VARIATION IN NITROGEN AND WATER RELATIONS TRAITS BETWEEN TWO BOREAL SPRUCE SPECIES FROM AN INTERACTING RESOURCE GRADIENT IN NORTHERN BRITISH COLUMBIA

by

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ABSTRACT

A comparative study of resource-use efficiency traits was made between two spruce species which grow across an interacting resource gradient in northern British Columbia. Near Fort Nelson BC, black spruce (*Picea mariana* (Moench) Voss) grows in the muskegs which are characteristically high in soil water content, and are extremely low in nutrient availability. In contrast, white spruce (*Picea glauca* (Mill.) B.S.P.) grows on drier sites which are not as limited in nutrient availability. Mixed stands containing these two species have not been reported in this area, suggesting strong selection pressures. Five contiguous populations of black and white spruce were sampled from the Fort Nelson region and grown in a growth chamber under varying water and nitrogen regimes. It was hypothesized that genetically based differences in character means, plasticity, and/or trade-offs associated with adaptation to nutrient or water limitation may contribute to maintaining these species' unique distribution patterns.

The results showed that black spruce was significantly less N-stress sensitive, exhibited greater phenotypic plasticity in nitrogen-use efficiency (NUE), and had a greater specific N absorption rate under high N conditions than white spruce. White spruce was more water-use efficient (as measured by carbon isotopic composition), more drought-tolerant, and allocated more biomass to roots than black spruce. When exposed to simultaneous limitations of N and water, both species exhibited intermediate levels of NUE and water-use efficiency (WUE), and hence were forced to utilize each resource with suboptimal efficiency. It was also found that, despite recent theories, a high relative degree of drought-tolerance did not trade-off with WUE or net photosynthesis rate (*A*) under well watered conditions, and that higher WUE was not associated with low *A* under ample or stressed water supply. On the other hand, specific N absorption rate and *A* were negatively correlated as expected. Water-use efficiency and NUE did not trade-off intrinsically (i.e., within treatments) for either species, but did trade-off plastically (i.e., across treatments). The species exhibited different slopes for the plastic WUE vs. NUE trade-off; this genotype by environment interaction effect may reflect specialization to their respective habitats.

Two main conclusions were derived: 1) certain trade-offs (such as WUE vs. *A*, drought-tolerance vs. *A*, WUE vs. drought-tolerance, WUE and drought-tolerance vs. root allocation, and WUE vs. NUE) may not be central to maintaining the distributions of black and white spruce, and thus may not represent costs, in all situations, to habitat specialization; and 2) physiological and morphological differences in black and white spruce may, in some instances, cancel at the whole-plant level, but could nevertheless have significant ecological consequences. Finally, the ecophysiological differences found between the two species imply that black spruce may be restricted to muskegs in areas near the southern border of the boreal forest because of an inability to compete on water-limited upland sites.
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<tr>
<td>Sb</td>
<td>black spruce</td>
</tr>
<tr>
<td>Sw</td>
<td>white spruce</td>
</tr>
<tr>
<td>WUE</td>
<td>water-use efficiency</td>
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<tr>
<td>NUE</td>
<td>nitrogen-use efficiency</td>
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<tr>
<td>PNUE</td>
<td>photosynthetic nitrogen-use efficiency</td>
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<td>PUE</td>
<td>phosphorous-use efficiency</td>
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<tr>
<td>$\delta^{13}C$</td>
<td>carbon isotope composition variable</td>
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<tr>
<td>A</td>
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<td>E</td>
<td>transpiration rate</td>
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<tr>
<td>$g$</td>
<td>conductance</td>
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<tr>
<td>$C_i$</td>
<td>intercellular CO$_2$ concentration</td>
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<tr>
<td>SAR</td>
<td>specific nitrogen absorption rate</td>
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<tr>
<td>RAC</td>
<td>root absorption capacity</td>
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<td>D-T</td>
<td>drought-tolerance</td>
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<tr>
<td>RGRmax</td>
<td>maximal relative growth rate</td>
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<tr>
<td>$\Psi_{xylem}$</td>
<td>xylem water potential</td>
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<tr>
<td>$\Psi_{soil}$</td>
<td>soil water potential</td>
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<tr>
<td>R/S</td>
<td>root:shoot ratio</td>
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<tr>
<td>R/LA</td>
<td>root:leaf area ratio</td>
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<tr>
<td>[N]</td>
<td>nitrogen concentration</td>
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<tr>
<td>PAR</td>
<td>photosynthetically active radiation</td>
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<tr>
<td>GxE</td>
<td>genotype by environment interaction</td>
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INTRODUCTION

Variations in resource supply are the major environmental factors that determine the distribution and abundance of plant species (Schulze and Chapin, 1987). Although the environmental regime that impinges upon each species is extremely complex it can often be broken down into the major components that most strongly affect plant growth and survival (Bryant et al., 1983). Two environmental factors of major importance in determining plant success, and hence in regulating species distributions, are nutrient and water availability (Schulze and Chapin, 1987).

In the boreal region, as a result of extreme climatological conditions, localized variations in slope, aspect, and drainage translate into large changes in nutrient and water availability over relatively short distances (Van Cleve et al., 1983ab). Characteristic forest types of the boreal region tend to be strikingly correlated with these microenvironmental patterns (Van Cleve et al., 1983a). Thus, comparative ecophysiological studies of nitrogen- and water-relations in boreal forest tree species may be ideal for applying and testing aspects of mechanistic theories in ecology (e.g., Grime, 1977; 1979; Tilman, 1988; 1990).

Black (Picea mariana (Mill.) B.S.P.) and white (P. glauca (Moench) Voss.) spruce are the dominant late successional climax conifer species of the boreal forest (Elliot-Fisk, 1988), and are sympatric throughout. Near the southern border (Map 1), however, they exhibit distinct microgeographic habitat preferences which conform to an interacting moisture and nutrient gradient. Black spruce is found predominantly in lowland muskegs which are poorly drained, and extremely low in available nutrients (Moss, 1952; Maycock and Curtis, 1960; Vincent, 1965; Roche, 1969; Small, 1972abc, Valentine, 1971; Weetman, 1979; Larsen, 1980; Van Cleve et al., 1983ab; Yarie, 1983; Grime and Anderson, 1986; Elliott-Fisk, 1988). White spruce, in contrast, grows on drier upland sites which are not limited in nutrient availability (Larsen, 1980; Van Cleve et al., 1983ab; Yarie, 1983; Grime and Anderson, 1986; Elliot-Fisk, 1988). Muskeg sites occur in level depressions which are often associated with permafrost (Van Cleve et al., 1983ab). Upland sites usually occur on steep slopes and display soil properties characteristic of semiarid regions (Van Cleve et al., 1983b). Although Sb and Sw exhibit similar levels of shade-tolerance (Elliot-Fisk, 1988) and stands are frequently found growing contiguously (DeLong et al., 1991), they do not form mixed stands (Valentine, 1971). This suggests that selection pressures acting on either species in the other's habitat may be extremely intense.

Despite the fact that most Sb is in the muskeg, it does not grow well there. Black spruce stands are considered the least productive boreal forest type (Larsen, 1980; Van Cleve et al., 1983b; Viereck et al., 1986). In contrast, Sw grows best on the upland sites where it is found, and Sw stands are often very productive (Maycock and Curtis, 1960; Vincent, 1965; Viereck et al., 1986; Elliot-Fisk, 1988; DeLong et al., 1991). Whereas Sw does not grow in muskegs at all,
MAP 1 - North American Boreal Forest Distribution

*from Elliot-Fisk (1988)
in the rare event that Sb gets established on upland sites (e.g., planted) it can grow very well, and often exhibits high productivity (Maycock and Curtis, 1960; Vincent, 1965; Black and Bliss, 1980; Elliot-Fisk, 1988; DeLong et al., 1991). Therefore, although Sb and Sw have contrasting realized (actual) niches, it appears that Sb may actually have the widest fundamental (potential) niche.

The habitats of Sb and Sw near the southern boreal border forest also differ in stand productivity. For this reason, the contrasting habitats may tend to favor species which differ in competitive ability (Grime, 1977; 1979; Grime and Anderson, 1986). For example, muskegs have open canopies suggesting that light competition is not very intense. In contrast, the well-drained upland sites exhibit a thick understory and are generally occupied by numerous tree species which form densely packed stands (Van Cleve et al., 1983a; Viereck et al., 1986). Thus, competition on upland sites is probably more intense than it is in muskegs (Grime, 1977; 1979; Grime and Anderson, 1986).

Current ecological theory predicts that the adaptive demands set by constraints in one type of resource-limited environment will trade off with the adaptive demands necessary for occupation of high resource environments or other types of resource-limited environments (Grime, 1977; 1979; Tilman, 1988; 1990). These unavoidable trade-offs may be the principal mechanism explaining species distributions across resource gradients. The adaptive demands required for occupation of the muskeg may contrast with those required in upland habitats.

Being congeneric species of similar growth form, gross phenological differences between Sb and Sw are not likely. Thus, adaptive responses in physiology and morphology may be the primary mechanisms by which these species cope with the environmental characteristics of their respective habitats. The goals of this study were to investigate the effects that nitrogen (N) and water supply have on various physiological and morphological traits that may be important to survival in environments limited in N or water availability. Because tree mortality peaks at the seedling stage (Franklin et al., 1987; Goldberg, 1990), the possession of particular adaptive characteristics in seedlings, or lack thereof, may be a critical determinant of later stand composition. Black and white spruce seedlings from adjacent populations located in northeastern British Columbia were grown under contrasting N and moisture regimes for a length of time approximating the first (establishment) growing season in their native habitats. An array of long-term, whole-plant N and water-relations traits were measured. Furthermore, because plants in nature are frequently faced with multiple limitations in resource availability, seedling responses to simultaneous N- and water-stress were also examined.
LITERATURE REVIEW

I. Nitrogen-Relations

Nitrogen is a critical element for plant growth and performance. Evans (1983; 1989) found that leaf N concentration ([N]) in wheat was positively correlated with photosynthetic rate per unit leaf area (A), which in turn is affected by soil N availability. High N supply has been shown to increase leaf production (Schulze and Chapin, 1987; Fichtner and Schulze, 1992; Nilsen 1992). Both greater A and leaf area will increase growth rate (Hunt, 1981; Robinson, 1991). In contrast, N deprivation induces a cascading sequence of events which may culminate in death. Decreased N-supply slows A and inhibits new leaf construction. Reduced growth rate produces a negative feedback on further N acquisition and hence growth. Eventually, leaf senescence and secondary nutrient deficiency symptoms, such as necrotic lesions and chlorosis, may arise (Chapin, 1980). This could be the primary cause of death, but mortality more likely results from increased susceptibility to herbivory, drought, frost, and other stresses.

As a result of its strong impact on plant growth, soil N supply can be a primary environmental control over species distributions (Tilman, 1984; Schlesinger et al., 1989; Berendse and Elberse, 1990). For example, Tilman (1984) constructed an artificial nutrient gradient and observed that species segregated across it within two years. Eutrophication of formerly nutrient-poor soils has led to the local extinctions of numerous plant species around the world (Berendse and Elberse, 1990); and nitrogen fertilization of wet heathlands in the Netherlands resulted in a species shift in proportional cover (Aerts and Berendse, 1988).

Adaptations that ameliorate the deleterious effects of N-stress could be important to survival in low N habitats. These adaptations fall into two categories. First are adaptations that act to increase a plant's internal N-status, and thereby slow or prevent severe internal N stress. These adaptations include: low RGRmax, high SAR, high R/S, and low phenotypic plasticity. The second type of adaptation is the ability to achieve greater rates of growth per unit internal N, and hence maintain physiological functioning with low internal N-status (i.e., increased nitrogen-use efficiency). These characteristics, although beneficial in low nutrient conditions, may be maladaptive or may trade off with performance under high nutrient conditions. Thus, variation in these characteristics may control species distributions across fertility gradients (Grime, 1977; 1979; Chapin, 1980; Tilman, 1988).
A. Adaptations that Increase Plant Nitrogen-Status

1. Significance:

   The maintenance of relatively high internal N-status has direct and indirect benefits in nutrient-poor habitats. Higher tissue [N] may be directly related to growth in nutrient poor environments because A increases with increasing leaf [N] (Evans, 1983; 1989; Field and Mooney, 1986). However, the chief benefits of improved N-status are derived indirectly, through risk aversion. First, a plant with higher tissue [N] may be less susceptible to further reductions in nutrient supply and other perturbations. For example, due to sufficient reserves, a plant with a higher tissue [N] may be able to rapidly recover from events such as herbivory, which are especially costly in nutrient limited environments (Bryant et al., 1983; Chapin et al., 1990). Second, higher tissue [N] may indicate a greater propensity for luxury consumption. Luxury consumption is the process by which N accumulates when absorption exceeds that used in growth (supply>demand). Luxury consumption allows a plant to store extra N during "good" periods to be utilized in future "bad" periods (Clement et al., 1979; Chapin et al., 1990). For example, Clement et al. (1979) showed that by repeatedly replenishing and depleting N stores, rye grass was able to maintain a constant growth rate despite being subjected to repeated cycles of N fertilization followed by deprivation (Clement et al., 1979).

   Luxury consumption is especially beneficial in habitats that are nutrient limited, but which experience periodic or seasonal nutrient flushes (Rorison, 1968; Chapin, 1988). Many environments are characterized by such conditions (Saebo, 1969; Chapin, 1988). For example, Saebo (1969) found that in boreal muskegs a large percentage of the total quantity of nutrients made available annually occurred during the spring as a result of freeze-thaw processes. Plants capable of luxury consumption could capitalize on spring nutrient flushes and then draw upon reserves to continue growth. On the other hand, plants that do not accumulate N may be at greater risk of experiencing extreme internal N-stress later in the season.

2. Maximal Relative Growth Rate:

   One of the most striking and commonly observed differences between plants adapted to soils of contrasting fertility is in maximal relative growth rate (RGRmax, defined as the efficiency with which each unit of plant biomass produces additional biomass under optimal conditions) (Rorison, 1968). For example, Grime and Hunt (1975) observed that species affiliated with highly fertile soils repeatedly had greater RGRmax than ones from nutrient stressed areas. This pattern has become a cornerstone of current mechanistic ecological theory (Grime, 1977; 1979; Tilman, 1988).

   There are several reasons why such variation may have arisen. The benefits of high
RGRmax in high nutrient adapted species are obvious. Habitats with high resource availability can support a large biomass and therefore are frequently highly competitive (Grime, 1977; 1979). Thus, individuals which can grow rapidly under high resource-competitive conditions (i.e., high RGRmax) will dominate areal and edaphic resources, and should be favored. Individuals with low RGRmax would be at a pronounced disadvantage in fertile habitats and would tend to be eliminated.

In contrast, low RGRmax in species from infertile soils may be a consequence of adaptations that increase growth under infertile conditions (Tilman, 1988), or may be an adaptation, in and of itself, to infertile habitats (Chapin, 1980). For example, allocative shifts that increase growth in low nutrient habitats may be deleterious on sites that are not limited in edaphic resources (see section I.A.4) (Chapin, 1993). On the other hand, low RGRmax may have evolved as a direct result of the benefits that it confers in sites of low fertility. Low RGRmax may be advantageous because it acts to allow the maintenance of greater internal tissue [N] relative to individuals with high RGRmax. For example, Chapin (1986) compared the RGR of several boreal species and found that species with low RGR tend to have greater nutrient concentrations than species with high RGR. Relative growth rate is negatively correlated with tissue [N] because plants with greater growth rates dilute N over a larger biomass (Lajtha and Klein, 1988). Moreover, because of less nutrient demand, plants with low RGRmax may be especially capable of luxury consumption in conditions of high N availability (Chapin, 1980). As this may be beneficial in low nutrient habitats that experience seasonal flushes, plants with high RGRmax may be less able to occupy such habitats. Whether low RGRmax evolved indirectly as a result of trade-offs, or directly as a result of the benefits is inconsequential. The fact that low RGRmax has advantages for survival in sites of low fertility means that individuals with low RGRmax may be more apt to inhabit them than ones with high RGRmax.

Low nutrient adapted species, due to lower RGRmax, are often less nutrient stress-sensitive (Shipely and Keddy, 1988). That is, growth rate in low nutrient adapted species may be less coupled to, or dependent on, soil nutrient supply than in species from high nutrient sites. For example, Gray and Schlesinger (1983) found that Salvia leucophylla Greene., a species associated with high N soils, decreased RGR in response to N-stress more than Ceanothus megacarpus Nutt., a species from low nutrient habitats (less N-stress sensitive). Moreover, Shipley and Keddy (1988) conducted a comprehensive survey of species from sites of contrasting nutrient availability, and found that species with low RGRmax consistently exhibited less reduction in growth as a result of N-stress than species with higher RGRmax. Low stress sensitivity is beneficial because it allows a plant to operate closer to its physiological optimum while stressed for N (Chapin, 1980). As a result, a plant's susceptibility to the effects of increased nutrient stress, as well as other potential stresses, will be reduced. Fichtner and Schulze (1992) argued that the
primary adaptation of non-nitrophilic annuals to their habitats may be low N stress-sensitivity resulting from low RGRmax. Their conclusions reveal the importance that this adaptation may have for occupying habitats of low fertility. However, low stress-sensitivity also means that a plant is less able to plastically respond to increased nutrient supply. Therefore, low stress-sensitivity may be disadvantageous in competitive and/or high nutrient habitats (Grime, 1977; 1979).

3. Specific N Absorption Rate:

The effects of RGR on plant internal N-status may be offset or enhanced by the rate roots are able to absorb N. Specific N absorption rate (SAR) is defined as the amount of N absorbed per unit root biomass per unit time (e.g., g N/g root/d) (Welbank, 1962), and will significantly influence plant tissue [N] (Gulmon and Chu, 1981; Gray and Schlesinger, 1983; Schlesinger et al., 1989; Birk and Vitousek, 1986). For example, Birk and Vitousek (1986) found that loblolly pine seedlings with greater SAR had a better internal N-status than seedlings with low SAR. In addition, during seasonal nutrient flushes a plant with a higher SAR may exhibit greater propensity for luxury consumption, and/or the ability to exploit soil nutrient reserves and thereby "starve" neighboring individuals (Chapin, 1980; Crick and Grime, 1987). Thus, by increasing N-status and avoiding deleterious effects of severe internal nutrient deprivation, plants with higher SAR may be more apt to survive in nutrient deficient habitats (Clarkson and Hanson, 1980).

Specific root absorption rate is controlled by environmental as well as physiological factors (Nye, 1977; Nye and Tinker, 1977). Several studies have demonstrated that SAR increases with increasing N-availability (Gulmon and Chu, 1981; Gray and Schlesinger, 1983; Birk and Vitousek, 1986). Nutrient cycling, mineralization, parent material, uptake, as well as environmental factors that influence microbial activity all play a role in defining the availability of nutrients in the soil of a particular habitat (Fitter and Hay, 1987). For example, the cold, acidic, and water-logged conditions of northern sphagnum peat bogs inhibit the breakdown of organic matter, causing the availability of nutrients in these bogs to be extremely low (Small, 1972abc; Marchand, 1975; Van Cleve et al., 1983ab).

Specific N absorption rate is also influenced by physiological capacity to absorb nutrients. This capacity, or root absorption capacity (RAC), affects SAR by regulating the amount of nutrients that pass into the plant (Fitter and Hay, 1987). Root absorption capacity is controlled by the quantity of active uptake sites a plant produces, mycorrhizal associations, and a plant's internal [N] (Chapin and Tryon, 1983; Chapin, 1980; 1986; 1988). Root absorption capacity is positively correlated with SAR, and therefore is beneficial for occupying low nutrient habitats (Chapin, 1986; 1988; Chapin and Tryon, 1983). However, Tilman (1988) suggested that plants are faced with a trade-off for constructing N-absorbing and carbon-acquiring enzymes. If so, a major cost of
increased RAC (and SAR) may be decreased photosynthetic capacity. Due to this potential trade-off, plants that adapt to low nutrient conditions through increased RAC may be less able to occupy habitats not limited in nutrients.

Root absorption capacity is highly plastic in response to nutrient availability (Chapin and Tryon, 1983; Chapin, 1986; 1988). Chapin (1986) and Chapin and Tryon (1983) found that the RAC for P increased with decreasing P availability in every species they examined. This suggests that reductions in SAR caused by low N-availability (above) may be partially off-set by plastic increases in RAC.

Evidence pertaining to the importance of SAR as an adaptation to habitats of low fertility is thus far inconclusive. For example, Chapin (1986) found that species from high nutrient sites had greater RAC for P than those from sites of less fertility (i.e., greater SAR when grown with similar supplies of P). Unfortunately, this study (Chapin and Tyron, 1983; Chapin, 1986) was conducted on roots collected directly from the field, and the results could reflect, at least partially, residual environmental and/or ontogenetic effects. On the other hand, Bloom (1985) found that varieties of barley adapted to soils of contrasting fertility had similar uptake kinetics (i.e., RAC) for N. In addition, the high N-adapted species, *Arrhenatherum elatius* (L.) Beauv. ex. J. and C. Bresl., had greater SAR than the low N-adapted species, *Festuca ovina* L., under high N-supply rates, whereas the reverse was true under low N-supply rates (Grime, 1991). Clearly, more research is required to fully understand the importance that increased SAR may have in promoting survival in nutrient limited habitats.

Although most species can plastically change RAC, species from low nutrient sites generally tend to be less able to reduce RAC when exposed to high levels of nutrients than species adapted to high nutrient environments (Chapin, 1980). Not decreasing RAC in high nutrient conditions may be a mechanism whereby low nutrient adapted species maximize luxury consumption (e.g., during seasonal flush periods). However, because of associated costs, lack of plasticity in RAC may be maladaptive where luxury consumption is not needed (Tilman, 1988).

4. Biomass Allocation:

Biomass allocation patterns may also affect a plant's propensity to occupy infertile habitats. It is well established that plants increase root allocation over shoot allocation (i.e., R/S) in response to N limitation (Davidson, 1969; Gulmon and Chu, 1981), and that plants from low nutrient habitats tend to have intrinsically greater R/S ratios than ones from fertile habitats (Grime, 1977, 1979; Chapin, 1980; 1988). Nevertheless, this tendency is not universal. Chapin (1986) reported no consistent difference in root:leaf ratio in taiga tree seedlings despite the fact that they naturally occur across a productivity (i.e., nutrient) gradient, and Chapin et al. (1982) found that plants from P-limited soils had lower R/S than ones from more fertile soils.
The chief benefit of increased R/S is an improvement in internal N-status. Internal N-status increases when the supply of nutrients exceeds demand. For example, larger roots will promote N uptake, but less shoot allocation means the absorbed N will be distributed over less shoot biomass. In some instances, however, a plant with a large R/S may exhibit a lower total tissue [N] if roots have lower [N] concentration than shoots (Chapin, 1980; Nilsen, 1992; but see Shaver and Melillo, 1984). As a result of less shoot biomass, a plant with a large R/S will have proportionally less photosynthesizing surface (e.g., leaf area). Hence, the major cost to a high R/S is that it trades off with RGR in nutrient rich habitats (Robinson, 1991; Chapin, 1993). This type of trade-off may be crucial in determining how species segregate across fertility gradients (Tilman, 1988).

Species from low nutrient soils tend to have less plastic R/S than high N adapted ones (Grime, 1977; 1979; Chapin, 1980; Crick and Grime, 1987). A "hard-wired" but large R/S may be an adaptation to capitalize on pulsed nutrient flushes by maximizing luxury consumption, instead of reducing R/S during flush periods as a high nutrient adapted species would. In fact, Crick and Grime (1987) argued that more stable patterns of root development in *Scirpus sylvaticus* L. (low nutrient species) compared to *Agrostis stolonifera* L. (high nutrient species) give it a selective advantage in the strongly nutrient limited environments, characterized by temporal pulses of higher availability, in which it lives.

5. Phenotypic Plasticity:

From the above discussion it is apparent that species from low compared to high nutrient habitats generally exhibit less plasticity in RGR, RAC, and R/S. Bradshaw (1965) argues that plasticity is selected for in environments characterized by temporal and spatial heterogeneity occurring on scales less than a single life span or within a habitat, respectively. Grime (1977; 1979) asserts that habitats of low resource availability are characteristically less heterogeneous than habitats of high resource supply (e.g., high resource habitats may exhibit local and temporal depletion zones due to high levels of crowding and competition). Thus, species from favorable habitats should, according to Bradshaw (1965), have greater plasticity than species from low resource environments. This pattern is generally found to be the case (Grime, 1977; 1979; Chapin, 1980).

Since they would be less competitive, it is easy to see why a lack of plasticity may prevent low nutrient adapted species from occupying sites of high fertility (Grime, 1977; 1979; Crick and Grime, 1987). However, it is not immediately apparent why high nutrient adapted species, being highly plastic, are not found in low resource environments. Two explanations may account for this. First, high plasticity does not necessarily equate to greater actual character expression. A plant with higher plasticity in R/S, for example, may have very low root allocation in nutrient rich conditions. Thus, despite a great ability to increase R/S under N-stress, it may nevertheless exhibit
a lower R/S in stressed conditions than a less plastic low nutrient adapted species. Second, low plasticity, in and of itself, may be adaptive and high plasticity may actually be maladaptive in low nutrient habitats. By not decreasing RAC and R/S, or increasing RGR, low nutrient adapted species may be better equipped to accumulate and store N during nutrient flushes. Plants that are highly plastic would respond strongly to nutrient flushes with significant decreases in RAC and R/S, and increases in RGR. This response may increase immediate growth, but would also decrease, or prohibit, luxury consumption (Grime, 1977; 1979; Chapin, 1980; Crick and Grime, 1987).

B. Nitrogen-Use Efficiency

A plant with higher nitrogen-use efficiency (NUE) than another will achieve greater growth per unit N taken up, and exhibit an enhanced ability to function under low internal N concentrations. Therefore, increased NUE may be a mechanism by which plants adapt to N-limited conditions (Clarkson and Hanson, 1980). Nitrogen-use efficiency can be estimated as the inverse of nitrogen concentration (g biomass/g N), or as the ratio of carbon-content to nitrogen-content in total plant tissue (g C/g N) (Chapin, 1980; Lajtha and Klein, 1988). A plant with a high NUE, then, will exhibit lower internal [N] than one with low NUE. Nitrogen-use efficiency is a complex characteristic which may be regulated by several physiological and morphological attributes.

All plants exhibit higher NUE when grown under conditions of low N availability (Chapin, 1980). Three processes have been proposed to account for this pattern. First, tissue N concentrations may be reduced as a result of a mobilization and utilization of stored N reserves, or, a prevention of luxury consumption (Chapin, 1980; 1988; Vitousek, 1982; Birk and Vitousek, 1986; Shaver and Melillo, 1984). Second, a larger proportion of biomass may be allocated to tissues with low N concentrations, such as roots over foliage (Chapin, 1980; Shaver and Melillo, 1984). Third, plants may shift from primarily stomatal to non-stomatal limitations on photosynthetic rate, enabling higher A per unit leaf N (high photosynthetic NUE (PNUE)) (Vitousek, 1982; Chapin et al., 1987; Reich and Schoettle, 1988).

Although each of these processes may, in theory, increase NUE, direct linkages between them and NUE are dubious. For example, Reich and Schoettle (1988) found a positive correlation between PNUE and NUE, and suggested the two are interchangeable measures of how efficiently a plant utilizes N. However, DeLucia and Schlesinger (1991) found no such correlation. Shaver and Melillo (1984) found that the root and shoot tissues of several marsh plants had equivalent nutrient concentrations; thus, observed increases in NUE resulted from processes other than changes in R/S. It appears that changes in NUE may be a function of some of these processes
some of the time, or possibly others yet to be defined. In fact, Clarkson and Hanson (1980) stated that it was not possible to explain how some genotypes produce a given amount of growth using smaller amounts of nutrients than are required by others. Nevertheless, the ecological consequence of increased NUE, regardless of the precise underlying mechanisms, will be greater growth on a particular soil N supply.

Due to the benefits of increased growth, selection in N limited habitats should favor individuals that require lower tissue N levels. In practice, however, when grown in common conditions, differences in nutrient-use efficiency between species from sites of contrasting nutrient availability do not always correlate with habitat. For example, Chapin (1983) grew a wide range of New Zealand tussock grasses, and several taiga tree species under contrasting P regimes. In general, it was found that species associated with infertile soils tended to have greater tissue P concentrations (lower PUE) under both high and low conditions than those from more fertile areas (Chapin, 1983). Similarly, Berendse and Elberse (1990) found that *Pinus sylvestris* L., which occurs in nutrient poor sites, had lower NUE than *Paulownia tomentosa* (Thunb.) Steud., which is characteristic of nutrient rich habitats. On the other hand, Schlesinger et al. (1989) found that American Great Basin species from low nutrient habitats had greater NUE than those associated with richer soils when grown under both high and low nutrient conditions. These conflicting findings suggest that selection for increased NUE may be environment and species specific.

The characteristics of the nutrient environment in which plants grow may influence the adaptive significance of NUE. In general it appears that under high nutrient growth conditions a low NUE is beneficial if individuals come from habitats with fluctuating levels of N availability, and a high NUE is adaptive if individuals are endemic to habitats with constant supplies of N. Because luxury consumption increases internal N reserves it decreases NUE. Thus, low NUE (in individuals grown under ample N supply) may reflect adaptation to infertile habitats that favor luxury consumption, i.e., ones with seasonal nutrient flushes (Chapin, 1980). If so, this may explain why low nutrient adapted species exhibit lower nutrient-use efficiency when grown in high nutrient conditions (e.g. Chapin, 1983; Berendse and Elberse, 1990). On the other hand, in conditions where nutrient supplies are high, storage is not necessary. In such situations, storing nutrients could reduce growth since latent N would be wasted N. Thus, high nutrient adapted species, when grown under high nutrient conditions, may exhibit higher NUE because it reflects a lack of luxury consumption. In environments that are chronically low in available nutrients, luxury consumption may not be possible, favoring increased NUE. In the Great Basin, seasonal flushes of nutrients are unlikely due to a lack of freeze-thaw processes and precipitation. This may explain why Great Basin species from low nutrient soils had intrinsically greater NUE (under high nutrient conditions) than ones from richer soils (above, Schlesinger et al., 1989).
When grown under low nutrient conditions, high nutrient-use efficiency, for the reasons outlined above, is clearly adaptive. This raises questions as to why high nutrient adapted species had higher PUE than those from less fertile soils in Chapin's (1983) study. One explanation for this anomalous pattern may be that other nutrient-relations traits may trade off with the ability to utilize nutrients efficiently. For example, N-stress avoiding mechanisms, such as low RGRmax, SAR, and R/S, each function to maintain high internal N concentrations (see above). Where as, increased NUE, by definition, is the ability to maintain greater growth per unit of internal N and thereby operate at lower internal N concentrations. This suggests that nutrient-use efficiency and mechanisms that increase internal nutrient-status may represent alternative modes for adapting to nutrient limited environments. If so, this may explain why species from low nutrient habitats in Chapin's (1983) study had lower PUE than species from high P sites.

C. Other Plant Characteristics

Other plant characteristics that influence the ability to occupy nutrient poor soils include: susceptibility to foliar leaching and herbivory, degree of resorption and ability for nutrient re-translocation, and, leaf and life longevity. Plants with sclerophyllous leaves are often associated with sites of low nutrient availability (Small, 1972abc). Sclerophyllly may reduce rates of leaching and herbivory, and thus could be extremely beneficial in sites of low nutrient availability due to extremely high costs of replacing lost nutrients (Bryant et al., 1983; Chapin et al., 1990). Plants adapted to low nutrients tend to have greater ability to resorb, and hence recycle, nutrients prior to leaf abscission than plants from high nutrient habitats (Chapin, 1980; Bryant et al., 1983; Birk and Vitousek, 1986; but see Chapin and Kedrowski, 1983). A large percentage of plants in low nutrient habitats are evergreen (Small, 1972ab; Chapin, 1980; Gray, 1983; Gray and Schlesinger, 1983). Greater leaf longevity associated with the evergreen habit is advantageous because every time a leaf is shed, it carries with it approximately half its N and P pool (Bryant et al., 1983). The conspicuous lack of annuals in low nutrient habitats suggests that long life spans may be important for survival under nutrient stress (Small, 1972; Chapin, 1980; Bryant et al., 1983). Long lived species (e.g., trees) may be able to internally recycle nutrients throughout their lives, and thereby have a proportionally lower annual demand for nutrients (Bryant et al., 1983). Many studies have incorporated the above characteristics into analysis of species nutrient relations across fertility gradients (Small, 1972ab; Vitousek, 1982; Bryant et al., 1983; Shaver and Melillo, 1984; Birk and Vitousek, 1986). However, when comparing closely related species of similar growth form, many of these characteristics will not differ. As a result, mechanisms contributing to the segregation of closely related species across nutrient gradients may be principally related to other types of adaptations as described in the preceding sections.
D. Conclusions

Apparent in this review is a conspicuous lack of consistent correlations between habitat preference, nutrient availability, and adaptive traits. These inconsistencies appear, at first, to question the adaptive significance of the reputed N-relations characteristics outlined above. However, if the patterns arose as a result of trade-offs amongst traits, they could be explained without detracting from their potential importance. For example, since characters that act to increase internal N-status (low RGRmax, high SAR, large R/S) are somewhat redundant, plants exhibiting high development in one attribute may not require high levels of the others. Or, because NUE appears to trade off with attributes that act to increase N-status, it is possible that some species could have high NUE and less well developed mechanisms for increasing N-status, whereas others may exhibit the opposite trend. Finally, if species in some studies had radically different growth form, or evolutionary history, it is possible they could employ other types of adaptations (i.e., long life, sclerophyll, etc.). As a result of these types of potential trade-offs, it is not surprising that species from nutrient limited habitats exhibit variation in N-relations traits. Not only may this possibility explain the inconsistencies in the literature, but it also exemplifies the necessity of measuring an array of potential adaptive traits in order to understand the means by which plants are adapted to nutrient limitation.

II. Water-Relations

Because air is generally dry relative to tissue, terrestrial plants are threatened with potential desiccation. Mechanisms that offset this potential vary in a manner related to the moisture environment to which species are adapted. Thus, the particular mechanisms that a species exhibits may allow persistence in certain habitats but, because of associated costs, may restrict survival in others.

Trees generally resist the deleterious effects of drought by 1) avoidance through enhanced water absorption and/or decreased water loss, or, 2) tolerance through adaptations that enable physiological activity at high levels of internal dehydration. Two indices that gauge a plant's relative ability to avoid or tolerate drought are water-use efficiency (WUE) and drought-tolerance (D-T). Water-use efficiency is the amount of carbon fixed by a plant per unit water lost (e.g., \( WUE = \frac{\text{carbon fixed}}{\text{water transpired}} = \frac{A}{E} \)). Drought-tolerance (D-T) is the relative ability to maintain positive rates of photosynthesis at low soil and internal water potentials (DeLucia and Heckathorn, 1989). Several interrelated physiological and morphological characteristics determine plant WUE and D-T. The relative degree of each can have profound ecological ramifications.
A. Mechanisms and Ecological Aspects of Water-Use Efficiency

High WUE can be advantageous in droughted environments because it enables a plant to acquire more carbon per unit water, and hence achieve greater rates of growth. Genotypic variation in WUE can be attributed to three mechanisms: leaf conductance, photosynthetic capacity, and biomass allocation. These mechanisms interact with each other to establish an individual's particular WUE. The relative contribution that each of these mechanisms makes to the particular plant's WUE may differ amongst species, and could have important ecological implications.

Conductance ($g$), the inverse of resistance, measures how easily CO$_2$ and H$_2$O molecules are able to pass in and out of a leaf. Conductance is positively correlated with net photosynthetic rate ($A$) (except at very high conductances) and transpiration rate ($E$) (DeLucia and Heckathom, 1989), and therefore has an important influence on WUE. Plant characteristics which affect $g$ include stomatal diameter, functioning and depth, as well as leaf shape, size, and degree of pubescence (Blake et al., 1984). Plants actively manipulate $g$ according to environmental stimuli through stomatal adjustment in the short term, or by altering other characteristics in the long term (Nobel, 1983). Most plants decrease $g$ in response to water stress (Nobel, 1983). Genotypes, as a result of specialization to habitats with contrasting water regimes, may exhibit genetic variation in $g$ (Blake et al., 1984; Condon et al., 1987; Martin and Thorstenson, 1988; Meinzer et al., 1990). Generally, species from xeric sites exhibit intrinsically lower $g$ than those from more mesic or hydric sites (Cowan, 1982). Low $g$, derived via either plastic manipulations or genetic variation, is advantageous in droughted situations because WUE is increased and $E$ is reduced.

Water-use efficiency increases with decreasing $g$ because $A$ is reduced proportionally less than $E$. This occurs for two reasons. The first is, CO$_2$ has a longer diffusion path length than H$_2$O and hence experiences a larger net resistance to flow. Carbon dioxide is fixed in the mesophyll so must pass into the mesophyll cells. Water vapor does not encounter this resistance and is only affected by the boundary layer and stomatal resistances. Hence, an increase in boundary layer or stomatal resistance causes the net resistance to CO$_2$ flow to be affected proportionally less than that of H$_2$O (Rawson and Begg, 1977). Another reason $A$ is decreased proportionally less than $E$ is that the intercellular concentration of CO$_2$ ($C_i$) will be less at low $g$ (due to uptake). This causes a greater diffusion gradient between the leaf and atmosphere, helping to maintain the flow of CO$_2$ into the leaf (Guy and Reid, 1986).

Although a low $g$ reduces $E$ and increases WUE, it also slows $A$ and hence growth (Condon et al. 1987; Martin and Thorstenson, 1988; Meinzer et al., 1990). Under conditions of ample water, a high WUE via reduced $g$ will confer no benefits (since water is not limiting to growth), but slowed $A$ could decrease competitiveness. Because of this trade-off (WUE vs. $A$),
species that have adapted genetically to low water conditions through decreased $g$ (i.e., increased WUE) could be excluded from sites with high water availability (Gerber and Dawson, 1990). On the other hand, plants adapted to high water conditions via increased $A$ (due to high $g$) may, because of lower WUE, exhibit less growth in droughted conditions. For these reasons, interspecific variation in WUE may cause differential species distributions across moisture gradients. Another cost to increased WUE may be lower photosynthetic N-use efficiency (PNUE) (Field et al., 1983; see section III).

Environmental and genotypic variation in photosynthetic capacity will also cause WUE to differ (Bennett and Rook, 1978; Condon et al., 1987; Hubick et al., 1986; Mastle and Farquhar, 1988). Photosynthetic capacity varies either because of greater or lesser ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) activity or because of differences in photorespiration (O'Leary, 1988). If the photosynthetic capacity of a plant is greater than another, and the two have similar $g$, then the one with higher photosynthetic capacity will exhibit a greater intrinsic WUE (due to greater $A$ and equal $E$). Therefore, a high WUE caused by a large photosynthetic capacity, in contrast to one caused by decreased $g$, should be positively correlated with CO$_2$ assimilation rate and dry matter production (Hubick et al., 1988; Meinzer et al., 1990; O'Leary, 1988). Photosynthetic capacity can also be influenced by environmental factors which will, in turn, affect WUE. For example, photosynthetic capacity may decrease with severe, or long term drought (Farquhar et al., 1989b). This can result from N re-allocation away from stomatally induced inactivity of photosynthetic machinery (e.g., decreasing RUBISCO), due to direct effects on metabolic functioning, or via increased susceptibility to photoinhibition (Farquhar et al., 1989b). Similarly, N availability may alter photosynthetic capacity, and hence WUE, by increasing or decreasing the quantity of photosynthetic proteins, particularly RUBISCO (Evans, 1989). Thus, it appears that in situations of extreme water-stress and/or N-stress WUE may be decreased.

Superficially, a high WUE derived from improved photosynthetic capacity should be beneficial in dry as well as wet habitats. This may not always be true because of associated costs or risks. For example, if $g$ is not lowered, a plant with high WUE (via improved photosynthetic capacity) could deplete soil water reserves rapidly and thereby experience symptoms of drought stress early (Bennett and Rook, 1978). In addition, by decreasing the internal concentration of CO$_2$, a high photosynthetic capacity can decrease PNUE (Field et al., 1983). These costs will put constraints on the adaptive value of high WUE, and could have important effects on species distributions.

Differences and changes in root:shoot ratio (R/S) between individuals and environments can cause variability in WUE (White et al., 1990; Cohen, 1970). Plants from low water habitats generally have intrinsically greater R/S than those from sites of high moisture, and, many species plastically increase root allocation (R/S) in response to water stress (Grime, 1977; 1979; Chapin,
By delaying water stress through increased uptake capacity and reduced transpirational leaf surface area, a large R/S makes physiological or morphological mechanisms for reducing transpiration (e.g., low $g$) less necessary. An empirical confirmation of this was made by White et al. (1990) who found that WUE was negatively correlated with root length density in *Phaseolus vulgaris* L. The benefit of developing a larger R/S is a postponement of dehydration which allows prolonged maintenance of relatively large $g$, and hence greater $A$ and growth during drought (Jackson and Spomer, 1979; White et al., 1990). A cost, though, is in partitioning resources to below ground structures instead of above ground structures for photosynthesis. Because of this cost, a larger R/S should primarily be adaptive in moisture limited environments in which above ground competition is not very intense.

Variation in WUE between individuals can be attributed to differences in $g$, photosynthetic capacity and R/S, alone or in combination. For example, Condon et al. (1987) found that differences among wheat genotypes in WUE were approximately equally attributable to variations in leaf conductance and photosynthetic capacity. Also, Blake et al. (1984) found that high WUE in poplar clones grown with ample water was correlated with lower rates of conductance, but failed to hypothesize how this could lead to the greater productivity observed by these clones relative to ones with low WUE. If theory is correct, then these poplar clones probably had higher photosynthetic capacity as well. Because each component of WUE may have unique costs and benefits (above), knowing how they contribute to the specific WUE that an individual exhibits is crucial for determining the manner by which it will interact with other individuals, and its propensity to survive in environments with contrasting moisture regimes.

A few studies have correlated relative degree of WUE in plants with habitat. In general, selection in more water limited environments seems to favor high WUE (Mooney et al., 1978; Field et al., 1983; Carter and Smith, 1988; Monson and Grant, 1989; Read and Farquhar, 1991). Monson and Grant (1989) sampled offspring derived from *Pinus ponderosa* Laws. populations from coastal habitats and from crosses between coastal (relatively wet) and interior (dry) populations. They found that the interior crosses had an intrinsically higher WUE (caused by low conductance) than the crosses from populations on the coast (Monson and Grant, 1989). Carter and Smith (1988) attempted to determine a causal explanation for the distribution patterns of trees in a subalpine forest in the central Rocky Mountains, where lodgepole pine (*Pinus contorta* spp. *latifolia* Engelm.) tended to grow on drier sites than Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.) and subalpine fir (*Abies lasiocarpa* (Nutt.) Hook.). Lodgepole pine had the greatest WUE (through reduced conductance) and the authors concluded that these distinct distribution patterns can be attributed to differences in water relations between the various species (Carter and Smith, 1988).

Although the above studies suggest that high WUE is adaptive in water-limited and xeric
areas it is not a prerequisite for survival and success in these types of habitats. For example, a plant with large roots will have the capacity to take up ample water and may not need a high WUE (Cohen, 1970; White et al., 1990; Read and Farquhar, 1991). In fact, high WUE may not always be adaptive in water-limited habitats. For example, Bennett and Rook (1978) observed that a Pinus radiata D.Don. clone with a high WUE had a lower relative survival rate when water was withheld than another less water-use efficient clone. This occurred because the clone with a higher WUE also had a high rate of transpiration (i.e., improved WUE was due to increased photosynthetic capacity); and the clone with low WUE had a low rate of transpiration but an even lower photosynthetic capacity (Bennett and Rook, 1978). In addition, if a high WUE is derived from a reduction in conductance, then the water that is saved could be lost either to uptake by a competing plant, or by evaporation or seepage (Cohen, 1970; Bunce et al., 1977; DeLucia and Heckathorn, 1989; DeLucia and Schlesinger, 1991). If soil water is depleted in this manner then the benefits of conserving water by employing a high WUE are diminished, and the costs of slowed growth are amplified.

Empirical studies have demonstrated that high WUE is not always a beneficial strategy in moisture limited environments. DeLucia and Heckathorn (1989) and DeLucia and Schlesinger (1991) found that Sierran pines were actually out-competed in certain areas of the Great Basin desert because they employed a higher WUE than their competitors. The Sierran pines, consequently, were restricted to places of higher moisture than species with lower WUE (DeLucia and Heckathorn, 1989). The adaptive significance of high WUE is questioned by these data.

B. Mechanisms and Ecological Aspects of Drought-Tolerance

The relative degree of D-T expressed by a plant is usually associated with its cell wall elasticity and/or its ability to undergo active changes in osmotic potential. Either mechanism will allow a plant to maintain turgor and positive rates of photosynthesis at decreasing water potentials (tolerance). Allocation patterns may also have direct and indirect effects on D-T. All three of these components will contribute to the relative degree of D-T that a plant possesses.

Decreasing osmotic potential is a common response of plants to water stress (Tyree and Jarvis, 1982; Robichaux, 1984; Abrams, 1988). Osmotic adjustment is achieved through ion accumulation (particularly K+) and through synthesis of osmotica such as proline, sorbitol, betaine, glutamic acid and others. The organic osmotica are called "compatible" solutes because they can be tolerated at high concentrations in the cytosol (Tyree and Jarvis, 1982). Osmotic adjustment lowers the water potential of the plant which enhances water uptake, and helps maintain turgor and stomatal conductance (Abrams, 1988). The major cost of osmotic adjustment is a considerable expenditure of carbon and energy to build solutes (Tyree and Jarvis, 1982).
The other general adaptation for tolerating different moisture regimes is a low bulk modulus of elasticity. In tissue with a low bulk modulus of elasticity (i.e., more elastic), cells shrink with water loss (Tyree and Jarvis, 1982), which maintains at least some turgor and passively contributes to osmotic adjustment. A low bulk modulus of elasticity will allow stomates to remain open longer than in plants not capable of elastic fluctuations under similar degrees of water stress. However, bulk modulus of elasticity can contribute only slightly to a plant's water absorption capability. The costs of a low bulk modulus of elasticity are unclear.

Allocation patterns may influence D-T by altering how rapidly water stress is felt by a plant. For example, during a drought a plant with a large R/S will avoid desiccation longer than a plant with a small R/S. Thus, for any particular level of external drought, a plant with a large R/S will tend to be less stressed (i.e., higher \( \Psi_{xylem} \)) and may be able to maintain a greater photosynthetic rate. A large R/S, though, can only arise by way of reducing shoot biomass and hence leaf area. A plant with a large leaf area (lower R/S) may acquire similar or greater quantities of carbon at any particular \( \Psi_{soil} \). Thus, enhanced photosynthesis rates achieved via high R/S may be offset by lower leaf area. Barton and Teeri (1993) explored the relationship between R/S and drought-resistance in tree species which inhabited a moisture gradient in the Chiricahau Mountains (Arizona), and found that the two were not correlated. Perhaps the plants in this study with high D-T require less root biomass because they are more tolerant, and thus could best increase biomass production by allocating carbon to leaves rather than roots.

Several studies have compared relative degrees of D-T between species on moisture gradients in attempts to explain distribution patterns (Tobiessen and Kana, 1974; Cline and Campbell, 1976; Bunce et al., 1977; Parker et al., 1982; Bongarten and Teskey, 1986; Abrams, 1988; Barton and Teeri, 1993; Lajtha and Getz, 1993). In general, these studies found that species from dry regions are more tolerant than ones from wet regions. Many studies conclude that the reasons species from wet sites are not found on drier sites are because 1) more tolerant dry site species can gain carbon at lower water potentials and hence grow faster when water is limited than less tolerant species (Tobiessen and Kana, 1974; Orians and Solbrig, 1977; Parker et al., 1982; Robichaux, 1984; Abrams, 1988), and 2) in competition, dry site species (more tolerant) can rapidly deplete soil water reserves and force less tolerant ones to cease photosynthesis earlier - this is the so-called "exploitation" or "profligate use of water" strategy (Bunce, 1977; Shainsky and Radosевич, 1986; DeLucia and Heckathorn, 1989; DeLucia and Schlesinger, 1991). Orians and Solbrig (1977) hypothesized that a cost of high drought-tolerance may be lower rates of photosynthesis in non-droughted conditions. This trade-off, they suggest, may limit highly drought-tolerant species from occupying high water sites. Two studies hypothesized possible mechanisms for this trade-off, and concluded that tolerance mechanisms, such as osmotic and elastic adjustments, could result in less efficient metabolic functioning (Cline and Campbell, 1976).
or reduced growth rate (Guy et al., 1989). These studies (Cline and Campbell, 1976; Orians and Solbrig, 1977; Guy et al., 1989) are exceptional because the authors attempted to explain why the species endemic to drier sites were not found on wet sites. Many studies examine why species with low drought tolerance (found on wet sites) can not grow in dry sites, or why drought tolerant species are found in dry areas; but they often fail to hypothesize whether the restriction of drought-adapted species from wet sites is due to costs of these adaptations, or for other reasons (e.g., Tobiessen and Kana, 1974; Bunce et al., 1977; Jackson and Spomer, 1979; Parker et al., 1982; Abrams, 1988). However, a few studies (e.g., DeLucia and Hechathorn, 1989; Robichaux, 1984) concluded that drought tolerant plants were restricted from sites inhabited by less tolerant individuals for entirely other reasons, such as nutrient availability, rather than a trade-off associated with increased D-T.

C. Water-Use Efficiency versus Drought-Tolerance

A plant which has a low degree of D-T, by definition, closes its stomates earlier during drought than a plant which is highly drought tolerant. By closing stomates early a plant reduces water loss and "saves" water. Therefore, an intolerant species could be considered a water saving species. Since high WUE derived by way of reduced conductance results in reduced transpiration, a species with high WUE (from limited conductance) can also be considered a water "saver". This suggests that a plant with high WUE via reduced conductance may also have a low degree of D-T, and vice versa.

If this is so, there appears to be a contradiction in the literature. For example, in the studies which examined relative degree of D-T, species with low tolerances (i.e., saved water and possibly had high WUE via reduced conductance) had distributions limited to moist sites (Tobiessen and Kana, 1974; Cline and Campbell, 1976; Bunce et al., 1977; Parker et al., 1982; Abrams, 1988). On the other hand, studies which examined WUE specifically (Field et al., 1983; Carter and Smith, 1988; Monson and Grant, 1989) have demonstrated that species from dry habitats had higher WUE via reduced conductance (i.e., they saved water and possibly had low tolerance). The implication is that both types of water relation traits are adaptive for xeric habitats. But as was pointed out in the paragraph above, they should be incompatible, at least in theory. A paradox is revealed.

The paradox could be explained away if species from these studies inhabited different types of moisture-limited environments. That is, if all the species that were examined for tolerance lived in environments of rapidly depleting soil moisture from external forces (e.g., competition, seepage, or evaporation) and all the species examined for WUE had a relatively stable (yet limited) water supply (i.e., not diminishing from external forces), then either strategy would be adaptive.
for its respective habitat. It is unlikely, though, that every species studied for either water relation trait (WUE and tolerance) inhabits the respective type of habitat necessary to refute the paradox. An example where this clearly does not occur was presented by Carter and Smith (1988). They found that lodgepole pine had a greater WUE and tended to grow on drier sites than other tree species which grew on moist sites, but in close proximity. Thus, as a result of seed dispersal into adjacent stands, competition for water is likely, especially at the seedling stage (Carter and Smith, 1988).

One already mentioned study examined both WUE and D-T (DeLucia and Heckathorn, 1989). The authors found that a combination of low WUE and high degree of D-T was highly adaptive for Great Basin species (DeLucia and Schlesinger, 1991). They also discerned that a high WUE derived primarily from decreased conductance was maladaptive in a water-limited and competitive environment, which is in accord with theory (see above, and Cohen, 1977; Cowan, 1982). These results are important because they demonstrate the possibility of a genetically based trade-off between WUE and D-T. A trade-off of this kind could be a significant driving force in evolutionary divergence, as well as in defining the types of habitats species can occupy. For example, depending on the specific characteristics of a plant's moisture environment, it may evolve to become more WUE or D-T, but apparently not both. Consequently, if habitats favor increased WUE or D-T (e.g., non-competitive or competitive water limited sites, respectively), then becoming more WUE or D-T may preclude occupation of the other type of habitat. These studies demonstrate the necessity of measuring both WUE and D-T in understanding how plants may interact in certain environments. Interpretation of earlier studies which fail to provide this information is difficult.

III. Interaction of Water and Nitrogen Relations

Plants in nature are faced with constant fluctuations in the supply of all resources necessary for growth and reproduction. Even in sites that are characteristically limited in N or water, the other resource may periodically become limiting. Plant survival strategies must deal with such periods when simultaneous stresses occur. However, the efficiency by which a plant utilizes N and water may be affected by the availability of both resources.

A. Nitrogen Effects on Water-Use Efficiency

It has been shown repeatedly that WUE decreases when plants are subjected to N-stress (Medina, 1972; Mooney and Gulmon, 1979; Wong, 1979; Longstreth and Nobel, 1980; Brown
and Wilson, 1983; Sage and Pearcy, 1987; Lajtha and Whitford, 1989; Reich et al., 1989; Toft et al., 1989). In each of these studies, except Toft et al. (1989) (see below), A decreased with increased N-stress. Since leaf $[\text{N}]$ is correlated with A (Evans, 1983; 1989), it is likely that decreased WUE resulted from reductions in carboxylating enzymes. However, for WUE to decrease with decreasing N-supply, $E$ must also increase or remain the same, or must diminish proportionally less than $A$. Each of these possible patterns have been observed (Toft et al., 1989), but the most commonly observed pattern is a reduction in both $A$ and $E$, but a greater decrease in $A$ than $E$ (Toft et al., 1989; e.g. Medina, 1972; Wong, 1979; Longstreth and Nobel; 1980; Brown and Wilson, 1983; Sage and Pearcy, 1987).

Some studies indicated that plants may decrease WUE in order to increase NUE (Toft et al., 1989; Gauslaa, 1990). For example, Toft et al. (1989) observed that Ceratoides lanata (Pursh) J.T. Howell, Elymus lanceolatus (Scribn. and J.G.Smith) Gould, and E. elymoides (Raf.) Swezey responded to conditions of low N and ample water by increasing stomatal conductance. Because of this, these species exhibited higher transpirational water losses and lower WUE, but presumably greater PNUE because of reduced stomatal limitation on A (Toft et al., 1989). This study is unique because it indicates that stomata may respond directly to N-stress, rather than decreased internal CO$_2$ concentration associated with reduced photosynthetic capacity. Most previous studies, as was pointed out above, found that WUE decreased (as NUE increased) with increasing N-stress because of greater reductions in $A$ than $E$ (Toft et al., 1989; Medina, 1972; Wong, 1979; Longstreth and Nobel; 1980; Brown and Wilson, 1983; Sage and Pearcy, 1987). In addition to increasing NUE, an increase in $E$ may increase mass flow, and thereby the flux of dissolved N to the roots (Fitter and Hay, 1987). Thus, although somewhat unusual, increasing $E$ and decreasing WUE may be a mechanism by which plants off-set the deleterious effects of N-stress.

A decrease in WUE, for any reason (greater NUE or mass flow), would likely be strongly constrained by soil water content. In fact, Chapin (1980) argues that nutrients in the soil solution in most environments are too dilute for the benefits of increased nutrient uptake via mass flow to outweigh the costs of transpirational losses. These costs may explain why such a response is rare. However, in low nutrient environments where water is not limiting, decreasing WUE to increase N absorption may be a viable strategy. In fact, this appears to be true for xylem tapping hemi-parasitic mistletoes (Ehleringer et al., 1985; Gauslaa, 1990). Gauslaa (1990) measured transpiration rates of Melampyrum pratense L. (a hemi-parasite) across a fertility gradient in the boreal forest. He found that individuals from low nutrient sites exhibited greater $E$ than those from high nutrient sites (Gauslaa, 1990). Many bogs and marshes are extremely limited in nutrients due to high water or flooded soil conditions (Small, 1972abc; Schlesinger and Chabot, 1977; Shaver and Melillo, 1984). In this type of environment, the benefits of improved NUE and mass flow
may be greater than the few costs that would be associated with maintaining a low WUE.

B. Water Effects on Nitrogen-Use Efficiency

Water-stress, on the other hand, decreases NUE. Most studies have focused on how PNUE is affected by water stress (Field et al., 1983; Lajtha and Whitford, 1989; Riech et al., 1989). It is generally concluded that decreased conductance reduces the flux of CO₂ into the leaf, causing conductance to be the primary limiting component to photosynthesis, thereby decreasing the amount of carbon taken up per unit N invested in the process (PNUE) (Field et al., 1983; Lajtha and Whitford, 1989; Riech et al., 1989). However, two studies that looked at the effects of water availability on whole-plant NUE found drought caused NUE to increase (Welbank, 1962; Day, 1981). In these studies extremely low soil water content apparently inhibited ion movement, reduced nutrient uptake, and thereby induced nutrient-stress (Welbank, 1962; Day, 1981). However, by decreasing PNUE and/or growth rate water stress may act to reduce NUE. For example, $C_1$ will decrease as a result of stomatal closure associated with drought causing lower $A$ per unit leaf [N] (decreased PNUE). Lower $A$ will decrease growth rate, and if N uptake is not affected, or is affected proportionally less than growth, internal nutrient concentrations will increase (decreased NUE). Clearly, more data are required to fully understand the effects of water availability on NUE.

Under high N and low water conditions it is conceivable that plants decrease NUE in order to increase WUE. Since NUE is negatively correlated with internal [N], a low NUE could improve a plant's WUE by increasing photosynthetic capacity. It follows that such a strategy would be adaptive only in habitats where water is limiting but N is not.

C. Nitrogen and Water Effects on Allocation

Allocation patterns will augment the physiological effects that water and N have on NUE and WUE. Since both water and N are obtained from the soil by roots, both water stress and N stress may induce a greater R/S (Grime, 1977; 1979; Chapin, 1991a). Increased R/S in response to either stress will coincidentally increase the absorption of the other resource (Chapin, 1991a). In this way, water stress may increase nutrient absorption, and hence decrease NUE; whereas nitrogen stress may increase water absorption and lead to a decrease in WUE. When both water and N are simultaneously limiting, R/S may increase proportionally more than in response to limitation in one resource. Or, if a negative feedback effect results, then R/S may only increase to the point where just one resource becomes most limiting.
D. Nitrogen-Use Efficiency versus Water-Use Efficiency Trade-Off

It is apparent from the above discussion that water and nitrogen have opposing effects on WUE and NUE. This trade-off is well established for instantaneous WUE and PNUE, and has been shown to occur both plastically (Lajtha and Whitford, 1989; Riech et al., 1989) and intrinsically* (Field et al., 1983; DeLucia and Schlesinger, 1991). For example, Lajtha and Whitford (1989) measured instantaneous WUE and PNUE in Larrea tridentata plants grown under various H2O and N regimes and found that they were negatively correlated across all treatments. This study demonstrates that environmental factors that elicit plasticity in WUE or PNUE, automatically force the other to change in the opposite direction. Furthermore, Field et al. (1983) analyzed species growing in a similar location, but whose distributions are normally associated with a moisture gradient. They found that species from xeric habitats had greater WUE and lower PNUE than ones from more mesic habitats (Field et al., 1983). DeLucia and Schlesinger (1991) found that Great Basin trees from low nutrient soils had intrinsically greater instantaneous WUE but lower PNUE than shrubs from higher nutrient soils. Both these studies reveal that evolutionary specialization in either WUE or PNUE generally precludes (at least for C3 plants) the possibility of similar directional changes in the other.

The trade-off between WUE and NUE may impose constraints on plant responses and adaptations to resource limitations. The plastic trade-off suggests that a plant may only be able to increase the efficiency by which it uses one resource (H2O or N) to the point where the cost to efficiency in the other becomes too high. This may place a ceiling on the degree to which a plant may respond to temporal and spatial fluctuations in water and N supplies. By preventing adaptation to low water and N environments simultaneously, the intrinsic trade-off could be a significant driving force for species divergence and in delimiting species distributions. The problem is further exasperated when both high WUE and NUE are favored (e.g., when both H2O and N are limited). In such a situation, a plant can increase NUE, or WUE, or exhibit intermediate levels of each. In each case it will always utilize one or both resources with suboptimal efficiency.

Studies thus far have not attempted to assess whether species differ in the manner by which WUE and NUE (or PNUE) trade off. These types of genotype by environment interactions, if they exist, could reflect selective forces acting directly upon the trade-off. For example, if the slope of NUE plotted against WUE differs amongst species, then costs in terms of increasing the efficiency of the use of one resource, could be lower or greater relative to the use of the other. A

*Intrinsic trade-off refers to a situation in which the expression of one trait is inversely related to the expression of another trait in a particular environmental context. Intrinsic trade-offs are located by comparing mean trait expressions (or mean character states) of two or more characters in one environment. They contrast with plastic trade-offs which involve comparisons of trait expression across environments.
species that could increase WUE and show less decrease in NUE than another (i.e., different slopes), may be better able to survive and compete in water limited sites which experience N limitation periodically. Conversely, the ability to increase NUE with less compromise to WUE could be favored in environments of low N that periodically experience water limitation. Thus, this type of interaction effect could have significant effects on species distributions, and ecological relationships.

To the extent that instantaneous PNUE and WUE are facets of whole-plant, long-term NUE and WUE, whole-plant, long-term measures should also trade off. Although a whole-plant, long-term trade-off is theoretically possible, DeLucia and Schlesinger (1991) failed to observe one in their study of Great Basin species. A whole-plant, long-term trade-off, or lack thereof, may be of greater ecological significance than a leaf-level, instantaneous one because individuals, not leaves, interact.

IV. Ecology and Autecology of Picea glauca and P. mariana

Despite the fact that Sw and Sb grow in contrasting environments near the southern edge of the boreal forest, in more northerly regions Sb is found on upland sites and hence forms mixed stands with Sw (Larson, 1980; Elliot-Fisk, 1988). The following table, established from data presented in Elliot-Fisk (1988), summarizes some salient features of the known ecology of the two species. The data are based on general species level silvicultural characteristics, and do not account for regional variations within or between species.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>White Spruce</th>
<th>Black Spruce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>needle/evergreen</td>
<td>needle/evergreen</td>
</tr>
<tr>
<td>Growth Rate</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td>Site Characteristics:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>substrate preference</td>
<td>basic loam</td>
<td>acidic</td>
</tr>
<tr>
<td>permafrost table</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>soil moisture</td>
<td>xeric-mesic</td>
<td>xeric-hydric</td>
</tr>
<tr>
<td>shade tolerance</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>nutrient requirements</td>
<td>moderate</td>
<td>low</td>
</tr>
<tr>
<td>optimal seedbed</td>
<td>mineral</td>
<td>organic/mineral</td>
</tr>
<tr>
<td>frost-free period (d)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Seral Status</td>
<td>late</td>
<td>early-late</td>
</tr>
<tr>
<td>Maximum Life-Span (yr)</td>
<td>600</td>
<td>300</td>
</tr>
</tbody>
</table>
A few previous studies have specifically examined the water relations of Sb and Sw. Water stress measurements on greenhouse grown Sb seedlings demonstrated that day time photosynthetic compensation point was reach at -2.5 MPa ($\Psi_{\text{shoot}}$), but germlings had a 80% mortality with a decrease in water potential to -2.5 MPa ($\Psi_{\text{shoot}}$) (Black and Bliss, 1980). The seeds in this experiment originated from Sb populations near the northern border of the boreal forest (Black and Bliss, 1980). In laboratory experiments on Sw seedlings, grown from seed collected in the tundra ecotone in Alaska, Goldstein et al. (1985) observed that stomatal threshold points ($\Psi_{\text{shoot}}$ where stomates begin to close) were at approximately -1.0 MPa, and complete stomatal closure (critical point) occurred at -1.8 MPa. Photosynthesis was not measured. When comparing Sw and Sb, Buxton et al. (1985) found that Sw tended to decrease soil water content more rapidly, but that both had similar threshold and critical stomatal closure points. The origin of these seeds could not be ascertained, and photosynthesis versus water stress was not measured (Buxton et al., 1985).

Several studies have also specifically examined the nutrient relations of Sw and Sb. Chapin (1983; 1986) and Chapin et al. (1983) analyzed several traits in Sw and Sb from the Alaskan interior in relation to variations in P supply. White spruce exhibited greater total biomass than Sb after a 9-month period (Chapin et al., 1983; Chapin, 1986), and exhibited a greater phosphorous-use efficiency (laboratory conditions). Although not measured for Sw, Sb showed a phosphate absorption rate between approximately 0.1 and 0.2 µmol P/h/day (grown under 10 and 20 µmol P/l supply rate) (Chapin, 1983). Interestingly, the rate of phosphate absorption by Sb and several other boreal trees (Sw was not examined) was unaffected by soil temperature (Chapin, 1986). The results of this study also showed no statistical difference in root-to-leaf weight ratio between Sb and Sw (Chapin et al., 1983).

Although past research has revealed some important characteristics of Sb and Sw (above), finer scale studies are required to understand their adaptations to the contrasting environments they inhabit near the southern border of the boreal forest (see Introduction, p. 1). Apparently no previous studies have compared genetically based variation in water- or N-relations characteristics between Sw and Sb populations from these regions.
OBJECTIVES

"The central goal in ecology is to understand the causes of the patterns we observe in the natural world." (Tilman, 1988)

I. Theoretical Framework

Pattern in ecology is caused by constraints imposed by the environment and the trade-offs that organisms face in dealing with these constraints (Tilman, 1988). The objective of this study was to examine how the interplay between such constraints and trade-offs may impact the mutually exclusive topographic distributions of Sw and Sb near the southern border of the boreal forest. The major environmental constraints to plant growth and survival in the poorly drained muskeg and well drained upland habitats include limitations in available nutrients or water. The types of trade-offs that may occur for plants faced with these constraints can be divided into two categories. The first type of trade-offs, or environmental trade-offs, are plant characteristics (or adaptive traits) that act to increase growth in resource limited conditions, but which reduce growth when resource availability is high. Environmental trade-offs are important mechanisms promoting the separation of species across quantitative resource gradients (i.e., from high to low availability of one resource). The second type of trade-offs, or trait trade-offs, occur when adaptive traits are physiologically or ecologically incompatible within a single individual or species. Trait trade-offs separate species across qualitative resource gradients. For example, an increase in one trait that promotes growth in one type of resource limited environment may trade off with other traits that are adaptive in other types of resource limited environments.

Due to the interacting nature of the resource gradient in the southern boreal forest there are, in essence, three independent gradients. These include a quantitative nutrient gradient (high vs. low nutrient availability), a quantitative moisture gradient (high vs. low water availability), and a qualitative nutrient and water gradient (low levels of each). Competition intensity may also have a major impact on the characteristics of resource supplies in either habitat. Therefore, variation in traits that trade off across these gradients, that trade off with traits that are adaptive in the other habitat, or that trade off with competitive ability may be a mechanism controlling the distributions of Sb and Sw.

II. Hypotheses

The central hypothesis of this study was that Sb and Sw will exhibit contrasting expressions of N- and water-relations traits and that this variation will correlate with the environmental characteristics of their respective habitats. At first this hypothesis seems self
evident. That is, species will be adapted to the habitats in which they occur because they occur there. However, once the trade-offs that these species may face in adapting to their environments are explicitly included, testing this hypothesis may reveal the mechanisms that control their distinct distribution patterns.

Specific hypotheses that are examined in this study are derived from results and implications of past research discussed in the literature review. The relevant section(s) of the literature review pertaining to each hypothesis are in parentheses below. The specific hypotheses include:

A. Trait Means, Phenotypic Plasticity, and Environmental Trade-Offs:

1. black spruce will exhibit lower RGR and plasticity (I.A.5), and greater NUE, SAR, and R/S than Sw (I).
2. white spruce will exhibit greater D-T, WUE, and R/LA (II) and more optimal plastic response of water-relations traits than Sb (II).
3. high WUE (II.A), D-T (II.B), R/S (I.A.4 and II.A), and SAR (I.A.3) will trade off with determinants of growth rate (e.g., net photosynthetic rate and/or leaf area ratio) under high resource conditions.

B. Trait Interactions and Trade-Offs:

1. the following N-relations traits will trade off intrinsically: NUE vs. R/S, SAR, and growth (I.B and I.D).
2. the following water-relations traits will trade off intrinsically: WUE vs. R/S (II.A), D-T vs. R/S (II.B), and WUE vs. D-T (II.C).
3. the following N- and water-relations traits will trade off intrinsically and plastically: WUE vs. NUE (III.D).

C. Suites of Adaptive Characteristics:

1. black spruce will have a more adaptive suite of traits than Sw for coping with high moisture, low nutrient conditions.
2. white spruce will have a more adaptive suite of traits than Sb for coping with high nutrient, low moisture conditions.
MATERIALS and METHODS

I. Seedling Material

A. Study Site-The Fort Nelson Resource Gradient

The seeds for the plant material (seedlings) utilized in this experiment were obtained from naturally growing populations of Sb and Sw near Fort Nelson, British Columbia (Map 2). Fort Nelson is located at the southern edge of the boreal forest at approximately 59° N latitude, at an elevation of 670 m, and is situated within the Boreal White and Black Spruce Biogeoclimatic Zone (BWBS) as described by DeLong et al. (1991). Black spruce and Sw are the dominant conifer species in the Fort Nelson region, and the landscape is characterized by a mosaic of level lowland (muskeg) and sloping upland forest ecosystems (Valentine, 1971; DeLong et al., 1991). The monthly temperature averages of this region remain below 0°C for 5-7 months of the year, and above 10°C for 2-4 months (DeLong et al., 1991). Fort Nelson receives on average 200-290 mm of rain a year (total precipitation = 446 mm) (DeLong et al., 1991), indicating that well-drained sites (upland) must be fairly droughted during the summer months.

B. Sampling Procedure

Open pollinated seeds from five pairs of adjacent and contiguous stands of Sb and Sw in the Fort Nelson area were obtained. Sampling sites were selected on the basis of road access, but were chosen such that they were all separated by more than 10 km. The latitudes and longitudes of the site locations were:

<table>
<thead>
<tr>
<th>Site #</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58° 25'</td>
<td>122° 55'</td>
</tr>
<tr>
<td>2</td>
<td>58° 46'</td>
<td>122° 11'</td>
</tr>
<tr>
<td>3</td>
<td>58° 15'</td>
<td>122° 45'</td>
</tr>
<tr>
<td>4</td>
<td>58° 08'</td>
<td>122° 47'</td>
</tr>
<tr>
<td>5</td>
<td>57° 51'</td>
<td>122° 57'</td>
</tr>
</tbody>
</table>

White spruce seeds were ordered from the British Columbia Forest Service Tree Seed Centre (seedlot #’s, by site: 1 = 37044, 2 = 35075, 3 = 8506, 4 = 8505, 5 = 8504). Black spruce seeds were collected by hand using an extension pruning pole. Three cones from 35 randomly dispersed trees in each stand were collected. Because Sb can reproduce by layering (Black
MAP 2 - British Columbia Biogeoclimatic Zone and Fort Nelson Study Site Location

Biogeoclimatic Zones of British Columbia
and Bliss, 1980), precautions were taken to avoid sampling genets more than once, thus only trees greater than 10 m apart were sampled.

The Sw seeds were stratified at 2°C for 3 weeks after soaking for 12 hours. The Sb cones were opened by heating at 30°C for approximately 48 hours and shaken to remove the seeds. Ten seeds from each tree were then selected randomly and randomized. Seeds from each population of both Sb and Sw were then selected randomly and planted into forestry containers (Stuewe and Sons, Inc., Corvallis, OR) which were filled with a peat moss, perlite, and dolomite mixture (2 parts peat, 1 part perlite, and 600 g dolomite) topped with forestry gravel (Target Forestry Sand, Burnaby, BC) and used in the two experiments described below.

II. Experiment I - Drought-Tolerance

A. Growth Environment

To estimate the relative degree of seedling drought-tolerance, seeds of each species were first germinated and grown in a greenhouse environment (UBC Plant Science Dept., Vancouver, BC). All the seedlings received ample water (4 times/week) and fertilization (2 times/week). Fertilizer nutrient concentrations were 150 ppm N, 60 ppm P, 150 ppm K, and 10 ppm S, with 1 ppm micro-nutrients added. The recipe for this mix included: .75g Plant Prod Forestry Seedling Special 20-8-20 (N-P-K), .05g K2SO4, and .005g Stem Micro-nutrients (Plant Products Co., Brampton, ON) added to each liter of water. The day/night temperature regime was approximately 20°C/14°C. Day time relative humidity was usually around 35%. Natural sunlight was supplemented with two halide high intensity lamps. On sunny days the seedlings received up to 1000μmol/m²/s PAR, and on cloudy days received as low as 350μmol/m²/s PAR. Due to the northern origin of the seed stock, the natural photoperiod was extended to 20 hours. However, after approximately 3 months of growth, the photoperiod was inadvertently changed to 16 hours. With the change in photoperiod approximately 20% of the the seedlings of each species (apparently evenly distributed amongst the provenances) set bud. These seedlings were removed from the experiment.

B. Measurements

After approximately 4 months of growth, the seedlings of each species were tested for relative drought-tolerance (D-T). To do this, drought was imposed and net photosynthesis and internal shoot water potential ($\Psi_{xylem}$) were monitored as the soil dried. At the point where the
seedlings had ceased positive carbon uptake the experiment was terminated. Net photosynthesis was measured with a closed infra-red gas analysis system (model LI-6200, LI-COR Inc., Lincoln, NB) during the course of the drought. The seedlings were exposed to saturating light during the measurements (650umol/m²/s PAR). At the start of a 30 s measurement period, the relative humidity inside the LI-COR chamber was maintained at approximately 25-30%, CO₂ concentration between 350 and 390 ppm, and leaf temperature and air temperature near 25°C. Immediately after the photosynthesis measurement, the Ψₓylum of each seedling was measured with a pressure bomb (Soil Moisture Equipment, Santa Barbara, CA). Leaf area was determined on a projected leaf area basis using the LI-3100 Area Meter (LI-COR Inc., Lincoln, NB). Initially, leaf area was determined on combined stem and foliage, then foliage area alone was calculated using a predetermined correction factor for the stem to foliage projected area ratio of each species (.9652).

III. Experiment II - Whole-Plant/Long-Term Nitrogen- and Water-Relations

A. Estimates of Nitrogen- and Water-Use Efficiency

The ratio of whole-plant carbon:nitrogen ratio (C/N) was calculated as an estimate of NUE. This measurement indicates the amount of carbon fixed per unit nitrogen, and suggests the efficiency by which N is distributed and utilized to acquire carbon. Carbon:nitrogen ratio is a more meaningful measure of NUE than the more commonly used 1/[N] (Chapin, 1980; Chapin and Van Cleve, 1990). A plant which sequesters, for example, sodium chloride (as in halophytes) may have a very high 1/[N] but low C/N. Clearly, in such circumstances C/N better represents the efficiency by which a plant utilizes N.

Relative whole-plant/long-term WUE was estimated at the population and species level using the carbon isotope method and expressed as δ¹³C values in per mil, where:

\[
\delta^{13}C = \frac{[^{13}CO_2/^{12}CO_2 \text{ (sample)}]/^{13}CO_2/^{12}CO_2 \text{ (standard)} - 1} \times 1000
\]

where the standard is a limestone, called PDB, from the Pee Dee formation in South Carolina (O'Leary, 1988). Farquhar et al. (1982, 1989a) identified the relationship between δ¹³C and the ratio of intercellular to atmospheric CO₂ concentration ([CO₂]) in C₃ plants. The ratio of intercellular/atmospheric [CO₂] has been shown to be highly correlated with WUE (Farquhar and Richards, 1984; Sharkey, 1985; Ehleringer and Cooper, 1988). Therefore, the fraction of ¹³C in the total plant tissue (δ¹³C) gives a relative value of the integrated WUE a plant operates at over the
course of its life (Farquhar and Richards, 1984; Ehleringer and Cooper, 1988; O'Leary, 1988). More positive δ^{13}C values reflect more water-use efficient plants.

B. Growth Environment

For this experiment (Experiment II) seedlings of each species were grown in a growth chamber at the University of British Columbia Forestry Nursery (Vancouver, BC). The rooting medium and seedling containers were the same as in Experiment I. The photoperiod was 20 h, radiation level approximately 350-400 μmol/m²/s PAR (from both fluorescent and incandescent lamps), relative humidity was approximately 35%, and day/night temperature was maintained at 24°C/14°C. Fans blew air over the seedlings continuously throughout the course of the experiment to ensure adequate ventilation and mixing of air. Cone-tainer trays were rotated in a random manner daily.

C. Experimental Design and Treatments

The experimental design for this study was a completely randomized 2x2x2 nested factorial. The five populations (nested term) of each of the two species (first factor) were subjected to two N levels (second factor) and two water levels (third factor), yielding four separate treatments: 1) +H2O+N, 2) +H2O -N, 3) -H2O+N, and 4) -H2O-N. Twenty seeds from each population (100 for each species) were randomly allocated to each treatment. A slight restriction on randomization was imposed by separating the treatments into individual cone-tainer trays (i.e., pseudoreplication). Although a minor deviation from a completely randomized design, this restriction was imposed intentionally for practical and biological purposes. Not to have separated the treatments would have increased the time required to water and fertilize the seedlings immensely, which could have 1) made the experiment physically impossible to carry-out, and 2) inadvertently added an unaccountable temporal factor. Because growth chamber environments are relatively homogeneous, the effects of spatial separation of treatments were probably very minor. Thus, it was decided that the impacts of spatial treatment separation would be less than those associated with the temporal separation of treatment applications. Nevertheless, precautions were taken to minimize any potential effects; trays were rotated daily and seedlings were spaced to minimize contact.

Germination occurred over a period of approximately one week, after which the germinants were grown for two weeks with ample moisture (watered four times/week) and nutrient supplies (fertilized two times/week as described below for the +N treatments). After two weeks time, when
the seedlings had reached approximately 2 cm in height, the four experimental treatments were
initiated.

The seedlings under the high moisture treatments (+H2O+N, +H2O-N) received approximately 5-10 ml of water daily. The 5-10 ml water application replaced what had been lost over the 24 hour period since last watering, but did not cause leaching of nutrients from the bottom of the cones. The low water treatments (-H2O+N, -H2O-N) were watered when the water potential of the soil (Ψsoil) in ten randomly selected cones dropped to approximately -1.0 MPa. This corresponded to 20% soil water content (see Appendix A). When Ψsoil reached the desired level of water-stress in the low water treatments, all the treatments were watered to the drip point and fertilized.

All four treatments received identical concentrations of phosphorus (62.5 ppm P), potassium (93.75 ppm K), sulfur (10 ppm S), and micro-nutrients (1 ppm). The high and low N treatments, however, received different supplies of N. The high N treatments (+H2O+N, -H2O+N) were fertilized with 225 ppm N, and the low N treatments (+H2O-N, -H2O-N) received 25 ppm N. The high N treatment concentrations were achieved by mixing .6 g NH4NO3 (34% N), .32g Plant Prod Forestry Seedling Finisher 8-20-30 (N-P-K respectively), .05 g K2SO4, .005 g Stem Micro-nutrients per each liter of water. The low N treatments were identical to the high N treatments except NH4NO3 was not added. The drought cycles for the water limited treatments, and hence the fertilization "cycles", usually lasted 4 days. The seedlings where subjected to 28 cycles over the course of 16 weeks.

At an age of approximately 8 weeks some seedlings where girdled by an unidentified insect (possibly the girdling weevil, Otiorhynchus sp., or the European marsh crane fly, Tipula paludosa; Lavender et al., 1990). The infestation was successfully thwarted utilizing a diazinon soil drenching procedure (10 ml diazinon:1 liter of H2O). Approximately 10 scattered individuals of both Sb and Sw in the -H2O+N, -H2O-N, and +H2O-N treatments were girdled. This did not affect the sample size of the experiment because extra seedlings were grown.

D. Morphological and Specific N Absorption Rate Analysis

At approximately 18 weeks of age (two week establishment period + 16 week treatment period), the seedlings were harvested. The roots and shoots of each seedling were separated at the root collar and the roots were carefully washed to remove soil. Both parts were then frozen at -70°C and freeze dried for approximately 60 hours. The dry weights of the roots and shoots were recorded for each seedling. Root:leaf area ratios were determined through first calculating leaf area by regressing leaf area over leaf weight for each species in each treatment, then dividing the
calculated leaf area value by root biomass. Specific N absorption rate was determined by simply dividing root biomass by total plant N-content.

E. Nitrogen and Carbon Isotope Composition Analysis

After the biomass measurements were complete roots and shoot were re-combined and pulverized in a planetary ball mill (Pulverisette, Fritsch GMBH, Germany) to sub-micon particle size. The pulverized tissue was combusted and nitrogen content determined using a Carlo Erba Elemental Analyzer Model 1106 at the UBC Department of Oceanography (Vancouver, BC). Liberated CO₂ then passed to a VG Spectrum Triple Collecting Ratio mass spectrometer for determination of δ¹³C value (as described by Hubick et al., 1986).

F. Determining Rates of Photosynthesis and Transpiration*

Gas exchange measurements were made on the same seedlings used in the δ¹³C and C/N analysis (above) just prior to harvest using an ADC LCA-3 open system (Hoddesdon, England) at saturating PAR (600 μmol quanta/m²/s), ambient CO₂ concentration (about 350 ppm), and a relative humidity of 35%. For the drought stressed seedlings (-H₂O+N and -H₂O-N), measurements were made both at the the beginning (12 hours after rewatering) and end of the last drought cycle. The net photosynthetic rate (A) and transpiration rate (E) values were averaged to obtain an integrated value of relative performance at the two extremes of the cycle (i.e., well-watered and droughted conditions). Measurements on well-watered seedlings (+H₂O+N and +H₂O-N) were made approximately 12 hours after watering. Shoot water potential was also measured at the beginning and end of the drought cycle using a pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, CA).

IV. Statistical Analysis

All statistical analyses were performed using SuperANOVA-Accessible General Linear Modeling Package and Stat View SE+ Graphics (Abacus Concepts Inc., 1990), designed for the Macintosh computer. The appropriate model for analysis of growth, SAR, C/N, δ¹³C, and morphological parameters was a three-way nested factorial analysis of variance (ANOVA). Proper error terms were determined using methods outlined by Hicks (1982) and are presented in Appendix B. The species were compared in each treatment using four 1-way ANOVA's (one for

* This portion of the experiment was conducted in collaboration with Dr. Qing-Lai Dang (Forest Sciences, UBC).
each treatment), with the species effect tested against the nested population term. Treatment means were compared using the Tukey's Compromise means separation procedure as recommended by SuperANOVA-Accessible General Linear Modeling Package (Abacus Concepts Inc., 1990). This method equally distributes the probability for Type I and Type II errors and therefore reduces the chances of both simultaneously. Analyses of covariance (ANCOVA) were performed for line comparisons. This procedure was done to determine significance of the regressions, slopes, and y-intercepts of $A$ versus $\Psi_{\text{soil}}$ (drought-tolerance analysis) and C/N versus $\delta^{13}$C regression data. The ANCOVA procedures were conducted as demonstrated by Hicks (1982) and SuperANOVA-Accessible General Linear Modeling Package (Abacus Concepts Inc., 1990). Probability plots and histograms were examined for normality, and residuals were also plotted against cell mean estimates to check for homoscedasticity. Data were appropriately transformed in order to meet assumptions of ANOVA.
RESULTS

The results demonstrate that the treatments in this study were, for the most part, effective. This is indicated by the ubiquity of significant differences between character values measured under low versus high water and N conditions. Moreover, significant species and population level differences were frequently detected.

I. Experiment I - Drought-Tolerance

Figure 1 shows the relationship between $A$ and $\Psi_{xylem}$ from the drought-tolerance (D-T) experiment. Second day measurements were biased and therefore are not presented on this figure. Photosynthetic induction was hampered on this day because the seedlings were not exposed to sufficient light levels prior to being measured, and stomates were slow to open.

The regression of net photosynthesis rate ($A$) on shoot water potential ($\Psi_{xylem}$) was highly significant ($P=.0001$) for each species (Fig.1). The general response pattern of the seedlings was little change in $A$ until a threshold $\Psi_{xylem}$ was reached (approximately -.07 MPa for Sb and -.11 MPa for Sw), after which $A$ decreased as internal moisture stress increased (lower $\Psi_{xylem}$). White spruce maintained a marginally greater $A$ than Sb across most $\Psi_{xylem}$ as indicated by the lack of overlap between the 95% confidence intervals of the regression lines. Black and white spruce also had significantly different photosynthetic compensation points (i.e., no overlap of confidence intervals at zero $A$). On average, the photosynthetic compensation point of Sb was reached at a water potential of approximately -2.15 MPa, and approximately -2.60 MPa for Sw.

II. Experiment II - Whole-Plant, Long-Term Nitrogen- and Water-Relations

A. Internal Stress Levels

Shoot water potential (MPa) measurements were taken at the termination of Experiment II. The treatment means are summarized below:

<table>
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<tr>
<th>Species</th>
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* note: measurements were made for only one well watered treatment, and droughted treatment measurements were made at the end of the cycle
Fig. 4. Relationship between net photosynthesis rate, A (μmol CO₂/m²/s) and shoot water potential, \( \psi_x \) (MPa) in *Picea mariana* (Sb, lower line) and *P. glauca* (Sw, upper line) seedlings. The linear regression equations and coefficients of determination are: for Sb \( y = 1.899x + 4.244, r^2 = .689, \) and Sw \( y = 1.818x + 4.782, r^2 = .641. \) The slope of each regression line was significantly different from zero \( (P = .0001) \). The x-intercepts of the species lines (Sb versus Sw) were significantly different \( (P = .0075) \), differences between y-intercepts were marginally insignificant \( (P = .0607) \), and the slopes were statistically equal \( (P \geq .05) \).
Percent of N in the seedling tissue ranged from 1.7% to 4.1%. These values match ranges observed in previous laboratory and field experiments (e.g. Welbank, 1962; Gray and Schlesinger, 1983; Birk and Vitousek, 1986; Berendse and Elberse, 1990). The effect of N-stress was to approximately halve N-content of both species. The species generally exhibited similar %N, except in the low water treatment (-H2O+N), where Sb exhibited 4.1% and Sw 3.1% N tissue N-content. Percent N of seedlings grown under water-stress was greater than for non-droughted plants.

B. Growth

The N and water effects on growth were highly significant, but the species did not differ (Table 1). Black spruce and Sw responded similarly to variation in water supply as indicated by the insignificant water by species interaction term, but differently to N supply as indicated by the significant N by species interaction term. Figure 2 shows that the responses of the species were in the same direction (i.e., decreased growth), but that Sw had a stronger response than Sb. The significant water by N interaction term indicates that growth (in both species) was affected differently by either stress while exposed to the other stress. From Figure 2 it is apparent that 1) growth was reduced less by N-stress in droughted compared to well-watered seedlings, and 2) growth was reduced less by drought in the N-stressed compared to high N seedlings.

Black spruce and Sw exhibited statistically equal growth in all treatments (Fig.2). Growth of each species was significantly lower in all stress treatments relative to the control (+H2O+N). Since seedlings grew less in the -H2O+N compared to the +H2O-N treatment (although not significantly so for Sw), it appears that water-stress was more severe than N-stress at the levels imposed in this study. Growth of plants under simultaneous N- and water-stress (-H2O-N) was not significantly lower than in the water-stress treatment (-H2O+N) for either species, but was significantly reduced compared to the N-stress treatment (+H2O-N) for Sb, but not Sw.

In contrast to Sb, Sw showed significant population level variation in growth (Table 2). However, no population level interactions were detected for growth in either species.

C. Specific N Absorption Rate

Variation in water supply had no effect on specific N absorption rate (SAR), but the effect of N was highly significant (Table 1). Averaged across all treatments the species exhibited significantly different SAR. The significant species by N interaction term indicates that N supply had a differential effect on SAR for the two species. From Figure 3 it is apparent that SAR was decreased more in Sb than in Sw. The effects of N-stress on SAR were the same whether the
Table 1. Main effect and interaction analysis of variance (ANOVA) summary table.

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*Calculations are for growth (g/plant); SAR = specific N absorption rate (mg/g); C/N = carbon-to-nitrogen ratio (g/g); δ13C = carbon isotope composition (per mil); A = net photosynthesis rate (μmol CO2/m²/s); E = transpiration rate (mmol H2O/m²/s); R/S = root-to-shoot ratio (g/g); and R/LA = root-to-leaf area ratio (mg/cm²) of Picea mariana and P. glauca seedlings. Probabilities of type I error for main effects and interactions are indicated by numbers.
Fig. 2. Mean growth (g/plant) ± SE of *Picea mariana* (Sb) and *P. glauca* (Sw) after approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species were statistically the same (P≥.05) in each treatment. Significantly different (P<.05) treatments within a species are denoted by separate letters above the bars.
Table 2. Population main effect and interaction analysis of variance (ANOVA) summary table.*

a) Black Spruce

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*Measurements on seedlings from five populations of a) *Picea mariana* and b) *P. glauca* grown under controlled conditions. Symbols as defined in Table 1. Probabilities of type I error for population and interaction effects are indicated by numbers.
Fig. 3. Mean specific N absorption rate, SAR (mg N/g root) ± SE of Picea mariana (Sb) and P. glauca (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species were significantly different at +H2O+N (P=.0005) and -H2O+N (P=.0066), but statistically equal at +H2O-N and -H2O-N. Significantly different (P<.05) treatments within a species are denoted by separate letters above the bars.
Specific N absorption rate in both species was significantly reduced by N-stress (+H2O-N) relative to the control (+H2O+N) (Fig.3). Black spruce maintained a greater SAR than Sw under both high N treatments (+H2O+N and -H2O+N), but the species had similar SAR at low N (+H2O-N and -H2O-N). Populations of Sb, but not Sw, exhibited significant differences in SAR (Table 2).

D. Tissue Carbon:Nitrogen Ratio

A natural log transformation of C/N data was necessary to meet assumptions of ANOVA (Hicks, 1982). All statistical calculations on C/N ratios used transformed values (ln(C/N)), but non-transformed results are presented on the figures. Tissue carbon-to-nitrogen ratio (C/N) was significantly affected by both N- and water-stress (Table 1). The species responded differently to both N- and water-stress as indicated by the significant species by N and species by water interaction terms. Figure 4 shows that Sb had a greater increase and decrease in C/N with N-stress and water-stress, respectively, than Sw. The lack of a significant water by N interaction term indicates that the effect of N-stress on C/N was similar whether the seedlings were droughted or well-watered. However, the significant species by water by N interaction term indicates that the species responses to N-stress differed from each other as a function of water supply.

In general N- and water-stress had opposing effects on seedling C/N, and combined stresses (-H2O-N) cancelled such that C/N was similar to the control. For each species, C/N was significantly greater under both low N treatments relative to both high N treatments (Fig.4). Water stress acting alone (-H2O+N) caused a significant reduction in C/N compared to control for Sb, but not Sw. Under low N supply, C/N was reduced by drought in both species, as demonstrated by the significantly lower C/N in the double stressed treatment compared to the N-stressed treatment (+H2O-N). The C/N ratio for both species was significantly greater in seedlings grown under simultaneous stress relative to those grown under the control conditions. The species had statistically identical C/N in each treatment except water-stress, where Sb exhibited a lower C/N than Sw.

Population level variation in C/N was absent for both species (Table 2). However, populations of Sw responded to the effects of variation in water supply in different ways, as indicated by the significant population by water interaction term.
Fig. 4. Mean carbon-to-nitrogen ratio, C/N (g/g) ± SE in *Picea mariana* (Sb) and *P. glauca* (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species were significantly different at -H2O+N (P=0.0001), but statistically equal at all other treatments. Significantly different (P<.05) treatments within a species are denoted by separate letters above the bars.
E. Carbon Isotope Discrimination

The effects of N- and water-stress on $\delta^{13}$C were highly significant (Table 1). Averaged across all treatments, the species differences in $\delta^{13}$C were also highly significant. The species responded to water-stress differently as indicated by the significant species by water interaction term. Carbon isotope composition in Sb was increased more by drought than in Sw (Table 3). The species also responded to N-stress differently (i.e., significant species x N interaction term), where it appears that Sb exhibited a greater decrease in $\delta^{13}$C than Sw. The lack of significance in the water by N interaction term indicates that drought had similar effects on $\delta^{13}$C whether the seedlings were stressed for N or not. However, the significant species by water by N interaction term shows that the species responded to water-stress while subjected to N-stress differently, compared to when not stressed for N.

Water- and N-stress acting independently (-H$_2$O+N and +H$_2$O-N, respectively) had opposing effects on $\delta^{13}$C, but in combination cancelled out (-H$_2$O-N vs. +H$_2$O+N). Carbon isotope composition was significantly less negative in both droughted treatments (-H$_2$O+N and -H$_2$O-N) compared to the well-watered treatments (+H$_2$O+N and +H$_2$O-N) for both species (Table 3). However, $\delta^{13}$C was more negative in both N-stressed treatments (+H$_2$O-N and -H$_2$O-N) compared to the high N treatments (+H$_2$O+N and -H$_2$O+N). For Sw the effect of combined stress (-H$_2$O-N) on $\delta^{13}$C was a significant decrease to more negative values relative to the control, whereas Sb did not show a significant change in $\delta^{13}$C between the double-stressed and control treatments. White spruce had less negative $\delta^{13}$C values than Sb in each treatment, however only marginally so in the double-stressed one.

Black spruce exhibited significant population level variation for $\delta^{13}$C, but Sw did not (Table 2). Moreover, populations of Sb were affected differently by N-stress as indicated by the significant population by N interaction term. Populations of both species responded to water-stress similarly.

F. Rate of Photosynthesis

Variations in N and water supply had a significant effect on net photosynthetic rate ($A$) (Table 1). Averaged across all treatments the species exhibited significantly different $A$. Although the species did not differ in response to N-stress, they did to water-stress, as indicated by the significant species by water interaction term. Drought caused a greater reduction in $A$ for Sw than Sb (Fig.5). The lack of a significant water by N interaction term demonstrates that the effects of N or water-stress on $A$ did not differ whether the species were stressed or not stressed for water or N, respectively.
Table 3. Carbon isotope composition, $\delta^{13}$C (per mil), of *Picea mariana* and *P. glauca* seedlings.*

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*Sb = Picea mariana; Sw = P. glauca. Species differences were significant at +H2O+N (P=.0004), +H2O-N (P=.0001), and -H2O+N (P=.0165), but marginally insignificant at -H2O-N (P=.0536). All treatments within a species were significantly different (P<.05) from each other.
Fig. 5. Mean net photosynthetic rate, $A$ (umol CO$_2$/m$^2$/s) ± SE of *Picea mariana* (Sb) and *P. glauca* (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species differences were significant at $+H_2O+N$ ($P=.0001$), $+H_2O-N$ ($P=.0006$), marginally insignificant at $-H_2O+N$ ($P=0.0527$), and not different at all at $-H_2O-N$ ($P>0.05$). Significantly different ($P<.05$) treatments within a species are denoted by separate letters above the bars.
Photosynthetic rates at both high and low water conditions correspond to those in the D-T experiment (Fig.1 and Fig.5). Drought reduced $A$ while the seedlings were not stressed for N ($+\text{H}_2\text{O}+\text{N}$ vs. $-\text{H}_2\text{O}+\text{N}$) as well as when they were stressed for N ($-\text{H}_2\text{O}+\text{N}$ vs. $-\text{H}_2\text{O}-\text{N}$). Although $A$ was not significantly lower in the N-stressed ($+\text{H}_2\text{O}-\text{N}$) treatment relative to control ($+\text{H}_2\text{O}+\text{N}$) for either species, it seemed to be slightly lower in $S_w$. Photosynthesis was significantly reduced for both species under the double-stressed treatment compared to the water-stressed treatment alone. As expected, $A$ in seedlings of both species grown under simultaneous N- and water-stress was significantly lower than in those grown under control conditions. While $S_w$ had significantly greater $A$ in the two well-watered treatments ($+\text{H}_2\text{O}+\text{N}$ and $+\text{H}_2\text{O}-\text{N}$) and a marginally greater $A$ in the droughted treatment ($-\text{H}_2\text{O}+\text{N}$) than $S_b$, the two species had statistically identical $A$ in the simultaneous stress treatment ($-\text{H}_2\text{O}-\text{N}$).

Population level variation in either species was not detected for $A$ (Table 2). However, populations of $S_w$ responded to drought differently as indicated by the significant population by water interaction term for $S_w$. Populations of $S_b$ responded to N-stress differently but only marginally so as indicated by the population by N interaction ($P=.0543$).

G. Rate of Transpiration

The effect of variation in N and water supply on transpiration rate ($E$) was highly significant, and the species were significantly different from each other (Table 1). Although the species responded similarly to N-stress (lack of significant species by N interaction term), they responded to water-stress differently (significant species by water interaction term). Figure 6 shows that $S_w$ exhibited a stronger decrease in $E$ than $S_w$.

Water-stress and N-stress had opposing effects on $E$. Transpiration was significantly lower in each species under the droughted treatment, but significantly greater under the N-stressed treatment compared to the control treatment ($-\text{H}_2\text{O}+\text{N}$ vs. $+\text{H}_2\text{O}+\text{N}$ and $+\text{H}_2\text{O}-\text{N}$ vs. $+\text{H}_2\text{O}+\text{N}$) (Fig.6). The effect of simultaneous N- and water-stress was a significant reduction in $E$ relative to control for each species ($-\text{H}_2\text{O}-\text{N}$ vs. $+\text{H}_2\text{O}+\text{N}$). Although $E$ in both species was lower in the double-stressed treatment compared to the N-stressed treatment ($-\text{H}_2\text{O}-\text{N}$ vs. $+\text{H}_2\text{O}-\text{N}$), $S_b$ exhibited a greater $E$ while $S_w$ showed no change in $E$ in the double-stressed treatment relative to the droughted treatment ($-\text{H}_2\text{O}-\text{N}$ vs. $-\text{H}_2\text{O}+\text{N}$). White spruce seedlings exhibited significantly greater $E$ in high water treatments than $S_b$ seedlings, whereas the two species had similar $E$ in the low water treatments.

White spruce, but not $S_b$, exhibited marginally insignificant population level variation for $E$ (Table 2). The significant population by water interaction term for $E$ in $S_w$ indicates that water-stress affected the populations differently. The marginally insignificant population by N by water
Fig. 6. Mean transpiration rate, $E$ (mmol H$_2$O/m$^2$/s) ± SE of Picea mariana (Sb) and $P$. glauca (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species were significantly different at +H$_2$O+N (P=.0006) and +H$_2$O-N (P=.0018), but statistically equal at -H$_2$O+N and -H$_2$O-N (P>.05). Significantly different (P<.05) treatments within a species are denoted by separate letters above the bars.
interaction term for $E$ in $S_b$ indicates that populations responded to water-stress differently while stressed for $N$ compared to when not stressed for $N$.

H. Biomass Allocation

Two allocation indices, $R/S$ and $R/LA$, were measured in this study. The effects of water and $N$ were highly significant for both parameters, and the species exhibited highly significant differences in each (Table 1). Although the response of the species to drought was different (significant water by species interaction term) for $R/S$, it was not for $R/LA$. The interaction was caused by the fact that $S_b$ increased $R/S$ with drought, but $S_w$ did not (Fig.7). Root-to-shoot ratio and $R/LA$ were affected by $N$-stress similarly for both species as indicated by the lack of significant species by $N$ interaction terms. The response to either stress was similar whether seedlings were stressed for the other resource or not (insignificant water x $N$ interaction term).

Both $S_b$ and $S_w$ showed significantly greater $R/S$ in both $N$-stressed treatments (+H2O-N and -H2O-N) compared to both high $N$ treatments (+H2O+N and -H2O-N) (Fig.7). However, only $S_b$ exhibited a significantly greater $R/LA$ in both $N$-stressed treatments relative to both the high $N$ treatments (Fig.8). Black spruce had a significantly greater $R/S$ and $R/LA$ under the droughted treatment compared to the control (-H2O+N vs. +H2O+N), but $S_w$ did not. Where $R/S$ was not altered by drought in $S_b$ while stressed for $N$, $R/LA$ was increased. On the other hand, $S_w$ showed no change in either $R/S$ or $R/LA$ with drought under both $N$ levels. Each species exhibited a greater $R/S$ and $R/LA$ in the double-stressed treatment compared to control (-H2O-N vs. +H2O+N). White spruce had a significantly greater $R/S$ in both high water treatments than $S_b$ (+H2O+N and +H2O-N), but the species had similar $R/S$ in the low water treatments (-H2O-N and -H2O-N). However, $S_w$ exhibited a significantly greater $R/LA$ than $S_b$ in each treatment, but only marginally so when double-stressed.

Black spruce showed significant population level variation in $R/S$ and marginally significant variation in $R/LA$, but $S_w$ did not (Table 2). Population by $N$ and population by water interaction terms in either species were not significant.

I. Correlations between Traits

Traits in every treatment were significantly correlated (Table 4), but it appears that the overall number of significant correlations in the double-stressed treatment was less than in the others. A positive relationship between $R/LA$ and $\delta^{13}C$ occurred in each treatment, but not significantly so in the simultaneous stress treatment. Generally, SAR was negatively related to the other $N$-relations traits (e.g., $C/N$, $R/S$); but $C/N$ and $R/S$ were not significantly correlated in any
Fig. 7. Mean root-to-shoot ratios, R/S (g/g) ± SE of *Picea mariana* (Sb) and *P. glauca* (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species were significantly different at +H2O+N (P=.0005) and +H2O-N (P=.0400), but statistically equal at -H2O+N and -H2O-N (P≥.05). Significantly different (P<.05) treatments within a species are denoted by separate letters above the bars.
**Fig. 8.** Mean root-to-leaf area ratio, R/LA (mg/cm$^2$) ± SE of *Picea mariana* (Sb) and *P. glauca* (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. Each bar represents the average of about 25 seedlings. Species differences were statistically significant at +H2O+N (P=.0001), +H2O-N (P=.0004), and -H2O+N (P=.0049), but marginally insignificant at -H2O-N (P=.0537). Significantly different (P<.05) treatments within a species are denoted by separate letters above the bars.
Table 4. Correlation matrices for nitrogen- and water-relations traits.*

<table>
<thead>
<tr>
<th></th>
<th>$\delta^{13}C$</th>
<th>C/N</th>
<th>R/S</th>
<th>R/LA</th>
<th>SAR</th>
</tr>
</thead>
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<td>0.57</td>
<td>0.63</td>
<td>-0.57***</td>
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<tr>
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<td>0.18</td>
<td>0.17</td>
<td>-0.06</td>
<td>0.62***</td>
</tr>
<tr>
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<td>-0.84</td>
<td>-0.68</td>
<td></td>
</tr>
<tr>
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<td>0.92</td>
<td>-0.82</td>
<td></td>
</tr>
<tr>
<td>SAR</td>
<td>0.42</td>
<td>0.47</td>
<td>0.77</td>
<td>-0.30*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$\delta^{13}C$</th>
<th>C/N</th>
<th>R/S</th>
<th>R/LA</th>
<th>SAR</th>
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</thead>
<tbody>
<tr>
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<td>0.50</td>
<td>0.44</td>
<td>-0.61***</td>
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</tr>
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<td>0.38</td>
<td>-0.72**</td>
<td></td>
</tr>
<tr>
<td>R/S</td>
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<td>0.03</td>
<td>0.68</td>
<td>-0.78**</td>
<td></td>
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<tr>
<td>R/LA</td>
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<td>0.09</td>
<td>0.41</td>
<td>-0.69**</td>
<td></td>
</tr>
<tr>
<td>SAR</td>
<td>-0.21</td>
<td>-0.47</td>
<td>-0.77</td>
<td>-0.30*</td>
<td></td>
</tr>
</tbody>
</table>

*Data based on seedlings of *Picea mariana* and *P. glauca* grown under various nitrogen and water treatments, including: a) above diagonal +H2O+N and below diagonal and +H2O-N, and b) above diagonal -H2O+N and below diagonal -H2O-N. Symbols as defined in Table 1. A correlation is significantly different from zero at *: P<.05, **: P<.01, ***: P<.001, ****: P<.0001.
treatment. Carbon isotope composition ($\delta^{13}C$) and C/N were not significantly related in any treatment, however, SAR and $\delta^{13}C$ were found to be negatively related within each treatment (not significant when double-stressed). As expected, R/S and R/LA exhibited a highly significant positive relationship in each treatment.

Carbon isotope composition and C/N were found to be negatively correlated when plotted across all treatments (Fig. 9). The regression lines for each species were highly significant ($P=.0001$). The difference between the species slopes was significant ($P=.0259$). For example for Sb, a 1 per mil increase in $\delta^{13}C$ is associated with a decrease in C/N of 14.1. For Sw, a 1 per mil increase in $\delta^{13}C$ is associated with a decrease in C/N of 9.2. Plants of both species grown in the droughted treatment (-H2O+N) had the least negative (highest) $\delta^{13}C$ and lowest C/N, whereas plants in the +H2O-N treatment exhibited the most negative (lowest) $\delta^{13}C$ and highest C/N. Plants in +H2O+N and -H2O-N tended to have intermediate $\delta^{13}C$ and C/N, but plants in the control treatment (+H2O+N) had slightly less negative $\delta^{13}C$ and lower C/N values than plants in the -H2O-N treatment. Intercepts and slopes for correlations between $\delta^{13}C$ and C/N for populations within either species (not presented) were not significantly different ($P<.05$).
Fig. 9. Relationship between carbon isotope composition, $\delta^{13}$C (per mil), and carbon-to-nitrogen ratio, C/N (g/g), in a) Picea mariana (Sb) and b) P. glauca (Sw) seedlings grown for approximately 16 weeks under various nitrogen and water regimes. The linear regression equations and coefficients of determination are: a) $y = -0.069x - 27.233$, $r^2 = 0.322$, and b) $y = -0.098x - 25.807$, $r^2 = 0.354$. The slope of each regression line was significantly different from zero ($P=.0001$). The y-intercepts and slopes of the species lines (i.e., Sb versus Sw) were significantly different ($P=.0001$ and $P=.0259$, respectively).
DISCUSSION

This discussion is divided into four sections. The first section compares the relative performance of Sb and Sw under high and low N and water regimes and discusses the implications in light of potential costs, benefits, and environmental trade-offs. The second section attempts to locate trait trade-offs and to determine their ecological significance. The third section integrates the findings of the first two sections by evaluating the suite of adaptive traits held by each species in terms of its performance, potential and realized, in the field and laboratory. Population level variation is discussed in the fourth section.

I. Trait Means, Phenotypic Plasticity, and Environmental Trade-offs

Mechanisms which improve a species' growth and chances of survival under resource limitation may reduce growth or survivability in non-stressed conditions. Because of these costs, or environmental trade-offs, adaptation to low resource environments may preclude successful occupation of high resource environments and vice versa. Through phenotypic alterations (plasticity) plants can ameliorate or eliminate the costs of particular traits across environments. Thus, variation in plasticity may also be important in determining the propensity of species to occupy habitats that differ in resource supply. This section examines the relative character means and plastic responses of several N- and water-relations traits in Sb and Sw to variations in N and water supply.

A. With Nitrogen-Supply

Because Sb and Sw grow across a nutrient gradient in the southern boreal forest, it is of interest to determine how they may be adapted and respond to variations in N supply. Adaptations that could be important to survival in infertile soils include: 1) low maximal relative growth rate, 2) high physiological capacity to extract nutrients from the soil, 3) high metabolic efficiency in the use of nutrients to produce new biomass, and 4) greater biomass allocation to roots. In addition, there is some indication that WUE may affect a plant's ability to acquire nutrients.

1. Maximal Relative Growth Rate:

A plant with low RGRmax may have an advantage in infertile habitats because of reduced need, or demand, for nutrients (Chapin, 1980; Shipley and Keddy, 1988; Fichtner and Schulze,
However, it may be less capable of occupying competitive, highly fertile environments that favor increased RGRmax (Grime, 1977; 1979). Such a trade-off could help to maintain species distributions across fertility gradients.

Despite the fact that the Sb and Sw populations tested come from habitats of presumably contrasting nutrient availability, they did not differ in final growth under optimal conditions (Fig.2, +H2O+N treatment). Since they germinated at approximately the same time and seeds were of comparable size, they probably had approximately equal RGRmax. One explanation for this counter-intuitive result is that Sw also comes from a habitat which is low in an essential resource (water). It may possess adaptive traits that trade-off with RGRmax, or a low RGRmax may be beneficial for surviving drought as well. However, because a low RGRmax is an adaptation in and of itself (see Literature Review section I.A.2), the underlying mechanistic causes for species relative values are irrelevant. In terms of this potential adaptation it appears that neither species is more or less suited to cope with low nutrient conditions.

2. Specific N Absorption Rate:

A high SAR may be an important mechanism by which plants cope with N-stress because it can enable: 1) efficient and rapid exploitation of soil N reserves, 2) maintenance of relatively high internal N-status, and 3) increased flux of N to the root system (by enhancing the concentration gradient between the bulk soil and root) (Chapin, 1980; Fitter and Hay, 1987). However, Tilman (1988) suggested that plants may be faced with a trade-off for allocating protein towards mechanisms that act to increase photosynthesis and those which increase nutrient uptake. If so, variation in SAR may be a factor contributing to the manner by which species segregate across fertility gradients.

The particular SAR expressed by an individual is a function of N availability in the soil and root absorption capacity (RAC) (Welbank, 1962; Nye, 1977; Nye and Tinker, 1977; Fitter and Hay, 1987; Chapin, 1991). Hence, the greater SAR exhibited by Sb in the high N treatment (Fig.3, +H2O+N treatment) suggests that it must also have greater RAC than Sw because N availability was constant. A high RAC may have arisen as a mechanism to capitalize on seasonal nutrient flushes characteristic of muskeg habitats (Saebo, 1969). With high rates of N uptake during flushes, Sb may be better able to survive subsequent periods of N deprivation.

Because Sb may have greater RAC (under high N) and lower A than Sw (Fig.5), the physiological ability to take up N and fix carbon may trade-off as Tilman (1988) suggested. Whether this trade-off is a function of competing enzyme allocation priorities, or not, is impossible to know with the measurements made in this study. Nevertheless, this trade-off suggests that, in the case of Sb and Sw, evolving a higher RAC (or SAR under high N) precludes high A, and vice versa. Such a trade-off may affect these species' abilities to occupy each other's habitats.
3. Nitrogen-Use Efficiency:

In theory, when plants are grown under high N conditions, both a low or high NUE may reflect superior adaptation. For example, a low apparent NUE can result from luxury consumption, and thereby represent an adaptation to infertile habitats that have periodic nutrient flushes (Chapin, 1980). Or, a high NUE may reflect adaptation to habitats which have relatively high and/or constant rates of N mineralization (Vitousek, 1982). On the other hand, when grown under low N conditions individuals with greater NUE will, by definition, achieve greater growth. The fact that the species in this study exhibited similar NUE under conditions of high (+H2O+N) as well as low (+H2O-N) N supply (Fig.4) indicates that neither is able to utilize N more efficiently than the other to maximize growth, and neither has a greater propensity for luxury consumption. Furthermore, it appears that when N is the chief limiting factor to growth, Sb and Sw should exhibit approximately equal rates of biomass accumulation. It seems unlikely, then, that mean NUE is an important mechanism influencing these species distributions.

The greater degree of plasticity for NUE exhibited by Sb (Table 1, Fig.4), however, may be an adaptation for coping with temporally pulsed nutrient supplies of muskegs (sensu Bradshaw, 1965). A highly plastic NUE may help maintain proportionally higher rates of growth under N-limited conditions, while achieving proportionally higher levels of luxury consumption during flushes.

4. Biomass Allocation:

Due to greater nutrient absorbing biomass (roots) and proportionally lower shoot biomass, plants with large R/S may be able to maintain higher internal N-status than ones with low R/S. A large R/S, then, may translate into greater growth and higher survival rates in nutrient limited habitats. However, as a result of reduced foliar allocation a large R/S may be maladaptive in conditions where nutrient supplies are not limiting.

Species from low nutrient habitats frequently exhibit intrinsically greater R/S than ones from sites with ample nutrients (Grime, 1977; 1979; Chapin, 1980). As Sw exhibited a higher R/S than Sb (Fig. 7, +H2O+N and +H2O-N), this generalization was clearly not supported by this study. However, since the sites where Sw is found may also favor high R/S (due to low water), this comparison does not refute the above generalization. Nevertheless, in terms of allocation, Sb should be less able to cope with nutrient-stress than Sw and, because of less allocation to leaf tissue, Sw may exhibit relatively slower growth in high nutrient conditions.

5. Water-Use Efficiency:

A genotype can derive a greater WUE than another by one of four possible mechanisms, including: 1) higher A and similar E, 2) similar A and lower E, 3) higher A and E, but
proportionally higher A than E, and 4) lower A and E, but proportionally lower E than A. The mechanisms that cause WUE may have associated costs and benefits dependent on the specific characteristics of the environment.

Figure 5 demonstrates that δ13C values were more negative under low N (+H2O-N) conditions compared to high (+H2O+N), which implies that WUE was decreased by N-stress (Farquhar et al., 1989a). While neither species exhibited a significant reduction in A, E in both was was greater under low N compared to high (Fig.5 and 6). The mechanisms for lower WUE in these species are interesting in that they conflict with the findings of most other studies. Generally, other researchers have observed that decreased WUE in response to N-stress resulted from a decrease in both A and E, but a greater decrease in A than E (e.g., Medina, 1972; Wong, 1979; Longstreth and Nobel, 1980; Brown and Wilson, 1983; Sage and Pearcy, 1987; Lajtha and Whitford, 1989; Reich et al., 1989). However, Toft et al. (1989) observed a similar response in cold desert species as that observed in this study. These species seem to have responded in an active manner to help avoid the deleterious effects of N-stress.

Decreasing WUE by increasing E may be beneficial for coping with N-stress for several reasons. First, decreased N availability probably led to lower levels of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) and hence decreased photosynthetic capacity (Evans, 1983; Sage and Pearcy, 1987). The seedlings could maintain A by opening stomata and increasing the flux of CO2 into the leaf to more fully saturate the photosynthetic machinery (Toft et al., 1989). In the case of the relationship between A and E exhibited by Sw, it appears that photosynthetic-capacity was reduced more than increased stomatal aperture could offset it. Second, lowering WUE via this mechanism could cause more carbon to be assimilated per unit leaf N, increasing PNUE (Field et al., 1983). Third, increasing E may increase mass flow of dissolved nutrients to the root system (Nye and Tinker, 1977; Fitter and Hay, 1987). Unfortunately, because of conflicting results of past research this third "benefit" requires more detailed study to establish its significance. For example, Schulze and Bloom (1984) found that large fluctuations in E had little influence on N absorption in radish and tomato, but Mastle et al. (1992) found that WUE across several species was negatively correlated with tissue nutrient concentration.

B. With Water-Supply

Traits that could be important adaptations to survival in water-limited habitats include: 1) high degree of drought-tolerance, 2) highly efficient utilization of water, and 3) greater biomass allocation to roots. Because soil water content may alter N-availability, absorption capacity, and/or N-utilization, a plant's SAR and/or NUE may also be affected by water-supply. Therefore, this section examines the effect water supply had on both water- and N-relations.
1. Drought-Tolerance:

A high degree of drought-tolerance (D-T) may be an important adaptation allowing plants to inhabit water limited environments. However, Orians and Solbrig (1977) suggested that the ability to withstand relatively high levels of internal desiccation (high D-T) may trade off with high photosynthetic rates (\(A\)) under optimal moisture conditions. If a trade-off between D-T and A exists, then variation in D-T may be a mechanism controlling plant distributions across moisture gradients.

Figure 1 shows that Sw was more drought tolerant than Sb and also maintained greater A while not water stressed. A high relative degree of D-T may be extremely advantageous in competitive water-limited habitats (Bunce et al., 1977; Shainsky and Radosevich, 1986; DeLucia and Hechathorn, 1989; DeLucia and Schlesinger, 1991), suggesting that Sw may be better equipped to occupy such habitats.

The results of this study do not support the hypothesis proposed by Orians and Solbrig (1977); that is, a high relative degree of drought tolerance did not trade-off with photosynthetic performance under non-stressed conditions. White spruce was more D-T than Sb, but also had higher \(A\) at high \(\Psi_{xylem}\). In a similar vein, DeLucia and Heckathorn (1989) and Robichaux (1984) found that more tolerant species were not found in areas where less tolerant species grew for entirely other reasons (such as nutrient availability) than costs of high D-T. These studies and the results of this one question the existence of photosynthetic costs associated with the evolution of high D-T, and suggest that, despite higher drought tolerance, Sw (in terms of A) will not be less apt to occupy wet sites than Sb.

2. Water-Use Efficiency:

The significantly less negative \(\delta^{13}C\) values exhibited by Sw under "optimal" conditions (+H2O+N) indicate that it had an intrinsically higher WUE than Sb (Farquhar et al., 1989a). High WUE is of no particular benefit when water does not limit growth and could actually be deleterious (Cohen, 1970; Cowan, 1982; Meizner et al., 1990). For example, Meizner et al. (1990) concluded that coffee genotypes with higher WUE, resulting from lower A and \(E\), would yield less when grown in well-irrigated soils than genotypes with lower WUE. However, if WUE is derived from higher A, no such trade-off exists. For example, Condon et al. (1987) found that wheat genotypes with higher WUE (less negative \(\delta^{13}C\)), due to greater A, exhibited more dry matter production when given ample water than genotypes with lower WUE. The greater WUE observed in Sw resulted from it having a higher A, and \(E\), than Sb (Table 3, Fig.5, and Fig.6), suggesting that Sw will not suffer from its higher WUE when growing in mesic or hydric conditions.

In low, as opposed to high, water conditions a high WUE may be beneficial because a more water-use efficient plant will assimilate more carbon per unit water utilized over time.
Moreover, if high WUE is derived from lower \( E \), the onset of severe internal water-stress may be slowed. However, rates of photosynthesis and growth per unit time will be reduced, and drought may not be delayed if water saved is lost to seepage, evaporation, and uptake by competing plants (Cohen, 1970). A high WUE, via lower \( E \), then, should be advantageous in non-competitive water-limited habitats but disadvantageous in competitive ones (Cohen, 1970; Bunce et al., 1977; Shainsky and Radosevich, 1986; Delucia and Hechathorn, 1989; Delucia and Schlesinger, 1991).

If, on the other hand, higher WUE is derived from greater \( A \), rates of photosynthesis and growth per unit time will not be reduced, eliminating the risk of water loss to external factors. However, if \( E \) is also greater drought will not be delayed. In fact, Bennett and Rook (1978) found that a clone with high WUE having greater \( E \) and proportionally greater \( A \), died earlier under severe drought than a clone with low WUE.

In this study, \( S_w \) achieved a higher WUE than \( S_b \) in the droughted treatment through having a higher \( A \) and similar \( E \) (Table 3, Fig.5, and Fig.6). Of the four possible combinations causing variation in WUE, this particular combination should confer on \( S_w \), relative to \( S_b \), the benefits of higher WUE with few of the potential costs. Since \( S_w \) had greater \( A \) it should be better able to maintain growth during drought; since it did not have greater \( E \) it should not be at greater risk of death under extreme drought; and, because it had a higher WUE it should acquire a larger quantity of carbon per unit water lost. This suggests that \( S_w \) may be better suited than \( S_b \) for inhabiting both competitive and non-competitive water-limited environments.

Although both species increased WUE (less negative \( \delta^{13}C \)) with drought, \( S_b \) had a stronger response than \( S_w \) (Table 1; Table 3). Gas exchange measurements indicated that this difference in plasticity resulted from \( S_b \) exhibiting proportionally less reduction in \( A \) than \( E \), relative to \( S_w \). Because it increased WUE more, while simultaneously exhibiting less reduction in \( A \), it must be concluded that \( S_b \) responded in a more optimal manner to drought than \( S_w \); but not enough to actually surpass the WUE attained by \( S_w \) during drought.

### 3. Biomass Allocation:

As a result of greater water absorption and less water loss, a plant with a large \( R/S \) can maintain relatively high \( \Psi_{xylem} \) at any particular \( \Psi_{soil} \). Thus, in water limited conditions a plant with a greater \( R/S \) may be able to exhibit greater \( A \) than one with low \( R/S \) (Chapin, 1993). However, because of less photosynthesizing surface area a large \( R/S \) may slow growth in hydric or mesic habitats.

Unfortunately, due to genotypic, developmental, and environmental variation in specific leaf area (SLA) and leaf:stem ratio, there is no consistent relationship between shoot biomass and leaf area in different species or across environments (e.g., Mooney et al., 1978). Since whole-
plant $E$ is a function of LA rather than shoot biomass, and simple comparisons of R/S could be misleading; R/LA was also calculated. Black spruce exhibited a significant increase in R/S and R/LA in response to moisture stress, which is a common response and has been observed previously in angiosperms (Davidson, 1968; Miao et al., 1992). White spruce did not show a significant change in R/S or R/LA with drought, causing the significant species x water interaction for R/S (Table 1). Intrinsically large root allocation may have become genetically fixed (less plastic) as a result of adaptation to habitats consistently low in soil moisture resources (Bradshaw, 1965; Chapin, 1991). Since Sw had greater R/LA, despite low plasticity, it should exhibit a proportionally "better" water status than Sb when growing in common droughted situations, but may be at a disadvantage when growing in high water conditions.

4. Specific N Absorption Rate:

Past studies have shown that water supply can strongly influence soil nutrient availability, as well as RAC (Welbank, 1962; Nye and Tinker, 1977; Fritter and Hay, 1987). For example, ion mobility can decrease by two orders of magnitude between −0 MPa and −1.0 MPa soil water potentials. Slowed microbial activity in dry soils may reduce N mineralization rates, and decreased soil water content can reduce mass flow of dissolved N in the rhizosphere (Welbank, 1962; Nye and Tinker, 1977; Fritter and Hay, 1987). Moreover, extreme soil desiccation can, through direct damage to root tissue, reduce a plant's physiological capacity to absorb N (Welbank, 1962).

Specific N absorption rate was not affected by water supply (Table 1, Fig.3), suggesting that neither N availability or RAC was reduced by drought in this study. It can be concluded, then, that the water treatments had direct effects on plant N-relations, as well as water-relations, rather than indirect effects mediated through alterations of the N environment. In contrast to these results, Welbank (1962) found that SAR was lower in droughted treatments, despite equal N supply. Perhaps differences in the degree or type of drought can account for this discrepancy. Soil was allowed to simply dry down to excessively low water content through one cycle in Welbank's study, but in this experiment water stress was moderate (-1.0 MPa) and soils were wetted to field capacity cyclically.

5. Nitrogen-Use Efficiency:

Welbank (1962) and Day (1981) found that plants grown with high water supply had lower nutrient-use efficiency than individuals subjected to drought. Their results are opposite to what happened for Sb in this study, and also contrast with the lack of effect on Sw (Fig.4). The possible cause of these discrepancies may be differences in drought severity. In the studies of Welbank (1965) and Day (1981), due to extreme drought, SAR and N uptake were likely reduced
proportionally more than growth such that supply was less than demand. In this study, because SAR was not altered and growth was reduced by water-stress (Fig.2), N absorption may have exceeded demand. If so, the proportion of latent to active N within the plant may have increased, thereby decreasing the NUE.

The low water treatment was the only one where Sb and Sw had divergent NUE, suggesting that Sw may be better able to allocate and utilize N to maximize carbon assimilation while exposed to drought. Greater NUE could be beneficial in droughted environments where rates of N mineralization are high and relatively constant, and Sw may out perform Sb under such conditions, as on the upland sites in which it occurs.

C. With Simultaneous Nitrogen and Water Stress

Most research to date has focused on plant responses and adaptations to single environmental factors, but plants in nature often encounter multiple stresses (Chapin et al., 1987). Although Sb and Sw grow in habitats with contrasting water and N availability, from time to time each habitat could exhibit simultaneous limitations in both N and water. The manner by which Sb and Sw respond to the effects of simultaneous N and water stress, then, may bear on their distribution patterns. This section examines how each N- and water-relations trait in Sb and Sw was affected by limitations in both N and water.

1. Specific N Absorption Rate:

The effect of N-stress on SAR was the same at high and low water (Fig.3). Thus, with low N availability, as was the case with high N (see section B, 4), it can be concluded that water stress had little affect on soil N availability or on the physiological propensity of the plants to absorb N.

2. Nitrogen-Use Efficiency:

Figure 4 shows that Sb and Sw used N with equal efficiency under combined water and N stress. Since both species had higher NUE relative to their respective control, it is apparent that each was able to assimilate more carbon per unit N taken up despite limitations on water supply. However, because NUE was lower in the double-stressed treatment compared to the N-stress treatment (+H2O-N), it appears that water-stress did reduce NUE.

Furthermore, because the level of N-stress in the two low N treatments was the same, drought apparently prevented the seedlings from realizing a NUE that such a level of N-stress might demand. Thus, although the seedlings were able to increase NUE with N-stress under drought they were forced, apparently due to drought, to operate at a reduced level of NUE.
Because SAR was not affected by drought (above), it seems likely that reduced growth rate (associated with water-stress) allowed N to accumulate above immediate demand, eliciting a decrease in NUE.

3. Water-Use Efficiency:

There was no change in $\delta^{13}$C for Sb when grown under simultaneous N- and water-stress compared to control, whereas $\delta^{13}$C was significantly more negative for Sw (Table 3). In agreement, Toft et al. (1989) found that $\delta^{13}$C was not statistically different for cold desert species grown under higher water and N supplies compared to individuals subjected to lower amounts of these resources. The differential response of Sb and Sw caused them to exhibit more similar $\delta^{13}$C, and hence WUE, in the double-stress treatment than in any other treatment. Gas exchange measurements indicate that the slightly greater WUE in Sw ($P=.0536$) resulted primarily from it having slightly lower $E$ and slightly greater $A$ (Fig.5 and 6).

The significantly lower WUE (more negative $\delta^{13}$C value) in the double-stressed compared to the water-stressed (-H2O-N vs. -H2O+N) treatment, suggests that N-stress restricted the ability of both species to increase WUE. It also appears that Sw increased WUE (less negative $\delta^{13}$C) with drought by a greater amount at high N than it did at low (+H2O+N vs. -H2O+N compared to +H2O-N vs.-H2O-N); whereas Sb appears to have increased WUE in response to drought more while stressed for N than not. This 3-way interaction suggests that in high N conditions Sw may be able to respond to drought better (through increased WUE) than it would in nutrient-limited conditions. In contrast, Sb may be more responsive to drought in low nutrient environments than it is in high. Interestingly, these response patterns seem to be correlated with habitat. Drought on upland sites is likely to arise most frequently without N-stress, and, in the event that drought occurs in muskegs, it probably would be accompanied by N-stress.

4. Biomass Allocation:

Under simultaneous water and N stress the species exhibited statistically equal R/S (Fig.7), but Sw had a slightly greater R/LA ($P=.0536$) (Fig.8) than Sb. The similar R/S ratios suggest that, in terms of allocation, neither species would be better able to cope with N deficiency when growing in simultaneous N- and water-limited conditions. As a result of greater R/LA, however, Sw may maintain higher internal water-status, and hence be better able to avoid drought under these conditions.

As was the case with WUE, the species differences in R/S and R/LA seem to have been lessened as a result of simultaneous water- and N-limitation. The apparent cause of this plastic convergence seems to be differences in response to drought. As can be seen in Figure 8, Sb exhibited a slight (but not significant) increase in R/S in the double-stress treatment relative to the
N-stress treatment, whereas Sw showed very little change. On the other hand, both species had greater R/S when grown under low water and N compared to just low water. The pattern was more or less similar for R/LA, but the species convergence was less extreme than was the case for R/S. These results suggest that Sw has little plastic root allocation in response to water-stress, but does respond well to N-stress. Black spruce, on the other hand, appears to be more plastic in response to both factors.

D. Conclusions

The results discussed in this section have revealed variation in N- and water-relations traits between Sb and Sw. In support of hypotheses A.1. and A.2., this variation generally correlated with the environmental conditions of the species' respective habitats, possibly indicating adaptive divergence. For example, Sb had greater SAR (under high N conditions) and possibly greater RAC. White spruce exhibited higher mean values of WUE and R/LA, and was more drought-tolerant than Sb. Because these traits may improve growth rate in low resource environments, the observed variation between Sb and Sw may influence their respective abilities to occupy low N or water habitats. On the other hand, the species did not differ in growth and NUE as hypothesized (A.1.), and Sw had greater R/S than Sb. The possible reasons for these findings are addressed in section IV.

The species responded plasticly in similar directions but frequently by different amounts. In rejection of hypothesis A.2, Sb exhibited greater and more "optimal" plasticity in WUE than Sw, and Sw showed little morphological plasticity in response to variation in water supply. Perhaps this pattern resulted from the fact that Sw possessed relatively higher mean WUE and R/LA values, and therefore did not need to plasticly increase them much in response to drought. On the other hand, NUE of Sw seemed to be less deleteriously affected by drought than in Sb, which could be beneficial in droughted high nutrient conditions. Each species seemed to respond to N-stress by increasing stomatal aperture, increasing E, and hence exhibiting lower WUE. Such a response may be an adaptation for ameliorating the effects of N-stress, but clearly could be dangerous in situations of combined water and N stress.

Overall, Sb was more plastic than Sw in most of the N- and water-relations traits measured, which is in direct opposition to hypothesis A.1. Such an occurrence may have resulted from the fact that Sb occupies both upland and muskeg habitats in more northerly portions of the boreal forest. Perhaps high plasticity evolved in these regions to compensate for the extreme environmental variation between the interbreeding muskeg and upland populations.

Potential environmental trade-offs were realized in R/S for Sw in terms of reduced photosynthesizing surface area, and in SAR for Sb in terms of reduced A. Such trade-offs could
reduce growth rate in high resource sites and hence affect the abilities of Sb and Sw to occupy each other's habitat. Potential trade-offs in terms of reduced A were absent for D-T and WUE, suggesting that improvement in these characteristics would not affect performance under high water conditions. These diverse findings suggest that environmental trade-offs may be species- and environment-specific.

The trend under simultaneous N- and water-stress was a general convergence in trait means between Sb and Sw. Perhaps their convergence resulted from 1) each responding more optimally to the resource which is most limiting in their respective habitat, and/or, 2) each being worse affected by the resource which is not limiting in their habitats. The results of section C seemed to support both these possibilities. Overall, the findings suggest that the two species would be more equally matched, in terms of growth and survivability, in conditions of simultaneous N- and water-stress than in either stress alone.

II. Trait Interactions and Trade-Offs

Physiological traits may augment or counter each other, and may similarly interact with morphological traits. If certain environments favor plants with one trait while other environments favor plants with another trait, then such trait trade-offs will translate into ecological trade-offs (Tilman, 1988). If in some species traits do not trade off, then they may have wide distributions, or may come to dominate certain types of environments (Grime, 1977; 1979). Therefore, trait "compatibility" within a species may be an important cause of its particular distribution. This section examines trade-offs within and between various N- and water-relations traits.

A. Nitrogen-Relations

The negative correlations between SAR and NUE, and between SAR and R/S at each treatment (except in the control for SAR and NUE) reveal two apparent physiological versus morphological trade-offs (Table 4). Similarly, Chapin et al. (1983) and Chapin and Tryon (1983) found that Sb had both higher P absorption capacity and lower R/S than Sw, and data from Schlesinger et al. (1989) indicate that Great Basin species with higher SAR tended to also have lower NUE. One possible explanation for these relationships is autocorrelation of axes, whereby the traits are correlated for purely mathematical reasons (Vitousek, 1982; Lajtha and Whitford, 1989). Alternatively, a biological explanation may be that high SAR and NUE, or high SAR and R/S, are incompatible trait combinations for any individual (species) to evolve.
B. Water-Relations

1. Physiology versus Physiology:

High degrees of D-T and WUE may be alternative strategies for coping with limitations in water availability (Cohen, 1970). For example, high WUE in many species is a conservative water-saving strategy achieved through reduced \( g \), and hence \( A \) and \( E \) (Field et al., 1983; Carter and Smith, 1988; Monson and Grant, 1989). A high WUE, derived by water conservation, is beneficial only in environments where water is not lost to external sinks (Cohen, 1970; Cowan, 1982). Conversely, a plant with a relatively high D-T, by definition, keeps its stomates open longer at low \( \Psi_{xylem} \) and will tend to lose more water (Tobiessen and Kana, 1974; Cline and Campbell, 1977; Bunce et al., 1977; DeLucia and Heckathorn, 1989; Lajtha and Getz, 1993). A high D-T is advantageous in competitive water-limited environments, but may be less beneficial than high WUE in situations where water is not lost to competition (Cohen, 1970; DeLucia and Heckathorn, 1989). DeLucia and Heckathorn (1989) suggested that a trade-off between D-T and WUE for Great Basin species was a factor contributing to plant distributions in that region.

In this study, Sw was both more drought tolerant and had a higher WUE than Sb. Thus, these results refute the concept that high D-T and WUE are mutually exclusive water-relations strategies. The data do, however, support the concept that water-saving and D-T (water-spending) are alternative modes for coping with water stress (Cohen, 1970; DeLucia and Heckathorn, 1989). The species which had lower \( E \) (Sb) was less drought tolerant, whereas the species that had greater \( E \) (Sw) was more drought tolerant. Since high WUE does not always represent a water-saving strategy, as demonstrated by this study and others (e.g., Bennet and Rook, 1978; Condon et al., 1978), the lack of a trade-off between WUE and D-T is purely semantic. Having greater D-T and WUE than Sb, suggests that Sw may be better equipped to persist in both competitive and non-competitive water-limited environments (Cohen, 1970; DeLucia and Heckathorn, 1989). Not only can Sw photosynthesize at lower water potentials than Sb, but it can acquire more carbon per unit water spent in the process.

2. Morphology versus Physiology:

Barton and Teeri (1993) found that more drought resistant tree species tended to allocate less biomass to roots. White et al. (1990) found that individuals with greater R/S tended to have lower WUE (more negative \( \delta^{13}C \)) than those with small R/S. These studies suggest that morphological (R/S) and physiological (WUE and D-T) mechanisms may be alternative modes for coping with water limitation. A large R/S acts to slow or prevent internal dehydration, making high D-T and/or WUE less necessary. A plant with high D-T and/or WUE may best increase growth by allocating more biomass to foliage.
There was no indication of a trade-off between morphological and physiological drought-resistance mechanisms in Sb and Sw. In each treatment, WUE (δ13C) and R/LA were positively correlated (not significantly in -H2O-N) (Table 4); and figures 4, 5, and 9 show, qualitatively, that Sw had a larger R/LA, as well as a higher degree of D-T and WUE than Sb. Greater R/LA in Sw, despite being physiologically more drought resistant, may be a mechanism to decrease whole-plant water loss and hence compensate for its higher E (see section I.B.2).

C. Nitrogen- versus Water-Relations

1. Physiology versus Physiology:

Water- and nitrogen-use efficiency (i.e., δ13C and C/N) were not significantly correlated within any treatment (Table 4). This demonstrates a lack of an intrinsic trade-off between the two traits, and suggests that for Sb and Sw, evolving a higher level of WUE or NUE does not preclude improved efficiency in the use of the other resource, respectively. These results are counter to what those of Field et al. (1983) suggested for co-occurring California evergreens after finding a significant negative correlation between WUE and NUE. However, their results were based on a correlation of species means and instantaneous gas exchange measures of WUE (A/E) and NUE (PNUE) (Field et al., 1983). The data on Sb and Sw from this study also contradict the results of DeLucia and Schlesinger (1991), who used similar measurements. They found that Great Basin species with relatively high WUE also had greater NUE than others (DeLucia and Schlesinger, 1991). These past studies and the results of this one demonstrate that WUE and NUE can be negatively, positively, or not correlated at all.

Despite the absence of an intrinsic trade-off between WUE and NUE, the traits were negatively correlated for each species when plotted across all treatments (Fig.9). This demonstrates a plastic trade-off and suggests that in order to increase WUE, NUE is sacrificed, and vice versa. Previous studies have observed a plastic trade-off between photosynthetic WUE (A/E) and PNUE (Reich et al. 1989; Lajtha and Whitford, 1989), but apparently none have demonstrated a significant trade-off for long-term, whole-plant measures. The levels of WUE and NUE in the double-stress treatment were intermediate to those of the single stresses alone. Thus, when Sb and Sw are exposed to simultaneous limitations in both water and N, they are forced to compromise the efficiency by which they utilize both resources.

The significantly different slopes of the species for the NUE vs. WUE (Fig.9) correlation and the occurrence of significant species by N by water interaction terms for both NUE and WUE (Table 1) are probably related. That is, the species responded differently to the effects of water- and nitrogen-stress, and hence exhibited differences in the plastic trade-off. Evidently, no other reports of 3-way interactions for WUE or NUE, or for differences in the slopes for the NUE vs.
WUE plastic trade-off have been made previously. To my knowledge, this is the only study that has looked for genotypic differences of this nature.

These species differences probably reflect underlying GxE effects, and are difficult to understand mechanistically. Ecologically, though, they have important implications. Because Sw exhibited a significantly lower slope, for any given increase in WUE it will experience less of a decrease in NUE than Sb, but any given increase in NUE will come at a greater cost in terms of WUE (see Results section II.B). For Sb, on the other hand, any given increase in NUE will result in less of a decrease in WUE than for Sw. This may reflect optimization of the trade-off, enabling each species to maximize the efficiency by which they use the resource which is most limiting (respective to their environments), while minimizing the subsequent reduction in efficiency of the use of the other resource. However, reducing the costs of increasing one trait automatically increases the costs for increasing the other which could result in a trade-off for optimizing the WUE vs. NUE plastic trade-off. Such a trade-off could influence the habitat preferences of these species.

2. Morphology versus Morphology:

Increased root allocation at the expense of shoot and leaf allocation is beneficial in both N and water limited habitats. This occurs because water and N are edaphic resources acquired by the root system, and demanded by the shoot system. This dual role of root allocation may be exceptionally important when both resources are limiting.

Because root allocation was greatest in the double-stressed treatment, Sb appears to have increased root allocation in response to each stress independently (Fig.7 and 8). This may improve the internal status of both resources simultaneously; and, since the water and N stress applied to the double and single stress treatments were the same, the individual impact of each stress should be proportionately less in the double- compared to single-stressed treatments. Similarly, Sw probably increased R/S in response to N-stress in the double-stressed treatment, suggesting that its water-status would be proportionally higher in the double-stressed than water-stressed treatment. However, because R/S in the double stress and single N-stress treatments were similar (due to a lack of allocative plasticity in response to water-stress, see section I.C.4), Sw, in contrast to Sb, would probably not have a proportionally "better" N-status.

D. Conclusions

Some trait trade-offs that were expected (hypothesis B) were located while others were not. For N-relations in Sb and Sw evolving a high SAR may prohibit increased NUE and R/S. This
may reflect optimization or balancing of the costs and benefits associated with each. However, due to autocorrelation the biological significance of these results is questionable. No trade-offs were located between water-relations traits suggesting that whole suites of drought resistance characters may arise together, which could have profound effects on the species distributions (see next section). Because Sw had higher values of each it may be more drought-resistant than Sb, but the cumulative effects of potential costs could, in theory, reduce its growth rate in high water conditions (but see above and below).

Nitrogen- and water-use efficiency traded-off plastically. This indicates that when Sb and Sw are exposed to limitations in both N and water they will be forced to use each resource with suboptimal efficiency. The species difference in the plastic NUE-WUE trade-off suggests that adaptation to one type of environment may limit a plant's ability to occupy the other type. However, the lack of an intrinsic trade-off between NUE and WUE, and the possibility that increased root allocation may improve adaptation to both types of edaphically limited environments, suggest that genetic improvement in these adaptations will not reduce, and may actually increase, the ability of a species to occupy both N and water limited habitats.

III. Suites of Adaptive Characters

A plant's suite of adaptive traits determines its integrated response to variations in resource supply, and hence its capacity to function in contrasting environments. This section attempts to identify the relative suites of adaptive traits of Sb and Sw by integrating the costs, benefits, and trade-offs of each N- and water-relations trait and response observed in the previous two sections. The suites of adaptive traits in Sb and Sw are examined in the context of their relative performance and sensitivity to stress in the experimental conditions, as well as in terms of their propensities to occupy separate muskeg and upland sites near the southern edge of the boreal forest.

A. In Relation to Experimental Conditions

Absolute growth under common conditions and growth sensitivity to stress gauge the relative quality of a species' suite of adaptive characters. Examining growth performance in the context of the experimental conditions, then, is useful for understanding how independent plant characters interact to determine the whole-plant response. The relationships between growth and the species' suites of adaptive characters are considered under the N-stressed, water-stressed, and simultaneous N- and water-stressed conditions imposed in this study.
1. Nitrogen-Stress:

The two species exhibited statistically identical growth under both high and low N conditions (+H2O+N, +H2O-N) (Fig.2). These results are surprising because they suggest that, despite being from habitats of contrasting nutrient availability, neither species has a more refined suite of adaptive traits for coping with the N-stressed conditions imposed in this experiment. In partial agreement with these findings, Fitchner and Schulze (1992) found that non-nitrophilic species did not grow more than nitrophilic species when subjected to limitations in N-supply. As a result of their findings, these authors concluded that greater absolute growth rate was less important for adapting to infertile habitats than N-stress-sensitivity (Fitchner and Schulze, 1992).

Shipley and Keddy (1988) define stress-sensitivity as the relative decrease in growth in response to a given decline in resource supply. In this study, Sb exhibited less decrease in growth than Sw (Table 1, Fig.2) and is therefore, using Shipley and Keddy's (1988) definition, less sensitive to N-stress than Sw.

The major differences between Sb and Sw in traits that may influence growth under high and low N supply appear to have cancelled each other out. Black spruce had a greater SAR but Sw had a greater R/S. Since each trait acts to increase internal [N] and the species had similar tissue %N (see Results section II.A), it appears their differences may have off-set each other. Black spruce exhibited a lower A than Sw, but Sw had a higher R/S. Since A and leaf area each contribute to whole plant carbon gain, it appears that, again, the species differences may have cancelled out. If so, this morphological-physiological balancing coupled with their similarity in RGRmax and NUE may explain why Sb and Sw did not differ in absolute growth.

Because of the species similarities discussed above, perhaps differences in plastic responses are responsible for black spruce's lower N-stress sensitivity. The greater plasticity for NUE and near perfect stability of A exhibited by Sb should act to decrease changes in growth across N levels. For example, efficient allocation and utilization of N when supplies are low should increase growth under stressed conditions, but a proportionally lower NUE (although it may reflect luxury consumption) would not improve growth under high and constant supplies of N. Not decreasing A with N-stress, for obvious reasons, would further contribute to low stress-sensitivity.

2. Water-Stress:

Unexpectedly, greater D-T, WUE, and R/LA (and R/S) exhibited by Sw did not amount to greater growth under droughted conditions (Fig.2), nor did these traits decrease growth compared to Sb under the high water conditions. Moreover, the lack of a significant water by species interaction (Table 1) demonstrates that Sb and Sw were both equally sensitive to the effects of drought. These results are surprising, but may be explainable.
Since the higher D-T and WUE in Sw was not associated with costs in terms of performance in non-droughted conditions, it is unlikely that species differences in these traits had any influence on performance under the well watered treatment. Moreover, although Sw exhibited greater A than Sb, this difference may have been countered by higher R/LA, which would further contribute to their similar growth responses under the high water conditions.

Growth in both species was definitely limited in the droughted treatment. It is strange that Sw did not out perform Sb since it was, in fact, more water-use efficient. It could be argued that perhaps costs of high WUE, such as slowed A, rendered high WUE less beneficial in the context of the particular characteristics of this experiment. However, this conclusion is unlikely since Sw achieved high WUE with no apparent costs (section I.B.2). The species difference in D-T, on the other hand, probably had no impact on growth in this study. The highest degree of internal stress experienced by the seedlings (in Experiment II) was a $\Psi_{\text{xylem}}$ of approximately -1.0 MPa, demonstrating that species differences in D-T would not have come into play. Finally, even though Sw had a higher R/LA than Sb at low water, it appears that it's greater A was not proportionally high enough to overcome the costs of reduced foliar allocation.

Overall, it appears that the degree of water stress imposed in this experiment was not strong enough for the potential assets and liabilities of high or low WUE, D-T, and R/LA to cause significant growth differences between species. It seems likely that with more severe drought the species differences in these water-relations traits would confer differential growth.

3. Simultaneous Nitrogen and Water Stress:

As was the case in the other treatments, Sb and Sw obtained statistically equal sizes in the double-stressed treatment (-H2O-N) (Fig.2). On the other hand, the significant N by water interaction (Table I and Fig.2) demonstrates that the addition of a second stress affected growth proportionally less than each did independently. It appears the seedlings where less sensitive to each stress while already stressed for the other.

As was pointed out above (sections I.C), the species seemed to converge on similar mean trait values under the double-stressed treatment more than in the others. In fact, they did not show statistically different values for any of the water or N traits measured (although WUE and R/LA were almost significant). This may account for the similarity in growth and sensitivity of the species in the low N and water treatment, but can not account for the significant N by water interaction effect.

Perhaps the effects of one stress were masked by the effects of the other. For example, N-stress may have set an upper limit to growth. As a result, the application of water-stress would have proportionally less effect on growth simply because growth was already limited. This explanation seems even more probable for the effects of N-stress under low compared to high
water conditions. Neither species exhibited a significant reduction in growth in the double-stressed treatment compared to the single water stress treatment. Perhaps water stress was so intense that the effects of N-stress were, in proportion, negligible. Clearly, more research is required to fully explain this intriguing phenomenon.

B. In Relation to Field Conditions

In this section relative suites of adaptive traits of the species are evaluated in terms of the abiotic and biotic factors that they may encounter in the field. Concern is often expressed over the relevance of extrapolating laboratory results to the field. Therefore, several caveats should be made concerning this study. First, the populations sampled in this study were located in the same general locality, and the results may not be representative of the species at large, nor necessarily elsewhere near the southern edge of the boreal forest. Second, experiments are by definition abstractions of reality, and can not account for every factor that may affect performance in the field. Other such factors might include soil temperature, flooding tolerance, availability of other nutrients besides N, susceptibility to herbivory, mycorrhizal associations, and germination physiology. Nevertheless, this study seemed to do a good job of isolating intrinsic differences between Sb and Sw in traits that will surely impact their relative performance in relation to two major limiting factors they encounter in the field.

1. Survival in Muskegs:

The species' similarities in RGRmax, NUE, and the off-setting balance between their differences in SAR, A, and R/S, raise questions as to what characteristics enable Sb but not Sw to grow in muskegs. One difference between the species that could be of major importance was the lower degree of N-stress sensitivity exhibited by Sb. Several authors advocate that low stress sensitivity is a chief factor promoting survival in low nutrient habitats (Chapin, 1980; Shipley and Keddy, 1988; Fichtner and Schulze, 1992). A plant that is less stress sensitive operates closer to its optimal level of physiological functioning while subjected to stress, making it less susceptible to further, or other, stresses (Chapin, 1980). Other advantages that Sb may have are its greater plasticity in NUE and greater SAR. A large percentage of the total quantity of nutrients are made available annually during flush periods in muskegs, which are followed by inter-flush periods of nutrient deprivation (Saebo, 1969). Highly plastic NUE may allow a plant to utilize N efficiently during inter-flush periods, while exhibiting proportionally high levels of luxury consumption during flushes. High SAR (under high N conditions) may be a mechanism to capitalize on flushes and possibly accumulate N stores to be used during subsequent N-stressed periods. Finally, the
ability of Sb to increase NUE with less of a decrease in WUE may improve its chances of survival in muskegs.

Although the species differences in the physiological-morphological balance may be offsetting at the whole-plant level (see previous section, III.A.1), the differences themselves could be of importance to their habitat separation. In contrast to conventional wisdom, a lower R/S, as exhibited by Sb, may be beneficial in muskeg habitats. High permafrost tables in muskegs could impose physical constraints on plant root size (Vincent, 1965), especially for large plants like trees. Hence, tree species that require proportionally lower R/S ratios could be favored. In addition, a low R/S may increase nutrient flux to the roots via mass flow as a result of higher rates of whole-plant water loss (Nye and Tinker, 1977; Fitter and Hay, 1987). Chapin (1988) argues that this is not an important adaptation for species growing in low nutrient habitats. His argument is based on the observation that low nutrient adapted species generally have large R/S ratios, lower transpiration rates, and the water "expense" may be too great (due to large quantities of water needed to significantly impact nutrient uptake via mass flow) (Chapin, 1988). However, it has been demonstrated previously that plants may exploit one resource to acquire another when growing in environments with extreme variation in resource supplies (Bloom et al., 1985; Ehleringer et al., 1985; Gauslaa, 1990). Thus, two of the three qualifications mentioned by Chapin (1988) are absent in Sb and the environment in which it lives.

The combination of lower R/S and higher SAR, and the environmental characteristics of muskegs, suggest that Sb may have few costs to its particular suite of adaptive characters. The major cost of low R/S is reduced nutrient absorption. However, Sb may be able to overcome this trade-off, at least during nutrient flushes (i.e., the most critical time periods), with its greater SAR. Moreover, because carbon is frequently not limiting to growth in open, infertile habitats (Bryant et al., 1983; Bloom et al., 1985), the trade-off between SAR and A (above, Tilman, 1988) may have little relevance in the nutrient limited muskegs.

The fact that Sw exhibited few of the potential trade-offs associated with drought-resistance traits (see above and below), suggests that adaptation to the low water conditions of upland sites would not prohibit it from growing in the high water muskegs. Therefore, one mechanism accounting for the absence of Sw on the muskegs might be related to an inability to cope with low nutrient availability. Its higher sensitivity to N-stress, lower plasticity in NUE, higher R/S, and lower SAR may be contributing factors to its absence from muskegs.

2. Survival on Upland Sites:

The combination of greater D-T, WUE, and R/LA exhibited by Sw overwhelmingly suggests that it is well suited (relative to Sb) to survive in the water-limited upland habitats. Each
of these traits independently, but more so together, would greatly increase and prolong growth during drought. Also, a combination of high D-T, large root allocation, and a lack of costs associated with high WUE means that Sw could capture and exploit soil water reserves while simultaneously maintaining high rates of growth. Not only should Sw be able to survive drought on upland sites, but it should also be a strong competitor for water there.

In addition, Sw did not exhibit any of the environmental trade-offs normally associated with high D-T or WUE, and therefore would not exhibit slowed growth rate when water levels are high. Because plant size (within a growth form) is often correlated with survival (McGraw and Wulff, 1983), rapid biomass accumulation during "good" times may improve a plant's chances of surviving subsequent drought. When water does become available on upland sites it is likely to be rapidly lost to run-off, seepage, and competing plants. The lack of trade-offs coupled with proportionally high root allocation could allow Sw to grow rapidly and fully utilize soil water reserves before they are removed. Finally, the ability of Sw to increase WUE with relatively little reduction in NUE as compared to Sb could also increase its chances of survival in competitive upland sites. Competition can reduce available N, particularly in the late summer (Bloom et al., 1985).

Black spruce, on the other hand, due to lower D-T, WUE, and R/LA coupled with relatively high associated costs, including the plastic NUE vs. WUE trade-off, would be at a distinct disadvantage compared to Sw on the competitive, dry upland sites. Because a large root system is the principle mechanism by which plants can increase water uptake, greater root allocation may be a requirement in upland sites. A high SAR may be less necessary due to higher N availability. Thus, it appears that the lack of occurrence of Sb on upland sites may be a function of lower drought-resistance, as well as costs associated with adaptations for survival in muskeg conditions.

Nevertheless, these factors can not fully account for the absence of Sb on upland sites. As was pointed out in the introduction, planted Sb grows very well on such sites (Vincent, 1965; Black and Bliss, 1980). A biotic factor, such as competition, may be responsible. Competing plants may reduce the soil water potential on upland sites by exploiting soil water reserves (Cohen, 1970; Bunce et al., 1977; Goldberg, 1990), perhaps to a level severe enough to prevent Sb from becoming established.

C. Conclusions

The species similarities in growth under the N-stress and double-stress experimental conditions were not surprising since they exhibited similar suites of adaptive traits at the whole-
plant level. The absence of differences in growth under the drought conditions, however, was surprising. These results raise questions concerning the benefits of drought resistance mechanisms in mildly droughted environments. Nevertheless, a decisive factor maintaining species distributions are periodic extreme conditions (Nobel, 1984), and most, if not all, water-limited environments will exhibit extreme drought periodically. This suggests that the growth responses to variations in water supply may not necessarily be representative of how they would behave in the field. It was also observed that seedling stress-sensitivity was lower while already stressed for either stress. This may have resulted from one stress masking the effects of the other, but nevertheless raises intriguing questions and possibilities for future studies.

As to why Sb and Sw do not form mixed stands near the southern edge of the boreal forest, this study has indicated that variation in N- and water-relations traits may play a role. The particular suit of adaptive traits exhibited by Sw suggests it would be more successful in the competitive water-limited upland environments than Sb. The suite of adaptive traits exhibited by Sb seems to be slanted towards better survivability in muskegs. The lack of these characteristics may be what prevents Sw from occupying muskegs. However, the evidence is a bit weaker and not as straight forward as that for the situation on upland sites. Since it does not grow well in muskegs (Van Cleve et al., 1983b), the fact that Sb did not exhibit greater adaptativeness for such conditions may not be surprising. Perhaps Sb is not adapted to muskeg habitats per se, but is restricted to them because of a competitive disadvantage on dry upland sites. The water-relations data support this hypothesis. The existence of Sb near the southern edge of the boreal forest, then, may be attributed to its ability to merely survive in the harsh muskeg habitats.

IV. Population Level Variation

Significant population level variation in mean trait expression and in plasticity for several of the N- and water-relations traits was detected (Table 2). Because the sampled populations were all from the same region (Fort Nelson, BC), this variation indicates intraspecific micro-geographic divergence. These results are of interest because they demonstrate that micro-evolutionary forces can influence the expression of these particular N- and water-relations traits in Sb and Sw. However, since the populations were selected randomly it is impossible to determine which mechanisms, selection or random genetic drift, are causal.

Overall, there were no emergent trends or anomalous populations. For example, mean separation (not presented) did not reveal any one population being consistently different from the rest; and interaction plots of significant interactions (not presented) generally indicated that one population or another out of the five did not respond to N-stress or drought.
Intraspecific variation for $\delta^{13}C$ has been observed previously in annuals (Gerber and Dawson, 1990), agricultural varieties (e.g. Ehleringer et al., 1990), and amongst coastal and interior varieties of *Pseudotsuga menziessii* (Mirb.) Franco (Zhang et al., 1993). However, this study may be the first to report of $\delta^{13}C$ variation amongst conifer populations within one limited geographic region. The population level interaction with N for $\delta^{13}C$ (Sb) may also be the first reported, although Toft et al. (1989) observed one at the species level. In general, there appears to be a conspicuous lack of intraspecific interactions for $\delta^{13}C$ and water supply in the literature (Ehleringer et al., 1990). For this reason, Ehleringer et al. (1990) suggested that $\delta^{13}C$ may be an ideal characteristic to select genotypes for agricultural purposes. The results of this study are in agreement with this general trend (i.e., no population by water interaction), suggesting that $\delta^{13}C$ may be useful for tree breeding in silviculture. But, due to potential effects of N, the results do indicate that site variation in N availability should be of concern.

It is intriguing that Sw exhibited significant population by water interactions for A and E, whereas populations of Sb exhibited marginally different A responses to variation in N supply. Likewise, Sb exhibited population level variation and a population by N interaction for $\delta^{13}C$, while Sw exhibited a population by water interaction for NUE. These patterns may reflect habitat preferences.

Future research on intraspecific variation in these N- and water-relations traits in Sb and Sw may be enlightening. Detailed analysis of specific characteristics of the sites of each population may reveal correlations between their relative variation and the N and water characteristics of the sites in which they occur. Such findings could increase understanding of the micro-evolutionary forces that act upon these traits.
SUMMARY and CONCLUSIONS

This study examined how two closely related species from contrasting N and water environments respond to variations in N and water supply. It was proposed that intrinsic variation in N- and water-relations traits between Sb and Sw, as well as the trade-offs associated with these traits, may contribute to the maintenance of their mutually exclusive habitat distributions. The hypotheses predicted several potential outcomes to the experiments conducted in this study. The results showed that some of the hypotheses were supported while others were not. Based on these findings, several general conclusions can be drawn.

This study demonstrated that trade-offs, either environmental or trait, should not universally be considered costs to increased adaptation to low resource environments. For example, the predicted environmental and trait trade-offs associated with improved water-relations traits were completely absent. On the other hand, SAR (or RAC) and A, and several N-relations traits were, in fact, observed to trade-off as expected. Moreover, while NUE and WUE did not trade off intrinsically, they did plasticly. The species differences in the NUE-WUE plastic trade-off correlated with their habitat preferences, suggesting adaptive divergence. These mixed results, although interesting and important, may thwart attempts to formulate generalized ecological theories applicable to all plant species in all situations, as have been recently advocated (e.g., Grime, 1977; 1979; Tilman 1988; 1990). Fine scaled experimentation may be required to fully understand the diverse ecological patterns that occur in the real world. It is imperative that such complexity is incorporated into the manner by which we perceive nature.

Another interesting implication made apparent by this study was that physiological and morphological differences may cancel out at the whole-plant level, but in some environments could, in and of themselves, have ecological significance. For example, in terms of growth in the experimental conditions the species differences in SAR, A, and E appear to have been off-set by differences in allocation patterns (R/S and R/LA). However, in terms of performance in the specific habitats in which the species occupy in the field, the differences themselves may be of greater importance than their combined effects on whole-plant processes. This implies that for future studies a reductionist approach, as taken here, may be necessary to fully understand how plants adapt to their environments.

Finally, the results indicated that Sb and Sw possess differences in N- and water-relations in a manner that is correlated with the habitats they occupy near the southern border of the boreal forest. Therefore, this study revealed mechanisms that may be partially responsible for their micro-geographic distribution patterns in the area.


Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115-555.


APPENDIX A - Percent Soil Water Content versus Soil Water Potential

Soil Water Potential, $\Psi_{soil}$ (MPa)

*This curve was established by measuring $\Psi_{soil}$ for the soil with the Westcor C-52 Sample Chamber Psychrometer/Hygrometer (Wescor, Inc., Logan, UT) as it dried from field capacity to equilibrium with the atmosphere.*
APPENDIX B - Example Analysis of Variance Model

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