Table 21).

When all three methods of expressing needle area were used to generate A/C$_i$ curves, apparent CE was negatively correlated with foliar N ($p \leq 0.05$). Foliar P was

correlated with PSA_{max} (Pearson's r = 0.23, p≤0.05), PNA_{max} (Pearson's r = 0.26, p≤0.01), and TNA_{max} (Pearson's r = 0.20, p≤0.05). Similar results occurred between foliar K and PSA_{max} (Pearson's r = 0.24, p≤0.05), PNA_{max} (Pearson's r = 0.32, p≤0.01), and TNA_{max} (Pearson's r = 0.29, p≤0.05). Carbon isotope discrimination (Δ) was strongly correlated with PSAmax (Pearson's r = 0.28, p≤0.01), PNAmax (Pearson's r = 0.31, p≤0.01), and TNA_{max} (Pearson's r = 0.35, p≤0.01).

Discussion

Multiple nutrient limitations and soil moisture deficits led to lower PSA_{max} at the HTRC in 2003 (refer to chapter two). Although PSA_{max}, PNA_{max}, and TNA_{max} increased progressively during the growing season at all locations, the greatest increases were at CHES and resulted from additional N application in July. Nitrogen application increased photosynthesis, and gas exchange measurements, and chlorophyll content in several tree species (Chandler and Dale, 1995; Warren et al., 2004). Transplant effects in *A. balsamea* and *A. balsamea* var. *phanerolepis* may have contributed to species × location variation. Trees were transplanted from KRF in 2003 and A_{max} was higher at KRF than other locations when compared among all species.

Net photosynthesis was strongly related to taxonomic relationships. Both *Laterales* and *Medianae* are two subsections of the section *Balsaemea* (Farjon, 1990), yet PSA_{max}, PNA_{max}, and TNA_{max} differed between these subsections. Grouping species by section and subsection also reduced the frequency of significant location × species interaction.

Water use efficiency was greatest at locations with sandy soils suggesting soil moisture was an influencing factor. Needle stomatal conductance declines before A_{max} or

as water becomes limiting, resulting in increased WUE (Cregg, 1994). In our study, WUE was closely related to Δ, and consistent with previous findings (Arndt et al., 2001; Cregg and Zhang, 2001; Farquhar and Richards, 1984; Silim et al., 2001). In hybrid crosses, WUE was lower and Δ was higher than in other species and subsections. In addition, hybrids had increased leader growth suggesting a trade-off between drought tolerance and increased foliar growth.

Needle cross-sectional thickness, width, and shape factor increased between late June and early September indicating needles were still growing during the first measurement. Needle thickness and shape factor were greatest at CHES and HTRC. Both sites are not shaded during the day, as opposed to KRF and NWHRS which are shaded either in the morning or late afternoon. Needle morphology parameters were closely related to section and subsection classification, which was expected given the importance of needle morphology to systematic classification (Liu, 1971, Farjon, 1990). Needle width and thickness increased with increased light exposure in several conifer species (Stenberg et al., 1999; Sprugel et al., 1996). However, in the present study needle width was greatest at KRF.

Needle thickness and shape factor increased at CHES following N application in July 2004. Needle thickness was correlated with increased N and P, while shape factor was correlated with foliar Mg and Ca, however N application increased both at CHES.

We found net photosynthesis (PSA_{max}, PNA_{max}, and TNA_{max}) increased with needle thickness and shape factor. In conifers, needle thickness was related to needle dry mass to projected area and photosynthesis increased as needle thickness increased

(Niinemets, 1999). Needle mesophyll increased with needle thickness resulting in more area to harvest light (Lin et al., 2001; Sprugel et al., 1996).

Carbon isotope discrimination (lower $^{13}\delta$) increased with PSA/TNA among all trees in our study. Shoots with a higher PSA/TNA ratio had fewer needles·cm^{-1} than shoots with a lower PSA/TNA ratio (Carter and Smith, 1985). Increased needle packing has resulted in boundary layer effects in conifers under calm conditions (Smith and Carter, 1988; Martin et al., 1999). Increased aerodynamic resistance resulting in boundary layer effects resulted in reducing transpiration more than photosynthesis and increasing WUE in *A. lasiocarpa* (Smith, 1980). Increased stand density in *A. amabilis* resulted in lower CO_2 concentrations and lower discrimination (more ^{13}C) (Buchmann et al., 1998). Similar effects on a smaller scale may explain the increased Δ (less $^{13}\delta$) as shoots became more vertically arranged.

Dark respiration from photosynthetic light response measurements was greatest at NWHRS which also had the lowest midday water potential. Block effects were highly significant ($p \leq 0.0001$) suggesting that time of day influenced Rd. This is logical since respiration increases rapidly with temperature (Zha et al., 2001) and measurements were carried out throughout the day. Net photosynthesis was closely correlated with foliar P and K and was highest at KRF, which coincided with the most foliar P and K of the four trial sites. Foliar N was not as closely correlated with PSA$_{max}$, PNA$_{max}$, and TNA$_{max}$ from photosynthetic light response measurements. This is presumably due to adequate foliar N from resulting from spring N fertilization. Net photosynthesis (PSA$_{max}$, PNA$_{max}$, and TNA$_{max}$) in *A. balsamea* was lowest at all locations except KRF. Reduced A$_{max}$ in *A. balsamea* at other sites may be from pH intolerance (refer to chapter two) or from

transplanting stress. Species selected for photosynthetic light response measurements represent trees from diverse geographical origins and with varying needle morphology and shoot architecture. Yet, net photosynthesis and the light compensation point were the only parameters to consistently vary among species when comparing all three methods of expressing needle area.

Net photosynthesis (PSA_{max}, PNA_{max}, and TNA_{max}) was greatest in *A. lasiocarpa*, *A. koreana* and *A. nephrolepis* when external CO_2 levels were increased to generate A-C_i curves. In both, needle shape factor was greater than the other four species included in the study. Low apparent CE corresponded with low PSA_{max} and PNA_{max} in *A. holophylla*, however the relationship varied among other species and locations. Similar to photosynthetic light response curves, foliar P and K were strongly correlated with PSA_{max}, PNA_{max}, and TNA_{max} in A/C_i curves while foliar N was not correlated presumably to adequate foliar N from spring fertilization.

In summary, net photosynthesis (PSA_{max}, PNA_{max}, and TNA_{max}) varied among species and taxonomic subsections. Instantaneous water use efficiency did not differ among species, however, carbon isotope discrimination (Δ) did differ among species and subsections. Strong relationships existed between PSA_{max}, PNA_{max}, TNA_{max} and Δ. Both needle morphology and needle architecture differed among species and subsection. Needle thickness was correlated with increased net photosynthesis. Differences in shoot architecture appear to influence boundary layer effects and increase carbon isotope discrimination. Photosynthetic light response and A/C_i curves differed among species, however main differences were to A_{max}.

Table 1. List of *Abies* species planted at four locations in Michigan.

Common Name	Scientific Name	Section/ Subsection[1]	Geographic Origin	Elevation (m)	Latitude (N)
Ernst fir	*A. chensiensis*	*Holophyllae*	Asia	2500 - 3800	25 - 33
Korean fir	*A. koreana*	*Medianae*	Asia	1000 - 2000	33 - 36
Needle fir	*A. holophylla*	*Holophyllae*	Asia	0 - 1400	33 - 49
Nikko fir	*A. homolepis*	*Homolepides*	Asia	600 - 2200	33 - 38
Siberian White fir	*A. nephrolepis*	*Medianae*	Asia	500 - 2000	35 - 55
Veitch fir	*A. veitchii*	*Medianae*	Asia	1200 - 3000	33 - 38
Balsam fir	*A. balsamea*	*Laterales*	N. America	0 - 1500	38 - 59
Cannan fir	*A. balsamea* var. *phanerolepis*	*Laterales*	N. America	0 - 1500	38 - 59
Corkbark fir	*A. bifolia*	*Laterales*	N. America	0 - 1700	37 - 39
Fraser fir	*A. fraseri*	*Medianae*	N. America	1100 - 2100	35 - 37
Noble fir	*A. procera*	*Nobilis*	N. America	100 - 2700	41 - 48
Sub-Alpine fir	*A. lasiocarpa*	*Laterales*	N. America	0 - 3500	32 - 64
Nordman fir	*A. nordmanniana*	*Abies*	Mediterranean	1000 - 2200	40 - 44
Turkish fir	*A nordmanniana* ssp. *equi-trojani*	*Abies*	Mediterranean	1000 - 2200	40 - 44
Fraser x Nikko Hybrid	*A. fraseri* x *homolepsis*	Hybrid	N. American/Asia	-	-
Korean x Balsam Hybrid	*A. koreana* x *balsamea*	Hybrid	N. American/Asia	-	-
Korean x Veitch Hybrid	*A. koreana* x *veitchii*	Hybrid	N. American/Asia	-	-

[1] Farjon, 1990.

Table 2. Thirty-year climate summary[1] and USDA plant hardiness zones[2] for four *Abies* planting sites in Michigan. For location descriptions see Figure 1.

Location	Average January Low (°C)[1]	Average July High (°C)[1]	Annual Precipitation (cm)[1]	Annual Snowfall (cm)[1]	Growing Season (days)[1]	USDA Hardiness Zone[2]
HTRC	-11	28	78.5	99	150	5A
NWHRS	-10	27	85.1	244	135	5B
CHES	-10	28	90.7	145	147	5B
KRF	-9	28	89.4	135	149	5B

1 Illinois Dept. of Nat. Res., 2005. 2 USDA Plant Hardiness Map, 1990.

Table 3. Soil properties of four *Abies* planting sites in Michigan

Location	Sand (%)	Silt (%)	Clay (%)	Soil Type	2003 pH	2004 pH
HTRC	83.1	8.7	9.3	Loamy Sand	6.25	5.72
NWHRS	83.3	7.6	9.1	Loamy Sand	7.11	6.66
CHES	61.3	23.5	15.1	Sandy Loam	6.76	4.62
KRF	72.4	17.1	10.5	Sandy Loam	4.63	4.12

Table 4. Mean volumetric soil moisture content at four *Abies* test plots in 2003 and 2004 using a portable TDR device.

	% Moisture			
Location	July 2003***	June 2004***	July 2004***	October 2004***
CHES	16.38 a	24.4 b	15.36 b	10.15 c
HTRC	7.19 b	NA	12.76 c	6.26 d
KRF	NA	28.86 a	18.05 a	16.10 a
NWHRS	16.38 a	11.19 c	6.78 d	12.73 b

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.001 respectively

Table 5. Net photosynthesis expressed using projected shoot area (PSA_{max}) of seven *Abies* subsections grown at four locations in Michigan in 2003 and 2004. Gas exchange was measured in late July 2003, and late June, July, and early September 2004.

	PSA_{max} ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)			
Subsection	July 2003**	June 2004***	July 2004**	September 2004***
Laterales	9.23 a	12.72 a	12.04 ab	14.16 ab
Homolepides	7.23 ab	7.78 c	11.50 ab	11.55 c
Hybrid	7.00 b	11.8 ab	9.92 b	13.13 abc
Medianae	6.24 b	9.55 bc	10.82 ab	13.02 bc
Holophyllae	6.16 b	10.31 b	11.66 ab	12.00 c
Nobilis	NA	13.72 ab	13.59 a	16.34 a
Abies	NA	10.66 ab	11.20 ab	12.09 bc

Location	July 2003	June 2004**	July 2004***	September 2004***
CHES	9.93	12.76 a	13.13 a	16.06 a
HTRC	NA	9.75 b	11.80 a	12.58 b
KRF	NA	10.64 b	11.65 a	12.71 b
NWHRS	9.50	10.59 b	9.55 b	11.40 b

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.001 respectively

Table 6. Mean photosynthetic rates expressed using projected needle area (PNA$_{max}$) of 17 *Abies* species grown at four locations in Michigan in 2004. Gas exchange was measured in late June 2004, late July 2004, and early September 2004.

	June 2004***	July 2004[x]				September 2004 ***
		Location				
Species		CHES	HTRC***	KRF	NWHRS**	
A. bal. var. *phanerolepis*	5.05 cdef	5.88 a	3.17 c	10.31 a	2.44 c	7.28 b
A. balsamea	6.86 abcdef	6.21 a	3.44 bc	7.99 a	3.58 bc	7.39 b
A. bifolia	9.21 a	8.12 a	6.82 a	8.78 a	9.89 a	9.55 a
A. chensiensis	7.08 abcde	8.68 a	8.35 a	7.43 a	7.26 abc	9.10 ab
A. fraseri	NA	6.44 a	6.51 abc	7.91 a	3.22 bc	7.34 b
A. fraseri x *homolepis*	6.58 abcdef	7.88 a	8.02 a	5.99 a	6.23 abc	8.44 ab
A. holophylla	7.08 abcd	8.65 a	7.16 a	9.31 a	5.49 abc	8.03 ab
A. homolepis	4.56 ef	8.29 a	6.24 abc	7.06 a	5.43 abc	7.64 b
A. koreana	4.77 def	8.62 a	5.34 abc	8.16 a	3.92 bc	8.11 ab
A. koreana x *balsamea*	6.15 bcdef	7.45 a	5.35 abc	7.27 a	5.35 abc	7.72 ab
A. koreana x *veitchii*	6.34 bcdef	6.60 a	7.86 a	7.23 a	4.74 abc	8.64 ab
A. lasiocarpa	9.20 a	9.70 a	8.26 a	9.42 a	7.60 abc	9.27 ab
A. nephrolepis	7.51 abc	6.95 a	6.03 abc	8.23 a	8.27 ab	9.15 ab
A. nord. ssp. *equi-trojani*	6.94 abcdef	8.60 a	7.83 a	8.03 a	5.34 abc	8.19 ab
A. nordmanniana	8.75 ab	9.60 a	7.91 a	8.41 a	3.42 abc	7.71 ab
A. procera	6.98 abcdef	10.29 a	9.44 a	8.16 a	6.24 abc	9.31 ab
A. veitchii	4.46 f	6.52 a	6.28 abc	5.02 a	5.25 abc	7.29 b

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.

[x] Location × species was significant at p≤0.001.

*, **, *** p≤0.05, 0.01, and 0.001 respectively

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Table 7. Net photosynthesis expressed using projected needle area (PNA$_{max}$) of seven *Abies* sections and subsections grown at four locations in Michigan in 2004. Gas exchange was measured in late June, late July, and early September 2004.

Subsection	PNA$_{max}$ (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)		
	June 2004***	July 2004**	September 2004[x]
Laterales	7.73 a	7.10 ab	8.43
Homolepides	4.56 c	6.75 ab	7.64
Hybrid	6.37 ab	6.67 ab	8.26
Medianae	5.49 bc	6.43 b	7.99
Holophyllae	7.07 a	7.73 a	8.56
Nobilis	6.98 ab	8.53 a	9.31
Abies	7.41 ab	7.48 ab	8.07
Location	June 2004**	July 2004***	September 2004[x]
CHES	7.35 a	8.33 a	10.36
HTRC	5.64 c	7.14 b	7.69
KRF	6.93 ab	7.86 ab	8.77
NWHRS	6.13 bc	5.65 c	6.47

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.001 respectively

[x] Sub-section and location were significant at $p \leq 0.05$, however Location × Sub-section effect was significant at $p \leq 0.05$.

Table 8. Pearson's correlation coefficients for net photosynthesis (PSA$_{max}$, PNA$_{max}$, TNA$_{max}$), water use efficiency (WUE), needle morphology, carbon isotope discrimination (Δ), and shoot architecture in 17 *Abies* species grown at four locations in Michigan.

	PNA$_{max}$	TNA$_{max}$	WUE	X-Sec Area	Needle Width	Needle Thickness	Perimeter	Shape Factor	Δ	PSA/ TNA	PSA/ PSA
PSA$_{max}$	0.75***	0.75***	-0.18***	0.17***	-0.03	0.36***	0.02	0.33***	0.14	-0.27***	0.27***
PNA$_{max}$		1.00***	-0.30***	-0.27***	0.01	0.48***	0.07	0.47***	0.38***	0.35***	-0.35***
TNA$_{max}$			-0.32***	0.28***	0.06	0.46***	0.11*	0.41***	0.41***	0.37***	-0.40***
WUE				0.06	0.04	0.04	0.05	0.04	-0.44***	-0.06	0.06
X-Sec Area					0.86***	0.78***	0.91***	0.07	-0.01	0.23***	-0.22***
Needle Width						0.39***	0.99***	-0.43***	0.11	0.18***	-0.12**
Needle Thickenss							0.49***	0.62***	-0.08	0.20***	-0.24***
Perimeter								-0.33***	0.09	0.17***	-0.12**
Shape Factor									-0.19*	0.15**	-0.22***
D										0.30**	-0.27**
PSA/TNA											-0.94*
TNA/PN A											-0.02

*, **, *** p=0.05, 0.01, 0.0001 respectively

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Table 9. Net photosynthesis expressed using projected needle area (PNA_{max}) of seven *Abies* sections and subsections grown at four locations in Michigan in 2004. Gas exchange was measured in late June, late July, and early September 2004.

Subsection	TNA_{max} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	
	June 2004***	September 2004
Laterales	3.12 a	3.41
Homolepides	1.98 b	3.26
Hybrid	2.69 ab	3.46
Medianae	2.31 b	3.35
Holophyllae	3.01 a	3.51
Nobilis	2.91 ab	3.80
Abies	3.13	3.45
Location	June 2004**	September 2004***
CHES	3.08 a	4.26 a
HTRC	2.34 b	3.21 c
KRF	2.93 a	3.70 b
NWHRS	2.59 ab	2.69 d

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.001 respectively

Table 10. Mean carbon isotope discrimination (Δ) in 10 *Abies* species grown at four locations in Michigan in 2004.

Species	Location[x]				Mean
	CHES*	HTRC*	KRF	NWHRS	
A. koreana x *balsamea*	20.08 a	17.51 ab	21.20	18.93	19.43
A. koreana	19.85 ab	17.11 b	21.58	18.22	18.86
A. koreana x *veitchii*	19.84 ab	19.19 a	22.18	17.88	19.77
A. fraseri x *homolepis*	19.56 ab	18.89 ab	21.87	19.56	20.04
A. nephrolepis	19.36 ab	18.22 ab	21.39	19.03	19.24
A. bifolia	19.29 ab	18.41 ab	21.38	19.82	19.58
A. holophylla	19.15 ab	18.18 ab	21.30	18.30	19.11
A. veitchii	19.06 ab	18.46 ab	20.24	18.62	19.00
A. fraseri	17.94 ab	17.89 ab	21.40	18.21	19.05
A. balsamea	17.80 b	18.51 ab	21.11	17.52	18.80
Average	19.19	18.24	21.36	18.61	

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.
* $p \leq 0.05$
[x] Location x Species interaction was significant at $p \leq 0.01$

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Table 11. Mean carbon isotope discrimination (Δ) in four *Abies* subsections grown at four locations in Michigan in 2004.

Subsection	Mean
Hybrid	19.72 a
Laterales	19.26 ab
Holophyllae	19.23 ab
Medianae	19.19 b

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

Table 12. Needle morphology traits for 17 *Abies* species growing at four locations in Michigan in 2004.

Species	mm²	mm			
	Cross-Sectional Area***	Needle Thickness^x	Needle Width***	Perimeter***	Shape Factor^x
A. bal. var. *phanerolepis*	0.52 hi	1.46	0.49 fg	3.51 ef	0.53
A. balsamea	0.49 i	1.42	0.45 g	3.40 f	0.52
A. bifolia	0.73 defg	1.43	0.67 ab	3.62 def	0.69
A. chensiensis	1.08 a	2.15	0.66 abc	5.06 a	0.53
A. fraseri	0.68 efgh	1.65	0.55 def	3.98 cd	0.53
A. fraseri x *homolepis*	0.77 cdefg	1.79	0.55 def	4.28 bc	0.53
A. holophylla	0.92 bc	1.86	0.61 abcd	4.43 b	0.59
A. homolepis	0.76 defg	1.90	0.49 fg	4.38 b	0.49
A. koreana	1.07 ab	2.23	0.60 cde	5.25 a	0.48
A. koreana x *balsamea*	0.85 cd	1.92	0.55 def	4.53 b	0.51
A. koreana x *veitchii*	0.64 ghi	1.62	0.51 fg	3.85 de	0.53
A. lasiocarpa	0.72 defg	1.55	0.61 abcd	3.79 de	0.61
A. nephrolepis	0.66 fgh	1.53	0.54 ef	3.72 def	0.60
A. nord. ssp. *equi-trojani*	0.83 cde	1.81	0.59 de	4.27 bc	0.55
A. nordmanniana	1.16 a	2.21	0.69 a	5.19 a	0.54
A. procera	0.71 defg	1.59	0.60 bcde	3.84 de	0.60
A. veitchii	0.79 cdef	1.99	0.49 fg	4.59 b	0.47

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, 0.0001 respectively

^x Location × Species interaction was significant at $p \leq 0.05$

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Table 13. Needle morphology traits for seven *Abies* subsections grown at four locations in Michigan in August 2004.

Subsection	mm²	mm			
	Cross-Sectional Area***	Needle Thickness***	Needle Width***	Perimeter***	Shape Factor***
Holophyllae	1.04 a	0.67 a	1.97 a	4.70 a	0.59 ab
Abies	0.97 ab	0.64 ab	1.95 ab	4.60 ab	0.56 bc
Medianae	0.88 bc	0.58 bc	1.86 ab	4.44 ab	0.55 cd
Homolepides	0.83 bc	0.54 c	1.88 ab	4.39 ab	0.54 d
Hybrid	0.79 bc	0.56 c	1.75 bc	4.18 bc	0.56 cd
Nobilis	0.73 cd	0.62 abc	1.56 cd	3.81 cd	0.62 a
Laterales	0.62 d	0.57 c	1.45 d	3.55 d	0.61 a
Location	Cross-Sectional Area*	Needle Thickness***	Needle Width	Perimeter	Shape Factor***
CHES	0.90 a	0.65 a	1.77	4.28	0.61 a
KRF	0.83 ab	0.57 bc	1.83	4.34	0.55 c
HTRC	0.83 ab	0.61 ab	1.75	4.19	0.59 b
NWHRS	0.78 b	0.56 c	1.75	4.15	0.56 c

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.
*, **, *** $p \leq 0.05$, 0.01, 0.0001 respectively

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Table 14. Mean projected shoot to total needle surface area (PSA/TNA), projected needle to projected shoot area (PNA/PSA), and total needle to projected needle area (TNA/PNA) ratios of 17 *Abies* species growing at four locations in Michigan in 2004.

Species	PSA/TNA[x]	PNA/PSA[x]	TNA/PNA[x]
A. bal. var. phanerolepis	0.204	2.05	2.41
A. balsamea	0.250	1.74	2.39
A. bifolia	0.275	1.47	2.54
A. chensiensis	0.292	1.51	2.35
A. fraseri	0.193	2.42	2.41
A. fraseri x homolepis	0.249	1.68	2.39
A. holophylla	0.305	1.45	2.37
A. homolepis	0.271	1.64	2.32
A. koreana	0.211	1.98	2.35
A. koreana x balsamea	0.226	1.85	2.37
A. koreana x veitchii	0.270	1.50	2.39
A. lasiocarpa	0.234	1.81	2.45
A. nephrolepis	0.311	1.33	2.43
A. nord. ssp. equi-trojani	0.284	1.61	2.36
A. nordmanniana	0.304	1.42	2.35
A. procera	0.236	1.77	2.42
A. veitchii	0.283	1.53	2.31

[x] Species effects were significant at $p \leq 0.0001$, however, Location × Species interaction was significant at $p \leq 0.05$

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Table 15. Mean projected shoot to total needle surface area (PSA/TNA), projected needle to projected shoot area (PNA/PSA), and total needle to projected needle area (TNA/PNA) ratios of seven *Abies* sections and subsections grown at four locations in Michigan in August 2004.

Subsection	PSA/TNA***	PNA/PSA***	TNA/PNA***
Holophyllae	0.30 a	1.41 b	2.38 bc
Abies	0.29 ab	1.53 ab	2.36 c
Homolepides	0.28 ab	1.52 ab	2.34 d
Hybrid	0.27 bc	1.61 ab	2.39 b
Medianae	0.26 bc	1.69 a	2.39 bc
Laterales	0.25 c	1.73 a	2.45 a
Nobilis	0.24 bc	1.75 ab	2.44 a
Location	PSA/TNA***	PNA/PSA**	TNA/PNA***
CHES	0.27 b	1.58 ab	2.43 a
HTRC	0.26 b	1.70 ab	2.40 b
KRF	0.30 a	1.48 b	2.37 c
NWHRS	0.25 b	1.78 a	2.38 c

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.
*, **, *** p≤0.05, 0.01, 0.0001 respectively

Table 16. Mean dark respiration (Rd), net photosynthesis expressed using projected shoot area (PSA_{max}), apparent quantum efficiency (ϕ), and light compensation point (LCP) in six *Abies* species grown at four locations in Michigan in 2004.

Species	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$		$\mu mol\ CO_2 \cdot \mu mol^{-1}$	$\mu mol \cdot m^{-2} \cdot s^{-1}$
	Rd**	PSA_{max}***	ϕ	LCP**
A. balsamea	2.15 ab	7.0 c	0.040	53.0 ab
A. bifolia	2.05 ab	11.6 a	0.046	40.8 ab
A. holophylla	1.98 ab	7.9 bc	0.046	43.9 ab
A. koreana	2.23 b	10.6 ab	0.049	45.2 a
A. koreana x *balsamea*	2.35 b	9.5 abc	0.051	46.7 a
A. nephrolepis	1.36 a	9.6 abc	0.045	29.6 b
Location	Rd***	PSA_{max}***	ϕ*	LCP***
CHRS	1.89 b	7.3 c	0.042 b	47.6 ab
HTRC	1.88 b	7.0 c	0.047 ab	37.4 bc
KRF	1.16 a	13.0 a	0.041 b	27.9 c
NWHRS	3.22 c	10.2 b	0.054 a	60.0 a

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

Table 17. Mean dark respiration (Rd), net photosynthesis expressed using total needle area (TNA_{max}), apparent quantum efficiency (ϕ), and light compensation point (LCP) in six *Abies* species grown at four locations in Michigan in 2004.

Species	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$		$\mu mol\ CO_2 \cdot \mu mol^{-1}$	$\mu mol \cdot m^{-2} \cdot s^{-1}$
	Rd	TNA_{max}***	ϕ	LCP**
A. balsamea	0.52	2.0 c	0.010	54.2 ab
A. bifolia	0.55	3.4 a	0.012	48.9 ab
A. holophylla	0.54	2.3 c	0.013	43.3 ab
A. koreana	0.52	2.6 bc	0.011	50.2 a
A. koreana x balsamea	0.58	2.5 bc	0.013	49.3 a
A. nephrolepis	0.42	3.1 ab	0.014	30.1 b
Location	Rd***	TNA_{max}***	ϕ	LCP***
CHRS	0.54 b	2.4 b	0.023	47.8 b
HTRC	0.46 b	1.8 c	0.012	40.3 bc
KRF	0.31 c	3.7 a	0.011	27.8 c
NWHRS	0.78 a	2.8 b	0.012	68.1 a

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \le 0.05$, 0.01, and 0.0001 respectively

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Table 18. Mean dark respiration (Rd), net photosynthesis expressed using projected needle area (PNA$_{max}$), apparent quantum efficiency (ϕ), light compensation point (LCP), and light saturation point (LS) in six *Abies* species grown at four locations in Michigan in 2004.

Species	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$		$\mu mol\ CO_2 \cdot \mu mol^{-1}$	$\mu mol \cdot m^{-2} \cdot s^{-1}$	
	Rd	PNA$_{max}$***	ϕ	LCP*	LS[1]
A. balsamea	1.32	4.66 c	0.025	55.4 a	1250-1450
A. bifolia	1.43	8.26 a	0.032	43.9 ab	1350-1550
A. holophylla	1.30	5.48 c	0.032	41.9 ab	1650-1850
A. koreana	1.27	5.78 bc	0.028	46.5 ab	1650-1850
A. koreana x balsamea	1.37	5.73 bc	0.032	45.7 ab	1200-1400
A. nephrolepis	1.03	7.29 ab	0.034	28.8 b	1500-1700
Location	Rd***	A$_{max}$***	ϕ	LCP***	
CHRS	1.30 b	5.43 bc	0.030	51.2 ab	
HTRC	1.14 ab	4.55 c	0.030	37.8 bc	
KRF	0.82 a	8.47 a	0.030	27.5 c	
NWHRS	1.88 c	6.35 b	0.031	58.2 a	

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.

*, **, *** p$\leq$0.05, 0.01, and 0.0001 respectively

[1] Light saturation ranges were calculated at 95% of mean A$_{max}$ at PPFD=2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Landhäusser and Lieffers, 2001).

Table 19. Mean respiration (R), net photosynthetic rate expressed using projected shoot area (PSA$_{max}$), and apparent carboxylation efficiency (CE) of six *Abies* species grown at four locations in Michigan is 2004.

Species	μmol CO$_2$·m^{-2}·s^{-1}		CE***
	R***	PSA$_{max}$[x]	
A. koreana	5.83 ab	70.5	0.060 a
A. koreana x *balsamea*	6.20 ab	64.4	0.060 a
A. holophylla	4.36 c	48.0	0.042 b
A. nephrolepis	5.05 bc	68.5	0.052 a
A. balsamea	6.65 a	54.0	0.059 a
A. bifolia	5.62 abc	68.1	0.059 a
Location	R***	PSA$_{max}$[x]	CE***
CHRS	4.83 a	59.2	0.043 b
KRF	5.03 a	70.2	0.051 b
HTRC	6.08 b	53.9	0.063 a
NWHRS	6.52 b	65.6	0.064 a

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.

*, **, *** p$\leq$0.05, 0.01, and 0.0001

[x] Significant at p$\leq$0.05, however, location $\times$ species effect is significant at p$\leq$0.01

Table 20. Mean respiration (R), net photosynthetic rate expressed using projected needle area (PNA$_{max}$), and apparent carboxylation efficiency (CE) of six *Abies* species grown at four locations in Michigan is 2004.

Species	μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$		CE***
	R	PNA$_{max}$***	
A. koreana	3.39	43.5 ab	0.035 ab
A. koreana x *balsamea*	3.65	36.3 ab	0.038 ab
A. holophylla	3.63	39.2 ab	0.034 b
A. nephrolepis	3.90	52.5 a	0.039 ab
A. balsamea	3.99	33.5 b	0.036 ab
A. bifolia	3.97	44.7 ab	0.041 a
Location	R	PNA$_{max}$***	CE***
CHRS	3.55	42.7 ab	0.031 b
KRF	3.65	48.0 a	0.038 ab
HTRC	3.76	36.0 b	0.040 a
NWHRS	4.06	39.7 ab	0.040 a

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.

*, **, *** p≤0.05, 0.01, and 0.0001 respectively

Table 21. Mean respiration (R), net photosynthetic rate expressed using total needle area (TNA_{max}), and apparent carboxylation efficiency (CE) of six *Abies* species grown at four locations in Michigan is 2004.

Species	$\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$		CE
	R	TNA_{max}	
A. koreana	1.42	18.1	0.015
A. koreana x *balsamea*	1.53	15.0	0.016
A. holophylla	1.52	16.3	0.014
A. nephrolepis	1.57	20.3	0.016
A. balsamea	1.67	13.8	0.015
A. bifolia	1.56	17.6	0.016
Location	R	$TNA_{max}{}^x$	CE**
CHRS	1.45	17.2	0.013 b
KRF	1.53	20.1	0.016 ab
HTRC	1.50	13.6	0.016 a
NWHRS	1.70	16.6	00.017 a

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

[x] Significant at $p \leq 0.05$, however, location × species effect is significant at $p \leq 0.01$
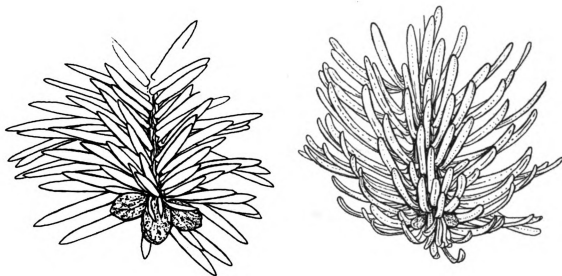
Figure 1. Two contrasting needle architecture arrangements. Left. Flat arrangement (*A. veitchii*). Right Bottlebrush needle arrangement (*A. procera*).
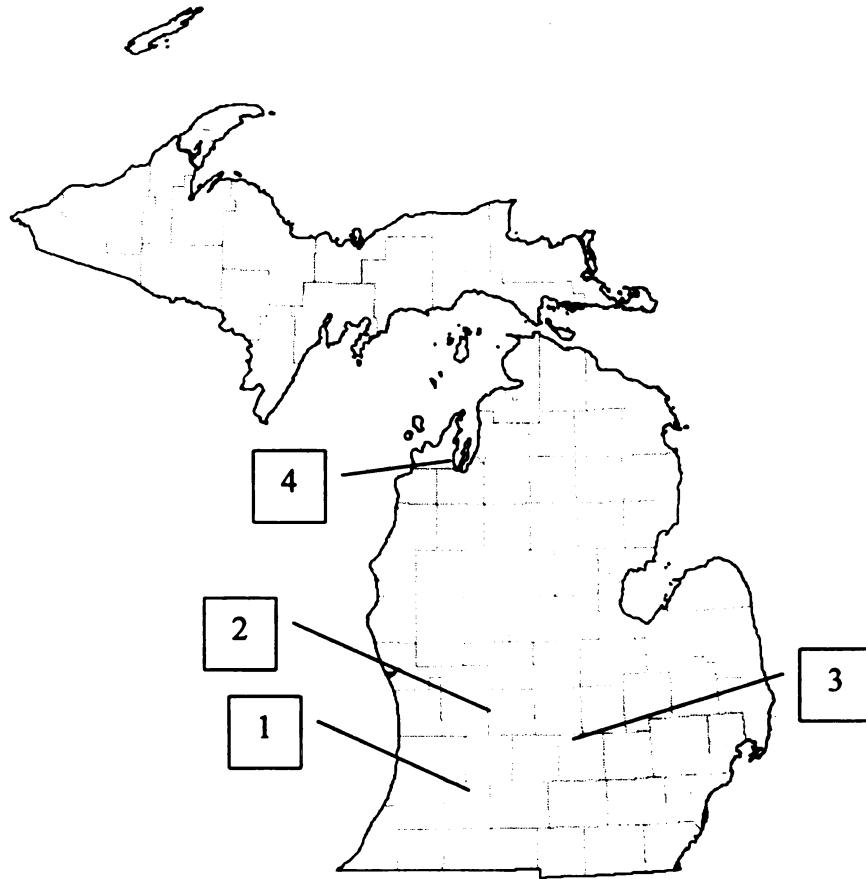
Figure 2. Location of four *Abies* trials in Michigan. 1) Kellogg Research Forest (KRF), 2) Clarksville Horticulture Experiment Station (CHES), 3) Horticulture Teaching and Research Center (HTRC), 4) Northwest Michigan Horticultural Research Station (NWHRS)
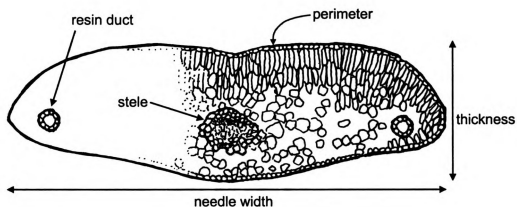
Figure 3. Needle cross-section displaying maximum needle width (horizontal), maximum needle thickness (vertical), and perimeter measurements.

Figure 4. Needle area measured as projected shoot area (left), projected needle area (center) when needles are plucked and scanned, and total needle area (right) when the perimeter:width ratio of cross-sections is multiplied by the projected needle area.
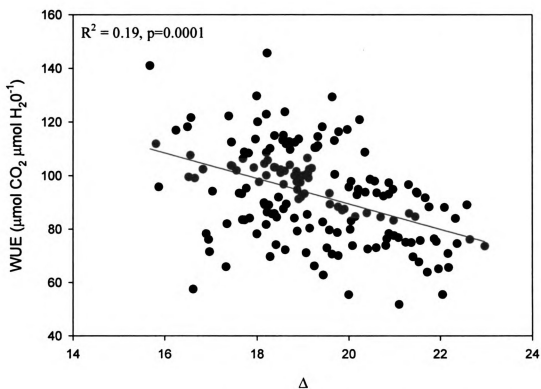
Figure 5. Relationship between water use efficiency and Δ in 10 *Abies* species grown at four locations in Michigan in 2004.
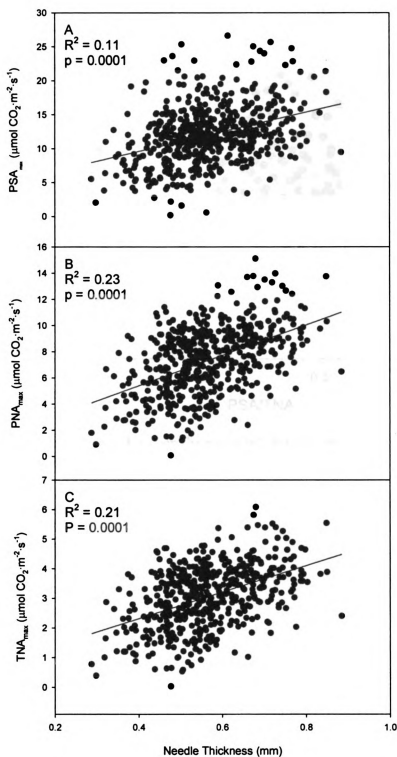
Figure 6. Relationship between A) PSA_{max}, B) PNA_{max}, and C) TNA_{max} and needle width of 17 *Abies* species grown at four locations in Michigan in 2004.
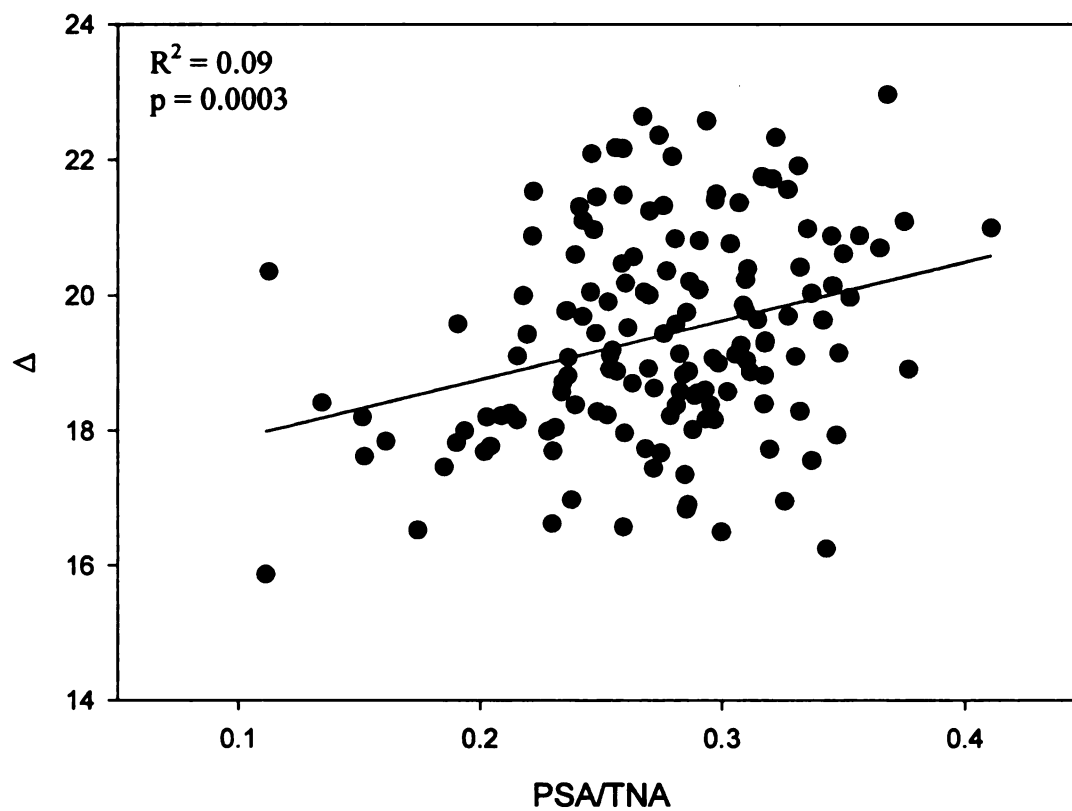
Figure 7. Relationship between the projected shoot to total needle area (PSA/TNA) ratio and carbon isotope discrimination (Δ).
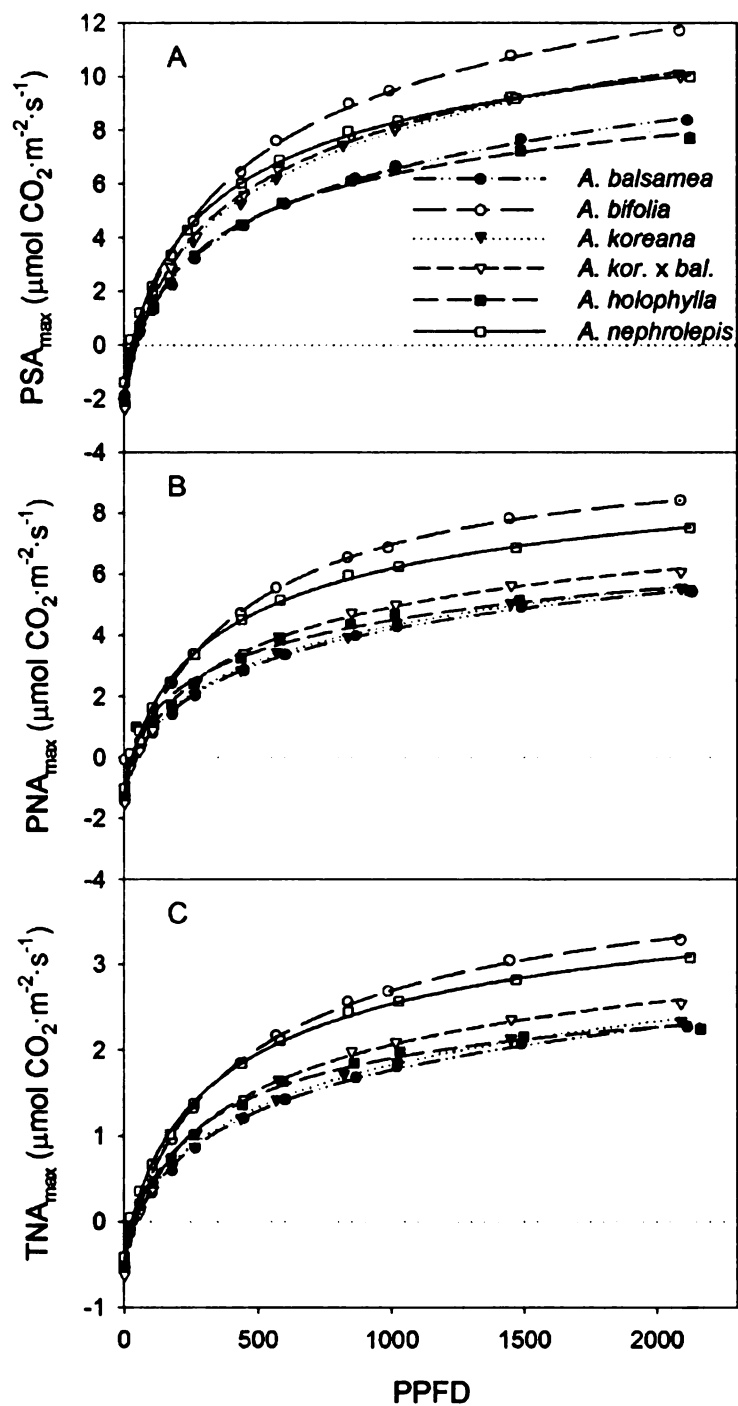
Figure 8. Photosynthetic light response curves for six *Abies* species expressed using A) projected shoot area, B) projected needle area, and C) total needle area and grown at four locations in Michigan in 2004.
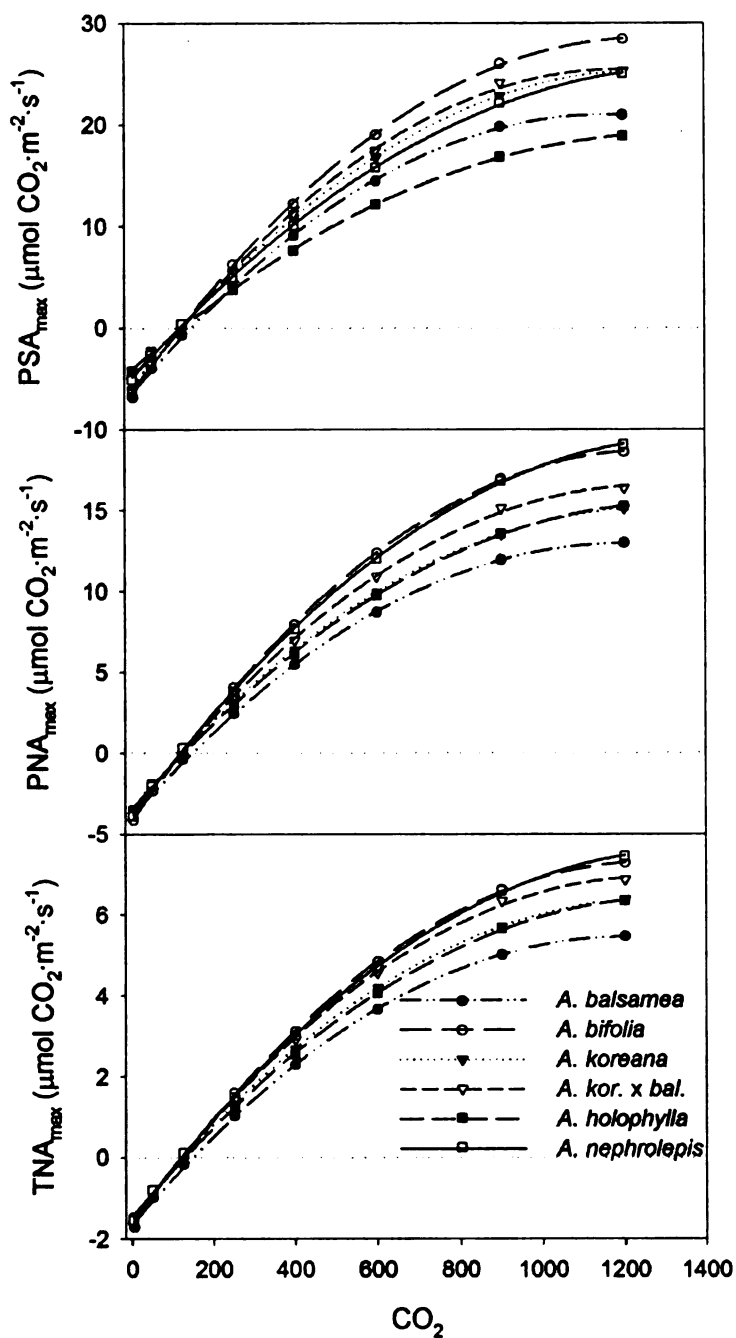
Figure 9. A/C$_i$ curves for six *Abies* species expressed using A) projected shoot area, B) projected needle area, and C) total needle area and grown at four locations in Michigan is 2004.

Literature Cited

American Standards for Nursery Stock. 2004. Amer. Nurs. Land. Asso. Washington, DC.

Arndt, S.K., S.C. Clifford, W. Wanek, H.G. Jones, and M. Popp. 2001. Physiological and morphological adaptations of the fruit tree Ziziphus rotundifolia in resonse to progressive drought stress. Tree Physiol. 21: 705-715.

Bernier, P.Y., F. Rauleier, P. Stenberg, and C. Ung. 2001. Importance of needle age and shoot structure on canopy net photosynthesis of balsam fir (*Abies balsamea*): a spatially inexplicit modeling analysis. Tree Physiol. 21: 815-830.

Brooks, J.R., T.M. Hinckley, and D.G. Sprugel. 1994. Acclimation responses of mature *Abies amabilis* sun foliage to shading. Oecologia. 100: 316-324.

Buchmann, N., T.M. Hinckley, and J.R. Ehleringer. 1998. Carbon isotope dynamics in *Abies amabilis* stands in the Cascades. Can. J. For. Res. 28: 808-819.

Carter, G. A. and W.K. Smith. 1985. Influence of shoot structure on light interception and photosynthesis in conifers. Plant Physiol. 79: 1038-1043.

Chandler, J.W. and J.E. Dale. 1995. Nitrogen deficiency and fertilization effects on needle growth and photosynthesis in Sitka spruce (*Picea sitchensis*). Tree Physiol. 15: 813-817.

Crafts-Brandner, S.J. and M.E. Salvucci. 2000. Rubisco activase constrains the phosynthetic potentialof leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. USA. 97(24): 13430-13435.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correlation factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim Acta, 12: 133-149.

Cregg, B.M. 1994. Carbon allocation, gas exchange, and needle morphology of Pinus ponderosa genotypes known to differ in growth and survival under imposed drought. Tree Physiol. 14: 883-898.

Cregg, B.M., J.M. Olives-García, and T.C. Hennessey. 2000. Provenance variation in carbon isotope discrimination of mature ponderosa pine trees at two locations in the Great Plains. Can. J. For. Res. 30: 428-439.

Cregg, B.M. and J.W. Zhang. 2001. Physiology and morphology of Pinus sylvestris seedlings from diverse sources under cyclic drought stress. For. Ecol. Mngt. 154: 131-139.

Farjon, A. 1990. Pinaceae drawings and descriptions of the genera *Abies, Cedrus, Psudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix*, and *Picea*. Champaign, IL.

Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. 33: 317-345.

Farquhar, G.D. and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with Water-use efficiency of wheat genotypes. Aust. J. Plant Physiol. 11: 539-52.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol., 40: 503-537.

Germino, M.J. and W.K. Smith. 1999. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. Plant, Cell, and Envir. 22: 407-415.

Grassi, G. and U. Bagnaresi. 2001. Foliar morphological and physiological plasticity in *Picea abies* and *Abies alba* saplings along a natural light gradient. Tree Physiol. 21: 959-967.

Guehl, J.M., G. Aussenac, J. Bouachrine, R. Zimmermann, J.M. Pennes, A. Ferhi, and P. Grieu. 1991. Sensitivity of leaf gas exchange to atmospheric drought, soil drought, and water-use efficiency in some Mediterranean *Abies* species. Can. J. For. Res. 21: 1507-1515.

Hallgren, S.W. and J.A. Helms. 1988. Control of height growth components in seedlings of California red and whit fir by seed sources and water stress. Can. J. For. Res. 18: 521-529.

Ishii, H., M. Ooishi, Y. Maruyama, and T. Koike. 2003. Acclimation of shoot and needle morphology and photosynthesis of two Picea species to differences in soil nutrient availability. Tree Physiol. 23: 453-461.

Knauf, T.A. and M.V. Bilan. 1974. Needle variation in loblolly pine from mesic and xeric seed sources. For. Sci. 20(1): 88-90.

Körner, C. 1991. Some often overlooked plant characteristics as determinants of plant growth: a reconsideration. Funct. Ecol. 5: 162-173.

Landhäusser, S.M., and V.J. Lieffers. 2001. Photosynthesis and carbon allocation of six boreal tree species grown in understory and open conditions. Tree Physiol. 21: 243-250.

Lin, J., M.E. Jach, and R. Ceulemans. 2001. Stomatal density and needle anatomy of Scots pine (*Pinus sylvestris*) are affected by elevated CO_2. New Phytol. 150: 665-674.

Martin, T. A., T.M. Hinckley, F. C. Meinzer, and D.G. Sprugel. 1999. Boundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. Tree Physiol. 19: 435-443.

Masle, J. and G.D. Farquhar. 1986. Effects of soil strength on the relation of water-use efficiency and growth to carbon isotope discrimination in wheat seedlings. Plant Physiol. 86: 32-38.

Monson, R.K. and M.C. Grant. 1989. Experimental studies of Ponderosa pine. III. Differences in photosynthesis, stomatal conductance, and water-use efficiency between two genetic lines. Amer. J. Bot. 76(7): 1041-1047.

Mori, A. and H. Takeda. 2004. Functional relationships between crown morphology and within-crown characteristics of understory saplings of three codominant conifers in a subalpine forest in central Japan. Tree Physiol. 24: 661-670.

Niinemets, Ü. 1999. Components of leaf dry mass per area – thickness and density – alter leaf photosynthetic capacity in reverse direction in woody plants. New. Phytol. 144: 35-47.

Paquin, R., H.A. Margolis, R. Doucet, and M.R. Coyea. 2000. Physiological response of black spruce layers and planted seedlings to nutrient addition. Tree Physiol. 20: 229-237.

Prioul J.L. and P. Chartier, 1977. Partitioning of transfer and carboxylation components of intracellular resistance to phosynthetic CO_2 fixation – Critical analysis of methods used. Ann. Bot. 41(174): 789-800.

Sakata, M. and K. Suzuki. 2000. Evaluating possible causes for the decline of Japanese fir (*Abies firma*) forests based on $\delta^{13}C$ records of annual growth rings. Environ. Sci. Technol. 34: 373-376.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, pp1243-1246. Nashville, TN, March 22-25.

Sellin, A. 2000. Estimating the needle area from geometric measurements: application of different calculation methods to Norway spruce. Trees. 14: 215-222.

SigmaScan Pro 5.0 Users guide. 1999. SPSS Inc. Chicago, IL.

Silim, S.N., R.D. Guy, T.B. Patterson, N.J. Livingston. 2001. Oecologia. Plasticity in water-use efficiency of *Picea sitchensis*, *P. glauca* and their natural hybrids.

Smith, W. K. and G. A. Carter. 1988. Shoot structural effects on needle temperatures and photosynthesis in conifers. Amer. J. Bot. 75(4): 496-500.

Smith, W.K., A.W. Schoettle, and M. Cui. 1991. Importance of the method of leaf measurement to the interpretation of gas exchange of complex shoots. Tree Physiol. 8: 121-127.

Smith, W. K. 1980. Importance of Aerodynamic resistance to water use efficiency in three conifers under field conditions. Plant Physiol. 65: 132-135.

Sprtová, M. and M.V. Marek. 1999. Response of photosynthesis to radiation and intercellular CO_2 concentrations in sun and shade shoots of Norways spruce. Photsynthetica. 37(3): 433-445.

Sprugel, D.G., J.R. Brooks, and T.M. Hinckley. 1996. Effects of light on shoot geometry and needle morphology in *Abies amabilis*. Tree physiol. 16: 91-98.

Stenberg, P., T. Kangas, H. Smolander, and S. Linder. 1999. Shoot structure, canopy openness, and light interception in Norway spruce. Plant, Cell, Environ. 22: 1133-1142.

Stenberg, P. 1996. Simulations of the effects of shoot structure and orientation on vertical gradients in intercepted light by conifer canopies. Tree Physiol. 16: 99-108.

Teskey, R.O., J.A. Fites, L.J. Samuelson, and B.C. Bongarten. 1986. Stomatal nonstomatal limitations to net photosynthesis in *Pinus taeda* L. under different environmental conditions. Tree Physiol. 2: 131-142.

Warren, C.R., N.J. Livingston, and D.H. Turpin. 2004. Photosynthetic responses and N allocation in Douglas-fir needles following a brief pulse of nutrients. Tree Physiol. 24: 601-608.

Webster, R., A. Rigling, and L. Walthert. 1996. An analysis of crown conditions of *Picea, Fagus* and Abies in relation to environment in Switzerland. Forestry 69: 347-355.

Woo, K., L. Fins, G.I. McDonald, D. L. Wenny, and A. Eramian. 2002. Effects of nursery environment on needle morphology of *Pinus monticola* Dougl. and implications for tree improvement programs. New For. 24: 113-129.

Zha, T., A. Ryyppö, K. Wang, and S. Kellomäki. 2001. Effects of elevated carbon dioxide concentration and temperature on needle growth, respiration and carbohydrate status in field-grown Scots pines during the needle expansion period. Tree Physiol. 21: 1279-1287.

APPENDIX

Project Summary

Cold Hardiness

The date of budbreak and growing degree days (GDD) necessary for budbreak differed by nearly a month among some species. Species native to colder regions such as *A. lasiocarpa*, *A. bifolia*, and *A. nephrolepis* were among the first to break bud when grown at a common site and were more prone to late frost damage. Early budbreak indicates a reduced GDD requirement in these species. Species such as *A. koreana* and *A. veitchii* were among the last to break bud when grown at a common site. Control freeze tests show species with greater mid-winter cold hardiness require fewer GDD to break bud than less cold hardy species. Future tree improvement research should identify provenances exhibiting late budbreak yet maintaining adequate mid-winter cold hardiness in species possessing other desirable ornamental characteristics and environmental tolerances such as *A. lasiocarpa* and *A. bifolia*. Future research should further investigate the relationship between mid-winter cold hardiness and GDD required for budbreak.

Nutrition

Tolerance of high pH soils varied widely among the species included in this study. Species such as *A. lasiocarpa*, *A. bifolia*, and *A. nephrolepis* were more tolerant of high pH soils than species such as *A. balsamea* or *A. balsamea* var. *phanerolepis*. Overall, net photosynthesis and foliar N, P, and K declined in trees grown in high pH soils as multiple nutrient deficiencies developed. Adequate foliar N was maintained following N fertilizer application in the spring. However, foliar P and K levels were strongly correlated with photosynthetic output indicating these nutrients became most limiting when foliar N requirements were met. Leader growth was greatest in the hybrid crosses, *A.*

nephrolepis, and *A. homolepis*. In all species, foliar N, P, and K were more important to leader growth than net photosynthesis. Future tree improvement efforts should continue to include tolerance of high pH soils as a selection criterion.

<u>Needle Morphology, Shoot Architecture, and Gas Exchange</u>

Species with similar needle morphology (cross-sectional area, needle thickness, width, perimeter, and roundness), shoot architecture, and net photosynthesis shared similar taxonomic classification (sections and subsections). Net photosynthesis increased with needle thickness and roundness. Species with vertical shoot arrangements were less drought tolerant presumably due to reduced boundary layer effects. Instantaneous water use efficiency was negatively correlated with carbon isotope discrimination (Δ). Hybrids had more leader growth than other species and subsections, yet were less drought tolerant (increased carbon isotope discrimination) suggesting trade-offs occur. Photosynthetic light response curves (light reactions) and A/C_i curves (dark reactions) were measured to further investigate differences in net photosynthesis among a subsample of five diverse species one hybrid. Curves differed depending on the method of expressing needle area, however, in all curves main differences were in net photosynthesis and not other curve parameters. Future tree improvement efforts should continue select species and provenances with increased drought tolerance. In addition, close relationships among species with similar taxonomic classification (subsections) should be used by plant collectors to identify species with potentially desirable characteristics for future introduction to the landscape industry.

Table 1. Analysis of variance of the date of budbreak and growing degree days (GDD) accumulated at budbreak in 17 *Abies* species at four locations in Michigan in 2004 and 2005.

| Source | df | Date of Budbreak | GDD |
		F Value	F Value
Species	16	73.39***	54.15***
Location	3	101.33***	33.68***
Year	1	5.41**	4.27*
Spp×Location	48	2.57***	2.66***
Spp×Year	16	1.57	2.62**
Location×Year	3	4.35***	7.51***
Spp×Loc×Year	44	0.84	1.08

*, **, *** p≤0.05, 0.01, 0.0001 respectively

Table 2. Analysis of variance of chlorophyll fluorescence values as a measure of cold hardiness in four *Abies* species near East Lansing, MI.

Source	df	F Value
Date	3	69.36***
Temperature	16	23.69***
Species	3	8.49***
Date×Temp	29	2.22**
Spp×Temp	48	6.54***
Spp×Date×Temp	87	0.91

*, **, *** p≤0.05, 0.01, 0.0001 respectively

Table 3. Equations depicting the relationship between net photosynthesis (PNA_{max}) and soil pH (Chapter 2, Figure 3).

Equation		R^2
CAN	$y = 14.45 - 1.67x$	0.70
ERN	$y = 11.34 - 0.69x$	0.34
NIK	$y = 9.75 - 0.66x$	0.44
SUB	$y = 13.65 - 0.96x$	0.32

$y = PNA_{max}$, $x = pH$

Table 4. Equations depicting the relationship between soil pH and A)N, C) P, and E) K and net photosynthesis (PNA_{max}) and B) N, D) P, and E) K (Chapter 2, Figure 5).

Graph	Equation	R^2
A	$y = 1.03 + 5.08x$	0.17
	$y = N$, $x = 1/pH$	
B	$y = 1.87 + 3.66x$	0.10
	$y = PNA_{max}$, $x = \sqrt{N}$	
C	$y = -0.97 + 1.30x$	0.20
	$y = Log\ P$, $x = 1/pH$	
D	$y = 10.26 - 0.60\ x$	0.17
	$y = PNA_{max}$, $s = \sqrt{P}$	
E	$y = 0.49 + 2.07x$	0.28
	$y = \sqrt{K}$, $x = 1/pH$	
F	$y = 1.26 + 6.46x$	0.26
	$y = PNA_{max}$, $x = \sqrt{K}$	

Table 5. Equations depicting the relationship between leader growth and foliar K (Chapter 2, Figure 6).

Equation		R^2
KxB	$y = 49.42 + 128.65x$	0.77
KxV	$y = 44.03 + 47.05x$	0.36
NIK	$y = 40.23 + 63.95x$	0.54
TUR	$y = 18.72 + 37.30x$	0.40

$y = Leader\ Growth$, $x = Log(K)$

Table 6. Pearson's correlation coefficients for soil pH, chlorophyll fluorescence, and 11 foliar nutrient elements of 17 *Abies* species at four locations in Michigan. Samples from October 2003 and 2004 were combined.

	F_v/F_m	PSA_{max}	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
pH	0.07	-0.25***	-0.47***	-0.43***	-0.46***	0.07	-0.19***	-0.28***	-0.39***	-0.37***	-0.07	0.03	-0.45***
F_v/F_m		0.01	0.35***	0.04	0.22***	-0.09*	-0.03	-0.06	-0.12**	-0.03	-0.07	0.07	0.14**
PSA_{max}			0.23***	0.36***	0.31***	0.18***	0.32***	0.39***	0.27***	0.34***	-0.03	-0.07	0.32***
N				0.46***	0.58***	0.01	0.36***	0.43***	0.21***	0.38***	-0.07	0.09*	0.59***
P					0.60***	0.22***	0.45***	0.50***	0.51***	0.51***	-0.02	0.002	0.45***
K						-0.06	0.28***	0.33***	0.36***	0.33***	-0.004	0.02	0.51***
Mg							0.49***	0.47***	0.26***	0.34***	-0.10*	0.12**	-0.004
Ca								0.47***	0.41***	0.56***	-0.15**	0.07	0.31***
S									0.45***	0.48***	-0.06	0.12**	0.33***
B										0.46***	-0.02	-0.04	0.30***
Zn											-0.04	0.18***	0.47***
Mn												-0.05	-0.02
Fe													0.08

*, **, *** Significant at $p \leq 0.05$, 0.01, or 0.0001, respectively

Table 7. Pearson's correlation coefficient for soil pH, chlorophyll fluorescence, and 11 foliar nutrient elements of 13 *Abies* species at four locations in Michigan sampled in October 2003.

	F_v/F_m	PSA_{max}	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
pH	0.01	-0.25***	-0.53***	-0.20**	-0.28***	0.31***	-0.06	-0.19**	-0.42***	-0.27***	0.05	0.12	-0.39***
F_v/F_m		0.0001	0.22**	0.11	0.17*	0.09	0.18**	0.29***	0.08	0.17*	0.001	0.02	0.12
PSA_{max}			0.23***	0.36***	0.31***	0.18***	0.32***	0.39***	0.27***	0.34***	-0.03	-0.07	0.38***
N				0.30***	0.39***	-0.18***	0.31***	0.54***	0.42***	0.38***	-0.08	-0.13*	0.49***
P					0.56***	0.25**	0.42***	0.60***	0.47***	0.45***	-0.01	-0.11	0.29***
K						-0.05	0.20**	0.47***	0.44***	0.37***	0.03	-0.11	0.38***
Mg							0.43***	0.28***	0.10	0.13*	-0.02	0.08	-0.13*
Ca								0.51***	0.39***	0.53***	-0.11	0.01	0.24**
S									0.54***	0.44***	-0.01	-0.04	0.40***
B										0.43***	-0.04	-0.09	0.40***
Zn											-0.09	0.05	0.38***
Mn												-0.09	-0.02
Fe													0.01

*, **, *** Significant at $p \leq 0.05$, 0.01, or 0.0001, respectively.

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Table 8, Tolerance of *Abies* species to soil pH levels at four test plots in Michigan in 2004. Tolerance based on decline in net photosynthesis with increasing soil pH.

<u>Intolerant of soils with pH > 5.0</u>

A. balsamea

A. balsamea var. phanerolepis

A. homolepis

<u>Intolerant of soils with pH > 5.5</u>

A. procera

<u>Intolerant of soils with pH > 6.0</u>

A. koreana x balsamea

A. koreana x veitchii

A. lasiocarpa

A. chensiensis

<u>Intolerant of soils with pH > 6.5</u>

A. fraseri

<u>Tolerant of soils with pH < 6.5</u>

A. bifolia

A. nephrolepis

Table 9. Mean photosynthetic rates expressed using total needle area (TNA_{max}) of 17 *Abies* species grown at four locations in Michigan in 2004. Gas exchange was measured in late June 2004 and early September 2004.

| | TNA_{max} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | |
| | Time | |
Species	June 2004***	September 2004**
A. lasiocarpa	3.76 abc	3.78 abc
A. nordmanniana	3.73 ac	3.37 abcd
A. bifolia	3.64 ac	3.72 abcd
A. nephrolepis	3.09 abcd	3.87 ab
A. chensiensis	3.02 abcd	3.82 a
A. nord. ssp. *equi-trojani*	2.94 abcdef	3.46 abcd
A. procera	2.91 abcde	3.80 ab
A. fraseri x *homolepis*	2.77 abcdef	3.51 abcd
A. koreana x *veitchii*	2.65 defg	3.68 abcd
A. koreana x *balsamea*	2.62 bdefg	3.17 abcd
A. balsamea	2.53 defg	3.10 bcd
A. bal. var. *phanerolepis*	2.16 defg	2.98 d
A. koreana	2.01 efg	3.45 abcd
A. homolepis	1.98 fg	3.26 abcd
A. veitchii	1.95 efg	3.12 bcd
A. fraseri	NA	3.06 bcd
A. holophylla	NA	3.32 abcd

Means within the same column and followed by the same letter are not significantly different α = 0.05, Tukey.

*, **, *** Significant at $p \leq 0.05$, 0.01, or 0.0001, respectively.

Table 10. F-values of PSA_{max} ratio of 17 *Abies* species grown at four locations in Michigan in 2003 and 2004.

Source	df	July 2003 F-Value	df	June 2004 F-Value	July 2004 F-Value	Sept 2004 F-Value
Loc	2	175.30 ***	3	9.13***	8.38***	25.28***
Species	13	6.78***	16	5.71***	4.37***	6.63***
S×L	22	1.50	48	1.15	1.82**	1.53*

*, **, *** p≤0.05, 0.01, and 0.0001 respectively

Table 11. F-values of PNA_{max} of 17 *Abies* species grown at four locations in Michigan in 2004.

Source	df	June 2004 F-Value	July 2004 F-Value	September 2004 F-Value
Location	3	9.07***	21.59***	43.90***
Species	16	7.36***	4.92***	3.57***
L×S	48	1.01	1.95**	1.37

*, **, *** p 0.05, 0.01 and 0.0001 respectively

Table 12. F-values of TNA_{max} of 17 *Abies* species grown at four locations in Michigan in 2004.

Source	df	June 2004 F-Value	September 2004 F-Value
Location	3	12.50***	40.04***
Species	16	7.98***	3.66**
L×S	48	1.35	1.46

*, **, *** p 0.05, 0.01 and 0.0001 respectively

Table 13. Analysis of variance for carbon isotope discrimination (Δ) and water use efficiency (WUE) of several *Abies* sub-sections grown at four locations in Michigan in 2004.

Source	Δ		WUE	
	df	F-Value	df	F-Value
Location	3	65.99***	3	21.66***
Sub-section	3	2.97*	6	1.78
L × S	9	0.55	18	0.78

Means within the same column and followed by the same letter are not significantly different $\alpha = 0.05$, Tukey.

*, **, *** p 0.05, 0.01 and 0.0001 respectively

Table 14. F-values of PSA/TNA ratio of 17 *Abies* species grown at four locations in Michigan in 2004.

Source	df	June 2004	September 2004
		F-Value	F-Value
Location	3	13.81***	17.69***
Species	16	12.26 ***	17.69***
L×S	48	1.47 *	1.66**

*, **, *** p 0.05, 0.01 and 0.0001 respectively

Table 15. F-values of TNA/PNA ratio of 17 *Abies* species grown at four locations in Michigan in 2004.

Source	df	June 2004	September 2004
		F-Value	F-Value
Location	3	7.25***	35.90***
Species	16	37.88***	25.04***
L×S	48	1.53*	1.59*

*, **, *** p 0.05, 0.01 and 0.0001 respectively

Table 16. F-values of PNA/PSA ratio of 17 *Abies* species grown at four locations in Michigan in 2004.

Source	df	June 2004 F-Value	July 2004 F-Value	September 2004 F-Value
Location	3	8.32***	8.32***	20.11***
Species	16	15.21***	9.64***	17.43***
L × S	48	1.41	1.46	2.43***

*, **, *** p 0.05, 0.01 and 0.0001 respectively

Table 17. F-values of photosynthetic light response curve parameters [dark respiration (Rd), apparent quantum efficiency (ϕ), net photosynthesis expressed using projected shoot area (PSA$_{max}$), and the light compensation point (LCP)] of six *Abies* species grown at four locations in Michigan in 2004.

Source	df	Rd	ϕ	PSA$_{max}$	LCP
Block(loc)	16	3.26***	2.10*	2.12*	2.35**
Species	5	3.86***	1.04	6.10***	4.46**
Location	3	27.12***	3.95*	25.54***	10.27***
S×L	15	1.07	1.36	1.60	0.95

*, **, *** p=0.05, 0.01, and 0.0001 respectively

Table 18. F-values of photosynthetic light response curve parameters [dark respiration (Rd), apparent quantum efficiency (ϕ), net photosynthesis expressed using projected needle area (PNA_{max}), and the light compensation point (LCP)] of six *Abies* species grown at four locations in Michigan in 2004.

Source	df	Rd	ϕ	PNA_{max}	LCP
Block(loc)	16	4.51***	1.77	2.76**	2.76**
Species	5	1.87	2.24	8.76***	3.05*
Location	3	25.98***	0.93	21.62***	12.07***
S×L	15	1.40	1.74	1.36	0.99

*, **, *** p=0.05, 0.01, and 0.0001 respectively

Table 19. F-values of photosynthetic light response curve parameters [dark respiration (Rd), apparent quantum efficiency (ϕ), net photosynthesis expressed using total needle area (TNA_{max}), and the light compensation point (LCP)] of six *Abies* species grown at four locations in Michigan in 2004.

Source	df	Rd	ϕ	TNA_{max}	LCP
Block(loc)	16	4.59***	1.82*	2.40**	11.22***
Species	5	1.53	1.60	7.79***	7.45***
Location	3	25.54***	0.66	29.18***	9.61***
S×L	15	1.00	1.81	1.52	1.45

*, **, *** p=0.05, 0.01, and 0.0001 respectively

Table 20. F-values of A/C_i curve parameters [respiration (R), apparent carboxylation efficiency (CE), and net photosynthesis expressed using projected shoot area (PSA_{max})] of six *Abies* species grown at four locations in Michigan in 2004.

Source	df	R	CE	PSA_{max}
Block(loc)	16	3.30**	2.14*	0.99
Species	5	6.88***	13.23***	3.19*
Location	3	9.98***	14.68***	3.00*
S×L	15	0.91	0.67	2.25*

*, **, *** p=0.05, 0.01, and 0.0001 respectively

Table 21. F-values of A/C_i curve parameters [respiration (R), apparent carboxylation efficiency (CE), and net photosynthesis expressed using projected needle area (PNA_{max})] of six *Abies* species grown at four locations in Michigan in 2004.

Source	df	R	CE	PNA_{max}
Block(loc)	16	2.04*	3.40**	1.07
Species	5	1.04	3.49**	3.03*
Location	3	1.20	4.26**	2.75*
S×L	15	1.40	1.38	1.79

*, **, *** p=0.05, 0.01, and 0.0001 respectively

Table 22. F-values of A/C_i curve parameters [respiration (R), apparent carboxylation efficiency (CE), and net photosynthesis expressed using total needle area (TNA_{max})] of six *Abies* species grown at four locations in Michigan in 2004.

Source	df	R	CE	TNA_{max}
Block(loc)	16	1.95*	5.40***	0.66
Species	5	0.59	1.55	1.83
Location	3	1.52	4.18**	4.13**
S×L	15	1.33	1.24	1.99

*, **, *** p=0.05, 0.01, and 0.0001 respectively

Table 23. Pearson's correlation coefficients for light response parameters [dark respiration (Rd), apparent quantum efficiency (ϕ), net photosynthesis expressed using projected shoot area (PSA$_{max}$), and light compensation point (LCP)] and soil pH, photosynthetic efficiency (F$_v$/F$_m$), 10 soil nutrients, shoot architecture, and carbon isotope discrimination (Δ). Response parameters are from six *Abies* species grown at four locations in Michigan is 2004.

	Rd	ϕ	PSA$_{max}$	LCP
pH	-0.32**	0.14	-0.04	0.22*
F$_v$/F$_m$	0.02	0.06	0.09	-0.04
N	-0.06	-0.01	0.08	0.10
P	0.21*	0.22	0.38***	-0.27**
K	0.24*	-0.06	0.27**	-0.21*
Mg	-0.28**	0.23*	-0.01	0.12
Ca	0.04	0.11	0.10	-0.13
Mn	0.23*	-0.22*	0.07	-0.19*
S	-0.13	0.20*	-0.15	-0.01
B	0.21*	0.03	0.18	-0.30**
Fe	0.07	-0.06	-0.32**	0.03
PNA/PSA	-0.28**	0.28**	0.11	0.22*
Δ	0.21*	-0.04	0.52***	-0.22*

*, **, *** p=0.05, 0.01, and 0.0001 respectively.

Table 24. Pearson's correlation coefficients for light response parameters [dark respiration (Rd), apparent quantum efficiency (ϕ), net photosynthesis expressed using projected needle area (PNA_{max}), and light compensation point (LCP)] and soil pH, photosynthetic efficiency (F_v/F_m), 10 soil nutrients, shoot architecture, and carbon isotope discrimination (Δ). Response parameters are from six *Abies* species grown at four locations in Michigan is 2004.

	Rd	ϕ	PNA_{max}	LCP
pH	-0.29**	-0.01	-0.11	0.04
F_v/F_m	-0.01	0.10	0.13	-0.01
N	-0.13	0.11	0.21*	0.12
P	0.13	0.25*	0.48***	0.12
K	0.16	0.16	0.46***	0.08
Mg	-0.30**	0.26**	0.08	0.01
Ca	-0.07	0.30**	0.30**	-0.02
Mn	0.25**	-0.15	0.08	0.03
S	-0.20	0.25*	-0.08	-0.21*
B	0.16	0.18	0.30**	-0.03
Fe	-0.08	0.17	-0.13	-0.19
PNA/PSA	-0.05	-0.23	-0.29**	0.01
Δ	0.20*	0.05	0.58***	0.25*

*, **, *** p=0.05, 0.01, and 0.0001 respectively.

Table 25. Pearson's correlation coefficients for light response parameters [dark respiration (Rd), apparent quantum efficiency (ϕ), net photosynthesis expressed using total needle area (TNA$_{max}$), and light compensation point (LCP)] and soil pH, photosynthetic efficiency (F$_v$/F$_m$), 10 soil nutrients, shoot architecture, and carbon isotope discrimination (Δ). Response parameters are from six *Abies* species grown at four locations in Michigan is 2004.

	Rd	ϕ	TNA$_{max}$	LCP
pH	-0.29**	0.14	-0.12	0.19
F$_v$/F$_m$	-0.01	0.10	0.13	-0.04
N	-0.14	0.11	0.25*	0.10
P	0.16	0.25*	0.43***	-0.29**
K	0.16	0.15	0.47***	-0.22*
Mg	-0.26**	0.24*	0.02	0.11
Ca	-0.06	0.32**	0.25*	-0.11
Mn	0.24*	-0.14	0.06	-0.24*
S	-0.18	0.26**	-0.13	0.04
B	0.18	0.15	0.23*	-0.29**
Fe	-0.06	0.18	-0.17	0.01
PNA/PSA	-0.07	-0.28**	-0.25**	0.18
Δ	0.18	0.09	0.59***	-0.19

*, **, *** p=0.05, 0.01, and 0.0001 respectively.

Table 26. Pearson's correlation coefficients for A/C_i response parameters [respiration (R), apparent carboxylation efficiency (CE) and net photosynthesis (PSA_{max})] expressed using projected shoot area and soil pH, photosynthetic efficiency (F_v/F_m), 10 soil nutrients, shoot architecture, and carbon isotope discrimination (Δ). Response parameters are from six *Abies* species grown at four locations in Michigan in 2004.

	R	CE	PSA_{max}
pH	-0.21*	0.37***	0.02
F_v/F_m	0.12	-0.12	-0.05
N	0.23*	-0.37***	0.07
P	0.04	-0.12	0.23*
K	0.19*	-0.23*	0.24*
Mg	-0.20*	0.22*	0.08
Ca	0.36***	-0.33**	0.06
Mn	0.05	-0.09	-0.06
Zn	0.19*	-0.18	0.05
S	-0.24	0.23	-0.12
B	0.02	0.09	0.03
Fe	0.15	-0.26**	-0.13
PNA/PSA	-0.44***	0.31**	0.07
Δ	0.26**	-0.20*	0.28**

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

Table 27. Pearson's correlation coefficients for A/C_i response parameters [respiration (R), apparent carboxylation efficiency (CE) and net photosynthesis (PNA_{max})] expressed using projected needle area and soil pH, photosynthetic efficiency (F_v/F_m), 10 soil nutrients, shoot architecture, and carbon isotope discrimination (Δ). Response parameters are from six *Abies* species grown at four locations in Michigan in 2004.

	R	CE	PNA_{max}
pH	-0.15	0.23*	-0.02
F_v/F_m	0.20	-0.11	-0.02
N	0.02	-0.21*	0.12
P	-0.12	0.08	0.26**
K	-0.03	-0.04	0.32**
Mg	-0.16	0.21*	0.07
Ca	0.01	0.01	0.27**
Mn	0.03	-0.02	-0.07
Zn	-0.10	0.09	0.20*
S	-0.19	0.25**	-0.09
B	-0.11	0.29**	0.09
Fe	-0.08	-0.09	-0.01
PNA/PSA	0.16	-0.17	-0.29**
Δ	0.07	-0.02	0.31**

*, **, *** $p \leq 0.05$, 0.01, and 0.0001 respectively

Table 28. Pearson's correlation coefficients for A/C_i response parameters [respiration (R), apparent carboxylation efficiency (CE) and net photosynthesis (TNA_{max})] expressed using total needle area and soil pH, photosynthetic efficiency (F_v/F_m), 10 soil nutrients, shoot architecture, and carbon isotope discrimination (Δ). Response parameters are from six *Abies* species grown at four locations in Michigan in 2004.

	R	CE	TNA_{max}
pH	-0.10	0.15	-0.06
F_v/F_m	0.10	-0.10	-0.02
N	0.01	-0.21*	0.12
P	-0.06	0.01	0.20*
K	-0.02	-0.05	0.29**
Mg	-0.08	0.12	0.01
Ca	0.07	-0.06	0.22*
Mn	-0.01	0.04	-0.01
Zn	-0.04	0.02	0.13
S	-0.13	0.16	-0.16
B	-0.04	0.20*	0.03
Fe	-0.03	-0.14	-0.08
PNA/PSA	0.17	-0.19	-0.27**
Δ	0.04	0.02	0.35**

*, **, *** $p \le 0.05$, 0.01, and 0.0001 respectively