Genetic variation for resource use efficiencies in lodgepole pine

by

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ABSTRACT

This study investigates genetic variation for resource-use efficiencies in lodgepole pine (*Pinus contorta* Dougl. ssp. *contorta* and ssp. *latifolia*). Because of the species' frequent occurrence on marginal sites, these resource-use efficiencies are expected to play an important role in its adaptation and evolution. A deeper understanding of the patterns of adaptation is needed to enable a more fine-tuned management of the existing genetic variation in the breeding program.

Since natural selection acts on phenotypes, both genotype and environment are considered jointly. Since the effects of genotypes, traits and environmental factors may not be separable, all of these elements are observed jointly in a controlled experiment. Under the null hypothesis, traits evolve independently to single environmental variables. Under the alternative hypothesis, there is an integrated physiological system that differs among genotypes, that reacts as a whole to multiple environmental variables and evolves as a whole in adaptation to those source variables.

Water-use efficiency and nitrogen-use efficiency were measured on one-year old seedlings of lodgepole pine in the controlled environment of the greenhouse. These traits were observed over a range of environments, created by controlled levels of available water and nitrogen. A preliminary experiment was set up in 1996 with provenances of lodgepole pine, mainly to proofrun the nursery techniques, to confirm the existence of genetic variation for resource-use efficiencies, to determine sources of variation in the experiment, and to investigate separability of the effects in general.

A second experiment was set up in 1997, using selected families incorporating a more continuous range of variation for source variables than is possible with a provenance structure. It had the added advantage that 10-year field site data are available for comparison.

Genetic variation exists for mean trait expression as well as for plasticity for both water-use efficiency and nitrogen-use efficiency. Genetic variability within populations tends to be high despite pronounced differentiation of populations. Populations are somewhat adapted to their local environments, but not very precisely: the breeder is not limited to specific seed sources in order to ensure adaptation to marginal sites. Genetic correlations do not indicate conflicts between selection for growth and adaptation.

Genotype-environment rank order change exists for several traits and no simple, consistent patterns for it emerge.

Patterns of trait integration vary across environments. Especially nitrogen deficiency can drastically change trait relationships. Thus, multiple environmental factors and multiple traits act jointly to create a large number of environmental niches, resulting in more opportunities for the maintenance of genetic variation. The result of this complex process is that patterns of adaptation for single traits, if present, are not narrowly defined.

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Δ	carbon isotope discrimination
α	level of confidence
$\delta^{13}C$	carbon isotope composition
A	Kamloops provenance
ADI	annual dryness index
ANOVA	analysis of variance
B	Salmon Arm provenance
BC	British Columbia
BGC	biogeoclimatic
bm	biomass (total plant dry weight)
BV	Bulkley Valley
BZ	breeding zone
C	Revelstoke provenance
c_i	intercellular partial pressure of CO_2
COI	cross-over interactions
d	diameter
df	degrees of freedom
ELEV	elevation
F	family effect
GxE	genotype-by-environment interaction
H	high (level of treatment)
h	height
h^2	heritability
hd	ratio of height over diameter or slenderness ratio
L	low (level of treatment)
M	medium (level of treatment)
MAP	mean annual precipitation
MAT	mean annual temperature
MOIST	ratio of summer precipication over summer temperature
MSP	mean summer precipitation (May to September inclusive)
MTWM	mean temperature of the warmest month
N	nitrogen, nitrogen level
nue, NUE	nitrogen-use efficiency

P	provenance effect
Q	Qualicum
r	Pearson correlation coefficient
rA	genetic correlation
REML	restricted maximum likelihood method
rP	phenotypic correlation
rt	root weight
RUE	resource-use efficiency
S	Squamish
SA	Shuswap Adams breeding zone
SDI	summer dryness index
sht	shoot weight
srr	shoot-root ratio
SUMP	mean summer precipitation (based on BGCsubzone data)
SUMT	mean summer temperature (based on BGCsubzone data)
SZMAP	MAP (based on BGCsubzone data)
SZMAT	MAT (based on BGCsubzone data)
TDIFF	difference between mean temperatures of the warmest and the coldest month
TO	Thompson Okanagan breeding zone
TOA	Thompson Okanagan Arid
TOD	Thompson Okanagan Dry
vol	volume
W	water, water level
WB	Willow Bowron
WK	West Kootenays
wue, WUE	water-use efficiency

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Michael Carlson suggested sites in the interior for harvesting the seeds of the 1996 experiment, provided the climate data for these sites and a helicopter and his personal time for the seed harvest. He made available seedlots from the Ministry of Forests' freezer for the 1997 experiment and provided the growth data of the existing progeny sites in the field. He also provided useful advice on stratification and growing the seeds in the greenhouse.

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INTRODUCTION

1. AIM OF THE STUDY

1.1 Justification and background

The environment has an important influence on the expression of traits. In recognition of this influence, provenance trials are established at an early stage in most breeding programs to test performance over different sites. These trials have often indicated genotype-by-environment interaction, sometimes resulting in rank order change. Such interactions are not just environmental 'noise' hiding the 'true gene effects'. Rather, they imply that gene effects vary with the environment or that different genes are active in different environments. It is often difficult to detect the biological causes. This is because so many environmental factors are integrated by each genotype in a manner specific for that genotype. The presence of genotype-by-environment interaction (GxE) complicates the selection process and reduces genetic gain. It is important to know to what extent GxE interaction reflects adaptation which may be managed and exploited, and to what extent it reflects random genetic factors, which can be ignored in a breeding program. A way to gain a deeper understanding of the causes of GxE is to relate it to explicit environmental variables of the planting site. This may be very complicated as more than one environmental factor may be involved. Interactions between factors may then make it impossible to detect the importance of single factors easily. Moreover, several correlated traits may be involved. The correlations between traits, which are caused by the integration of traits into a functioning phenotype, may make it impossible to detect the importance of single traits easily. In short, it may be impossible to find the causes of GxE if only simple explanations in two dimensions are considered.

1.2 Approach

This study addresses these problems by looking for patterns of adaptation to two important environmental variables: water levels and nitrogen levels. The efficiency with which trees use these resources is thought to play an important role in their fitness and adaptation. This is especially so for a species like lodgepole pine, which grows on a large range of sites, including marginal sites. Thus, measurements of water-use efficiency and nitrogen-use efficiency, as well as growth traits, in a controlled experiment over a range of water and nitrogen levels could provide an idea of the presence of interactions working between these factors and traits. Doing

so for a series of genotypes will result in a better understanding of the dynamic interactions of the environment and population genetics. These interactions will result in a pattern of adaptation that must be preserved in a breeding program.

1.3 Hypotheses

The basic hypotheses are as follows:

H_0: there are no patterns of adaptation to any of the environmental variables investigated

H_1: single traits evolve independently and respond to independent single environmental variables

H_2: single traits evolve independently and respond to multiple, interacting environmental variables

H_3: multiple traits evolve as an integrated physiological system in response to single environmental variables

H_4: multiple traits evolve as an integrated physiological system in response to multiple interacting environmental variables.

Thus, from H_0 to H_4 the level of complexity increases and more dimensions are needed to understand the pattern of adaptation, the characterisation of environmental niches, and the importance of genetic variation in fitness-related traits in preserving adaptation.

1.4 Objectives

The specific objectives of this study are:

- to clarify whether genetic variation exists for resource-use efficiencies (RUE) and other component traits of growth, either in mean genotype performance or in plasticity or both.
- to explain the observed variation as a function of source environmental variation and evolution, i.e. to clarify possible patterns of adaptation.
- to investigate if genotypes actually change rank from one environment to another.
- to investigate the relationships among RUE and between RUE traits and growth.
- to investigate if and how genetic correlations change over environments.
- to compare the study with field data and demonstrate the relevance of the chosen fitness-related traits and environmental factors in explaining the genotype-by-environment interactions in the field, at least for the chosen sites.

2. LITERATURE REVIEW

In this section, I review the literature pertaining to maintenance of genetic variation, multiple niche selection and genotype-environment interaction. The choice of traits and environmental factors for the experiment is justified and the appropriate measurements for these traits are discussed. Documentation of correlations between the traits points out that they do, to some extent, evolve jointly. Lodgepole pine is shown to be a suitable species for this study, since it often grows on and has adapted to marginal sites.

2.1 Maintenance of genetic variation in natural populations

Genetic variation is the result of the dynamic processes of mutation, drift, migration and selection. The effects of some of these processes are random, the effects of others are systematic. The selectionist theory emphasises the importance of natural selection as a force for maintaining polymorphisms (Lewontin 1974). This would imply that the resulting genetic variation has some functional significance: it is needed to maintain population fitness at a high level. The neutral theory (Kimura 1983) emphasises the importance of random processes and unique historical events in creating polymorphisms, which are then maintained because they are selectively neutral. In this latter scenario, genetic variation is not indicative of the existence of adaptive variation, though it is still necessary for long-term evolution.

When designing breeding and conservation programs it is important to know whether or not existing genetic variation reflects adaptation. However, this is not an easy question to answer. Strictly speaking, in order to prove that selection takes place on a certain trait, selection coefficients must be measured (Endler 1986). For most real-life examples the statistical power to accurately measure and compare selection coefficients is lacking. A large number of traits are quantitative, i.e., determined by many loci, each with a small effect. Selection coefficients on each locus will then be even smaller, and consequently difficult to detect, let alone to quantify. To properly model natural selection, the variation of selection coefficients and selection regimes over the life span of an organism would have to be known.

Yet modelling approaches have revealed that, at least theoretically, there are several forms of 'balancing selection' or selection that can maintain stable polymorphisms within and among populations (reviewed by Hedrick 1983 and Ennos 1983 among others).

The first and simplest single-locus model, assuming constant fitness, showed that heterozygous advantage maintains polymorphisms in a population. Experimental evidence for heterozygous

advantage is rare (Ennos 1983). The existence of several ecological niches, with different alleles favoured in each niche, can lead to the maintenance of polymorphisms without heterozygote overdominance. This case is worked out in models assuming variable fitness, such as that of Levene (1953), who showed that environmental dependency of selective values combined with temporal or spatial variations in the environment can, under certain conditions, lead to maintenance of stable polymorphisms. These conditions are more restrictive for temporal than for spatial variation. Random mating within populations homogenises the gene pool for each generation. Between populations, migration acts as a genetic glue, restricting genetic divergence. Another series of models has considered the influence of several migration patterns on the selection-migration balance (reviewed by Felsenstein 1976). A further development in this direction was taken into account in the models of Gregorius and Namkoong (1984; Namkoong and Gregorius 1985), who considered the differences in migratory behaviour and in selection between the two sexes.

With each of these extensions, new opportunities for the maintenance of polymorphisms appeared. Variable selection, intergenotypic competition, types and patterns of migration, sex-dependent selection and migration and frequency-dependent selection can all play a role (Ennos 1983). Thus, many single-locus models indicate opportunities for the active maintenance of polymorphisms. Extensions to multiple locus models are rare, but are likely to reveal an increased number of possibilities for the maintenance of genetic variation.

There are many studies in the literature relating genetic variation to environmental variation. Establishing a cause-effect relationship requires excluding all alternative hypotheses, and is often impossible. Nevertheless, it is believed that environmental heterogeneity plays an important role in the maintenance of genetic variation (Hedrick 1986).

When the expression of an individual genotype can be modified by environmental influences in a consistent or repeatable way, this is termed 'plasticity' (Bradshaw 1965). The specific shape that the relationship between phenotypes and environments takes is called a norm of reaction. Plasticity is expressed for specific traits and in response to particular environmental factors. It is not necessarily adaptive, but it is under genetic control and can be altered by selection. Though the importance of phenotypic plasticity in plant evolution was recognised early (Bradshaw 1965), it is only recently that reaction norms have been incorporated into evolutionary models. Schlichting (1986), Via (1987), Stearns (1989) and Scheiner (1993b) independently made this step. Genetic variation for plastic traits occurs when genotypes have

different reaction norm functions. In other words, a genotype is characterised not by its mean but by its norm of reaction.

Spatial variation for several environmental factors simultaneously can result in multiple niches with strong selection and the maintenance of large amounts of genetic variation among niches. The conditions for multiple niche selection are commonly found in plant populations (Ennos 1983).

When several traits are correlated, more opportunities arise for the maintenance of genetic variation in any one of them. Firstly, the contribution of each single trait to fitness may vary over environments. Secondly, each single trait can be influenced by different environmental variables, resulting in more environmental variation. Thirdly, trait correlations may change from environment to environment and the relative contribution of traits to fitness may vary.

Schlichting (1986) called into question the utility of examining the plasticity of single traits. Traits are correlated, both genetically as a result of pleiotropy and linkage disequilibrium, and phenotypically, because of physiological limitations of the plant on the way it can integrate its processes in producing a phenotype. Selection acts not only on response functions of single traits, but also on the interrelationships among traits. Therefore, the concept of phenotypic integration of multiple traits becomes important in understanding genotype-by-environment interactions. The adaptive significance of genetic variation may thus only become clear when high-level interactions are investigated. Not only may this variation need to be maintained in a breeding program, but such fine-tuned adaptation will likely result in genotype-by-environment interactions, which complicate selection procedures.

2.2 Analysis of genotype-by-environment interaction

Most of the methods for analysis of genotype-by-environment interaction (GxE) come from the field of plant breeding. All of these methods do not permit a causal analysis and therefore fall short of providing a deeper insight into the GxE interaction. To further explain this, two characteristics of these methods are elaborated: (1) the fact that the environment is described by the average performance of certain genotypes, and (2) the fact that a specific mode of interaction (mostly additive) is presumed, which consequently determines the result of the analysis.

(1) Most methods describe the environment by the average performance of certain genotypes. The environment may be composed of many different effects that may be inseparable

(Namkoong et al. 1988). To simplify this complex pattern of causality, most analyses have characterised the environment by the average performance of all genotypes tested or the average performance of a separate set of genotypes. The idea behind this is that genotypes would integrate all factors in a meaningful way via their physiology, reducing a multivariate problem to a univariate one. However, this procedure has been criticised (Knight 1970; Gregorius and Namkoong 1986 and 1987; Namkoong and Ades 1995; Nissilä 1996). In order to provide real insight into GxE, independent measures of environmental variables should be used to describe response functions. If the environment consists of multiple factors that interact in complex ways, reductionist techniques will obscure the picture, rather than clarify it.

Most of these methods of analysis were developed in an attempt to manage GxE in breeding programs, not to understand it. Initially, GxE was considered 'noise', and its management consisted chiefly of avoiding it. In agriculture, environmental variation can often be reduced by the choice of sites and by intensive management, which makes breeding for generalist genotypes easier. However, techniques and computer packages have vastly improved since then, and such a simplified approach is no longer necessary.

(2) Most methods presume a specific mode (operator) of the effects. The most commonly used model is a linear additive model of effects. The existence of GxE in this case implies a deviation from linearity and additivity. Such an interaction is not necessarily biologically relevant. For example, rates of response may differ while patterns of response and ranking of genotypes remain the same.

Gregorius and Namkoong (1986; 1987) proposed a wider concept of biological interaction, consistent with the mathematical theory of separable functions. In order to attribute phenotypic variation to genotypic versus environmental causes, genotypes and environments must have consistent effects. Any operator that produces consistent effects is feasible. If genotypic effects cannot consistently be separated from environmental effects or vice versa, interaction or 'inseparability' is said to exist. In that case the next step is to define a range or group of genotypes or environments where separability can be achieved and effects can be defined. In the mathematical sense, consistency of genotypic effects means that two genotypes either produce the same response in all environments or a different response in all environments. Consistency of environmental effects implies that two environments have either the same effect on all genotypes or a different effect on all genotypes. For quantitative traits, however, causal variables are continuous and only a finite sample of genotypes and environments is possible. In

this case it is necessary to consider the ranking of response functions. If the ranking changes, it is inferred that some point of intersection exists and that the effects are inseparable.

Gregorius and Namkoong (1986) and Gallo et al. (1995) further clarified how suitable operators can be found within groups of genotypes that show no crossing-over after scale effects have been removed. Knowledge of the operator and of one type of effect is sufficient to deduce the second type of effect.

Namkoong and Ades (1995) further stated that, regardless of the operator, it is the presence or absence of cross-over interactions (COI) between response curves for different genotypes and environments which determines whether G and E effects are inseparable or separable. Thus, the crucial issue becomes testing for COI.

The term 'GxE' is used here in its traditional sense and refers to the ANOVA concept of interaction (non-parallelism of response). The term 'COI' is used to refer to interactions resulting in rank order change of genotypes due to crossover of the norms of reaction, regardless of how the environment is quantified. Specifically, however, the COI that we are testing for are those of norms of reaction plotted against explicit environmental variables, so that significant COI indicate a lack of separability.

Crossover interaction (COI) can easily be established visually: whenever response curves intersect, there is a crossover. However, all means are estimated with a certain error, and so the crossover may be a result of estimation errors rather than real. Several tests to detect COI have been developed and were reviewed and compared by Truberg (1996). He suggested that first a test for classical GxE be carried out, e.g. an F-test in an analysis of variance. If that is positive, the test of Azzalini and Cox (1984) should be used to test for rank order interaction. Two other, less sensitive, non-parametric tests are available if the data are not normally distributed.

2.3 Choice of traits

Resource-use efficiencies were studied because the efficiency with which trees use resources that are available only on a limited basis will significantly influence their survival and thus their fitness. Both water and nitrogen shortages impose important constraints on seedling survival and growth in the forest.

Drought stress is a common cause of seedling mortality in both natural and regenerated forest stands (Cleland and Johnson 1986). Genetic variation in drought resistance has been reported for several species (e.g. Pharis and Ferrell 1966, Dykstra 1974, van Buijtenen et al. 1976). Genetic variation in water-use efficiency has likewise been reported (e.g. Holowachuk

1993; Patterson 1994; Zhang and Marshall 1994, 1995; Zhang and Cregg 1996; Zhang et al. 1993, 1994, 1996, 1997; Sun et al. 1996; Aitken et al. 1995; Lauteri et al. 1997)

The most pervasive nutrient deficiencies in coniferous plantations in temperate regions are phosphorus (P) and nitrogen (N) (Nambiar 1984). For lodgepole pine stands in British Columbia, N is the most important limiting element, followed by sulphur (S) and sometimes boron (B) (Brockley 1990). Nitrogen stress can be acute in older stands at high latitudes where most nitrogen is immobilised in litter and soil (Miller 1984). Genetic differences for nutritional characteristics have been reported for several tree species (e.g. Namkoong et al. 1992, Nambiar 1984, Brown 1970, Sheppard and Cannell 1985, Pope 1979). Significant genetic variation for nitrogen-use efficiency has been found among Monterey pine (*Pinus radiata* D. Don) families (Cotterill and Nambiar 1981), and loblolly pine (*Pinus taeda* L.) families (Li et al. 1991).

Based on the above observations, two indices of resource-use efficiency were chosen: water-use efficiency (WUE) and nitrogen-use efficiency (NUE). These indices are expected to jointly provide a measure of resource-use efficiency.

2.4 How to measure water-use efficiency

Species can realise adaptation to drought through different 'strategies' (Ludlow 1989). Lodgepole pine seems to be very sensitive to water stress, closing its stomates quickly and avoiding desiccation altogether (Bassman 1984; Lopushinski 1973). This, together with a high photosynthetic rate, results in a high overall water-use efficiency for the species, as compared to Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) or white spruce (*Picea glauca* (Moench) Voss) (Bassman 1984; though Smit and van den Driessche (1992) found a higher WUE for Douglas-fir than for lodgepole pine). Root growth (reviewed by Critchfield 1980; Smit and van den Driessche 1992), stomatal density (Illingworth 1975), drought resistance (Dykstra 1974) and water-use efficiency (Holowachuk 1993), all of which are elements of performance under drought conditions, have been studied and significant genetic differences have been found. Dykstra (1974) found variation in drought resistance among six provenances covering the whole range of the species. Differences in WUE among populations in British Columbia were shown to exist by Holowachuk (1993).

Since within-species variation for drought resistance is the result of multiple adaptive mechanisms (Newton et al. 1991), interpreting variation in terms of a single response can be misleading. However, drought avoidance and drought tolerance in Douglas-fir were shown to be positively correlated (Larsen 1981) and some responses are more important than others over the

range of the most frequent drought levels. Early stomatal closure appears to be an important dehydration avoidance mechanism in woody species (Newton et al. 1991). Population differences for stomatal closure have been observed for *Pinus taeda* (van Buijtenen et al. 1976).

WUE refers to the amount of carbon fixed per unit of water utilised. In plant physiology, it is often measured as the ratio of the photosynthetic rate over the transpiration rate and called 'instantaneous WUE'. Direct field measurements of seasonal WUE are labour intensive and cumbersome, as calculating the amount of water taken up by the plants requires knowledge of the quantity of water added by irrigation as well as water lost through the soil. For C_3 plants, however, a relationship has been found between the carbon isotope composition ($\delta^{13}C$) of plant tissue and the ratio of intercellular over atmospheric CO_2 partial pressures during the time the carbon was assimilated (Farquhar et al. 1982) (see also appendix 1). The carbon-fixing enzyme of C_3 plants, ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), partially discriminates against the heavier ^{13}C isotope, but as the stomates close and less CO_2 becomes available, ^{13}C will be assimilated anyway. As such, the carbon isotope composition ($\delta^{13}C$) of the whole plant tissue integrates transpiration efficiency at the leaf level over the whole growing season and is highly correlated with WUE (Ehleringer and Osmond 1991). In fact, $\delta^{13}C$ reflects seasonal WUE better than gas exchange measurements (Condon and Richards 1993, Hall et al. 1993). Carbon isotope composition, together with root biomass and root/shoot ratio should give a good idea of the relative ability of families and provenances to grow under drought.

In this experiment, traits are measured at the juvenile stage, because this is where most selection takes place. Trait expression may change as trees mature. The expression of WUE at mature age may result in large growth differences, which are relevant to a breeding program. However, Holowachuk (1993), who investigated WUE using carbon isotope composition of several lodgepole pine provenances in British Columbia, found good correlations between nursery results and WUE measured on 15-year-old trees grown from the same seedlots.

2.5 How to measure nitrogen-use efficiency

Large differences in nutritional characteristics exist within forest tree species, but insight into the mechanisms underlying these differences is lacking (Nambiar 1984).

There are two basic mechanisms of nutrition: uptake and utilisation. Nambiar (1984) was of the opinion that there is greater potential for manipulating uptake of nutrients by genetic means than there is for manipulating nutrient utilisation. Uptake is determined to a large extent by root

growth and morphology (Wheeler and Critchfield 1984). This is especially so for nutrients with low mobility, but less so for nitrogen, which is fairly mobile. Given the practical set-up of this experiment, where each tree grows in a separate cell, the importance of root morphology is low. Root length was found to affect uptake efficiency significantly in loblolly pine (Li et al. 1991), but is much more difficult to assess for large numbers of plants. Root biomass will give an indication of uptake capacity. However, the focus is on utilisation.

NUE was measured by the C/N ratio of the total plant. Significant differences in nitrogen-use efficiency have been found between Sitka spruce (*Picea sitchensis* (Bong.) Carr) and lodgepole pine, as well as among clones of lodgepole pine (Sheppard and Cannell 1985). Li et al. (1991) found significant family variation and a reasonably high heritability (0.84 in low N and 0.69 in high N levels) in loblolly pine for "nitrogen-use efficiency", which they defined as stem biomass per unit of N applied in the soil medium ("NUE"). At low N, both uptake and utilisation efficiency contributed equally to the variation in "NUE". At high N, uptake contributed relatively less to variation in "NUE". GxE was also found for "NUE".

2.6 Trade-off between WUE and NUE

An inverse relationship between WUE and NUE has been found. This section further details the plastic and genetic components involved in this inverse relationship and their evolutionary implications.

Firstly, theory suggests that WUE and NUE should trade off plastically across environments. Approximately half of the protein in leaves is found in the chloroplasts, whose main function is photosynthesis. One quarter to one-eighth of leaf protein is present in Rubisco, the carbon-fixing enzyme of C_3 plants (Salisbury and Ross 1992). When nitrogen and thus the enzyme is in short supply, the amount of substrate would have to increase to maintain the reaction speed of photosynthesis. Increasing the substrate (CO_2) concentration would require opening the stomates more, which would result in more water loss, decreasing the intrinsic water-use efficiency. Hence, when a plant develops a high NUE in response to a nitrogen shortage in its environment, and is capable of producing the same amount of biomass with less nitrogen, that would result in a low WUE. When a plant develops a high WUE in response to drought in its environment, this results in a decreased NUE. Evidence for a plastic trade-off (across treatments) was found in the experiment of Patterson (1994) with spruce and the experiment with American elm (*Ulmus americana* L.) of Reich et al. (1989). This trade-off

implies that, within a given genotype, NUE and WUE can't simultaneously be maximised. Improving WUE implies decreasing NUE and vice versa.

Secondly, a genetic component of this trade-off has been found in ecological studies. The evidence, though, is ambiguous. Field et al. (1983) compared WUE and NUE for five Californian evergreen species whose usual habitats differ in drought level. Within a single field environment, the ranking of species according to their WUE was almost exactly the opposite of their ranking according to NUE. However, De Lucia and Schlesinger (1991) found a positive relationship between a species' WUE and its NUE, and Patterson (1994) found no significant correlation between WUE and NUE for white spruce and black spruce (*Picea mariana* (Mill.) B.S.P). Patterson (1994) also investigated the genetic trade-off within each of these two spruce species in several experimental treatments. The within-species correlations were not significant either. More detailed information about actual genetic correlations between WUE and NUE within species is not available. Extrapolating from relationships across species, however, it seems that the genetic correlation between WUE and NUE might be either positive, negative, or zero.

Not much is known about the genetic trade-off between WUE and NUE. However, if families with high WUE automatically have low NUE and vice versa, that would imply restrictions with regard to the adaptation of genotypes to their environments, as well as with regard to breeding, at least in the short term. Over many generations genetic correlations can and do change: mutations and a change of the genetic background will provide new optimal trait combinations. On an evolutionary scale, then, the genetic correlation need not necessarily be seen as a constraint.

A plastic trade-off and genetic variation in this plastic trade-off, however, also have implications with regard to adaptation and evolution. Depending on the levels of N and W in the environment, fitness may reach its maximum value at varying combinations of WUE and NUE. Strong selection combined with reproductive barriers and environmental variation can then lead to the maintenance of large amounts of genetic variation with regard to WUE and NUE between and within populations. Genotypes may differ in the exact way they trade off these traits across environments, resulting in a three-way interaction among genotypes, environments and traits. In fact, a plastic trade-off between traits is likely to result in genotype-by-environment (GxE) interaction at the multiple trait level (Namkoong 1985; McKeand et al. 1997). Since WUE and NUE are important in determining overall plant performance, GxE interactions at the multiple trait level may provide insight into adaptation patterns, existing genetic variation and its

evolutionary significance. This in turn will help guide decisions for breeding optimal groups of genotypes for specific environments.

2.7 Lodgepole pine

Lodgepole pine spans a wide environmental range, from the Pacific Coast to the Rocky Mountain range, from Alaska to Baja California. Four subspecies are recognised (Critchfield 1957):

ssp. *contorta* (Pacific coast), also called shore pine,

ssp. *latifolia* (Rocky Mountain and intermountain regions, Northern Cascades), also called Rocky Mountain lodgepole pine,

ssp. *murrayana* (Southern Cascades, Sierra Nevada, mountains of southern and Baja California), also called Sierra Nevada lodgepole pine, and

ssp. *bolanderi* (Mendocino White Plains), or Bolander pine.

Considering that the range of *Pinus contorta* spans 33° in latitude, 35° in longitude and 3900 m. in elevation, it is little wonder that the species exhibits considerable genetic variation (Illingworth 1975; Wheeler and Critchfield 1984). Its unusually wide ecological amplitude is not only climatic, but also edaphic. In its coastal range it occupies a variety of extreme habitats, like bogs, muskegs, sand dunes, and rocky sites. Outside the coastal region, it is an important seral species, but its successional status is more permanent on marginal sites (Critchfield 1980).

The adaptational patterns observed today in lodgepole pine reflect at least partly the evolutionary history of the species (Ying and Liang 1994). Lodgepole pine has played an important role as a pioneer species colonising disturbed lands after the ice age (Critchfield 1985). The species is capable of rapid expansion due to its precocity, serotiny, small seed size, rapid juvenile growth rate, limited edaphic requirements, easy germination and relative frost-hardiness (Wheeler and Critchfield 1984). During the last ice age, an ice sheet is believed to have covered most of British Columbia (B.C.) down into Washington, Idaho and Montana (Critchfield 1985). South of the glaciated area, the principal races (ssp. *contorta* and ssp. *latifolia*) of lodgepole pine are geographically isolated, and their differences in botanical traits suggest that they have been genetically isolated for many millennia (Wheeler and Critchfield 1984). Populations north of this line have achieved their present distribution in the past ten to fourteen thousand years. Morphological observations, isozyme analysis, and monoterpene analysis support the hypothesis of two or more large refugia south of the ice, one refugium in

the Yukon and several refugia on Vancouver island and the Queen Charlotte islands (reviewed by Critchfield 1985, Wheeler and Critchfield 1984).

Narrow local adaptation was found within the coastal range of the species (ssp. *contorta*) (Ying and Liang 1994), in the northern range of the species (Ying and Illingworth 1986) and in the U.S. Rocky Mountain region (Rehfeldt 1988). Narrow local adaptation implies that seed cannot be moved far from its source without risking maladaptation. Narrow adaptation in the coastal range may reflect an adaptive pattern, which existed before the last glaciation. In the interior of B.C. the species shows broad geographic and elevational adaptation (Ying et al. 1984, 1989), indicating that the transfer of seed is possible over a wider area there. In the interior, environmental fluctuation due to frequent disturbance (primarily fire) is common, which can render natural selection for adaptation to specific sites less effective (Ying and Liang 1994).

A study by Rehfeldt et al. (1999) compiled all of the most recent data available on provenance tests in B.C. and regressed growth and survival on climate data. Response functions of provenances to climate variables and elevation illustrate that natural populations of lodgepole pine occupy suboptimal environments. Populations of ssp. *latifolia* occur in climates that are colder than their optima. Populations of ssp. *contorta* occur in climates that are much warmer and wetter than their optima. Most populations are competitively excluded from their ecological optima, with the exception of populations from the centre of the species' distribution. Despite having a very broad fundamental niche where they are able to survive, populations are actually growing and successfully competing in a much smaller niche. The steep clines in lodgepole pine may be caused by density dependent selection rather than by the physical environment. That would imply that adaptation is not nearly as narrow as some earlier studies (e.g. Rehfeldt 1988) have implied.

Provenance tests in B.C. have shown a regional pattern of genetic differentiation. Within geographic regions, genetic variation is largely associated with elevational gradients (reviewed by Ying and Liang 1994). Xie and Ying (1995) explained about 80% of among population variation using elevation and geographical patterns. Elevational gradients are fairly steep in the south of B.C. but not in the north. Clines become steeper as trees age (Xie and Ying 1995). In the U.S. Rocky Mountains (ssp. *latifolia*), Rehfeldt (1988) also found steep elevational clines.

Interior lodgepole pine (ssp. *latifolia*) demonstrated less genetic variation in the central region of B.C. than in the northern and southern regions at both the population and family levels (Xie and Ying 1995). Broad adaptation is believed to exist in the interior of the province, where the species is commercially most important. However, maladaptation may take a long time to

manifest itself, especially if it is not extreme (Ying and Liang 1994). In most initial analyses of provenance trials, GxE interaction variance has been either insignificant or small and has been ascribed to the inclusion of unsuitable sites and genotypes. Still, more detailed investigations of older trials, 'difficult sites', or traits other than height have resulted in an increased awareness that GxE should not be ignored and that its biological causes are not well understood (Ying et al. 1989; Ying 1991).

Based on the seed planning zones (British Columbia Ministry of Forests 1986), seven breeding zones have been delineated for breeding of interior lodgepole pine: Kootenays, Thompson-Okanagan, Shuswap Adams, Bulkley Valley, Willow Bowron, Central Plateau and Finlay. They have recently been updated but the old zones are still used to refer to the location of previously collected seedlots and established field trials. Seed was collected in the 1970s from 1846 plus trees spread over the range of ssp. *latifolia* in B.C. Progeny trials were established in the 1980s. The seedlots were grown in two to four sites in each of the seven breeding zones. Each site contains selections from its own zone, neighbouring zones and sometimes a few widely removed selections, as well as a few regional and local controls. Five- and in many cases ten-year growth and survival data are now available for these progeny tests. They provide data for a more detailed assessment of genetic variation, adaptedness and genotype-environment interaction at a scale relevant to breeding programs.

2.8 Summary of the literature

Fitness related traits are, by definition, under strong selection. Yet genetic variation for these traits within populations is often large in conifers. The present selectionist models are not detailed enough to explain the observed amounts of genetic variation. If the existing variation has an adaptive function, this must be taken into account when designing breeding and conservation programs. Patterns of environmental variation and correlation structures between different traits may increase the number of niches and genotypes, thus resulting in more opportunities for the maintenance of genetic variation, but also in more genotype-by-environment interaction.

Independent measures of environmental variables should be used to describe response functions of genotypes in a realistic way. The concept of 'separability of genetic and environmental effects' can provide a deeper insight into the nature of genotype-environment interactions and into the consequences of GxE in breeding programs. To this aim, crossover interactions are tested for response functions. In general, GxE cannot be ignored for single traits

when evaluated over a large range, and for multiple traits we must assume the amount of GxE will increase. Within well-chosen subsets of genotypes and environments, consistency of both genotypic and environmental effects should exist. Plantation failure can be minimised at the same time as genetic gain is optimised.

The efficiency of use for resources that have restricted availability will significantly influence plant survival. Both water and nitrogen shortages impose important constraints on seedling survival and growth in the forest. Two indices of resource-use efficiency were chosen: water-use efficiency (WUE) and nitrogen-use efficiency (NUE). These indices are expected to jointly provide a measure of resource-use efficiency. Some studies have found a negative correlation between WUE and NUE. Such negative correlations among traits increase the probability of multiple trait - environment interactions.

Variation for drought resistance is the result of multiple adaptational mechanisms. However, rapid stomatal closure appears to be one important dehydration and cavitation avoidance mechanism in woody species. Early stomatal closure results in an increased WUE. An indirect measurement of WUE is available: $\delta^{13}C$ of the whole plant tissue integrates transpiration efficiency at the leaf level over the whole growing season and is highly correlated with WUE. Though it is an expensive technique, it allows us to assess large numbers of genotypes relatively easily.

Large differences exist for nutritional characteristics in forest tree species. There are two basic mechanisms of nutrition: uptake and utilisation. Root biomass gives some indication of uptake efficiency, but our focus is on NUE, calculated here as C/N ratio of the total plant.

Lodgepole pine spans a wide environmental range. Therefore, differential adaptation among environments should be expected. Though ssp. *latifolia* seems to demonstrate broad adaptation when the main factors, geographic location and elevation, are taken into account, provenance trials have revealed inconsistent performance of provenances across sites. No clear pattern for this interaction has emerged, though, and its biological causes are not well understood. Until they are, management of the genetic resource will be sub-optimal.

MATERIALS AND METHODS

1. NURSERY EXPERIMENTS

Two experiments were carried out. The first experiment was set up in 1996 to gather practical experience with the techniques, using a choice of genotypes that was very likely to detect any genetic variation for water-use efficiency if it was present in the species. The main aim of the second experiment, carried out in 1997, was to relate resource-use efficiencies to source climatic variation. Other than that, the second experiment served to:

- improve the practical implementation of the nursery experiment at all stages but especially the water- and nitrogen treatments
- obtain more precise estimates for resource-use efficiencies and correlations with resource-use efficiencies
- obtain information about the resource-use efficiency of plus trees already in the breeding program of the B.C. Ministry of Forests and for which 10-year field data on height growth and survival are available
- evaluate families considered 'stable' in the field as well as 'unstable' families
- compare GxE in the field with GxE in the experiment
- relate seedling (1-year nursery) performance to sapling (10-year field) performance
- obtain a better picture of trade-offs among traits using more precise estimates.

Other than their choice of genotypes, the experiments differ somewhat in their set-up, the germination of seedlings, the resulting plant size before start of treatments and the total plant size, the climate of that year, the fine details of the treatment application, and their analysis. These differences will be pointed out where relevant.

1.1 Plant material

1.1.1 Seed sources for the 1996 experiment

Seedlots for this experiment were collected in the fall of 1995 (figure 1). Three interior (*Pinus contorta* ssp. *latifolia*) and two coastal (*Pinus contorta* ssp. *contorta*) populations were sampled. These populations differ with regard to their source environment (table 1). At each of the five locations, twenty families were sampled. In total, 100 open-pollinated families were sampled.

1.1.2 Seed sources for the 1997 experiment

Open-pollinated seedlots, harvested in the late 1970s and early 1980s by the B.C. Ministry of Forests from plus trees of the Thompson Okanagan (TO) and the Shuswap Adams (SA) zones, were used. The 565 selections from these two zones were of special interest because their source sites are distributed along a moisture gradient. Progeny trials have been established in the zone of origin as well as in some neighbouring zones. Only the 129 families which were planted in both the TO and the SA trials were considered, such that for all families, field data would be available over a range of field sites with different moisture regimes. Thus, information from the nursery experiment can be compared to the ten-year old performance of the same families in the field. It was felt that the nursery experiment would help to explain some of the GxE interactions observed in the field, provided that the same environmental factors were operating. Available seedlots were then ranked according to stability in the field progeny trials across the two zones, using Shukla's (1972) stability variance, based on five year height growth for the TO field sites and ten year height growth for SA field sites. 'Unstable genotypes' (with high stability variance) are those that deviate from the linear and additive predictions by the model.

Of the chosen 49 open-pollinated seedlots, 22 are unstable and 22 are stable. The seedlots span the range of available elevations and include good as well as poor performers. The selection may not be a random sample but can still be considered fairly representative of the material from the TOA-TOD and SA zones. The remaining five seedlots were chosen on the basis that they were planted in field trials in yet two other zones, namely on Willow-Bowron (WB) and Bulkley Valley (BV) sites. These five include one genotype from Bush (BSH) and one from West Kootenays (WK). See figure 2 for the locations and table 2 for details of these families.

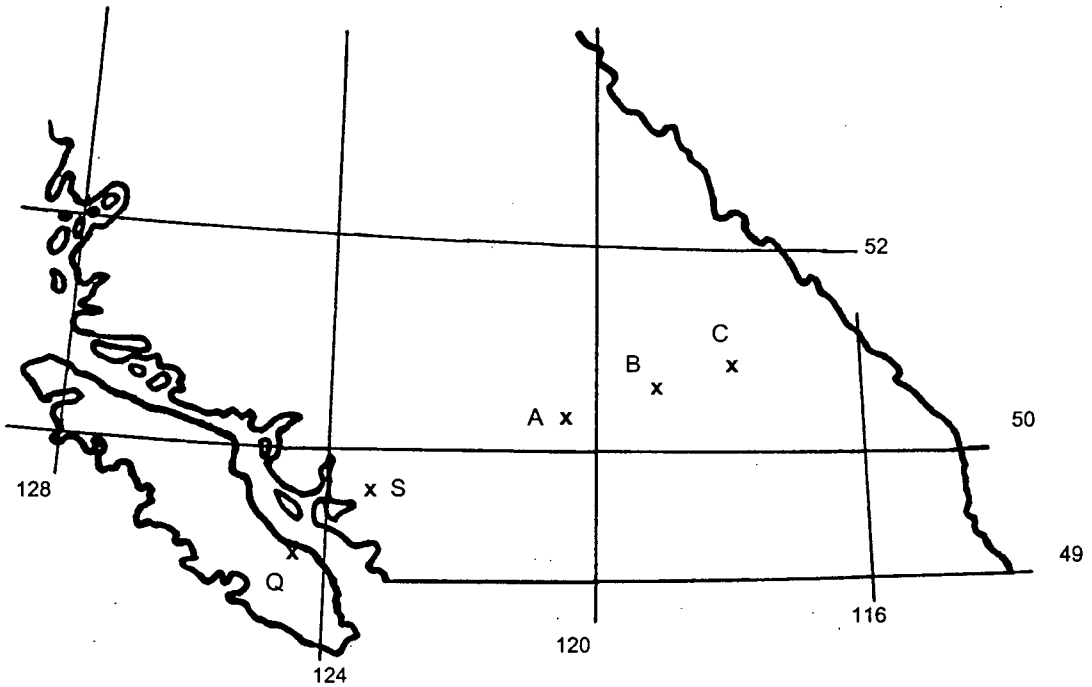

Figure 1. Locations of the five provenances used for the 1996 experiment.

Table 1. Seed sources for the 1996 experiment.

Site	ssp. *latifolia*			ssp. *contorta*	
	Kamloops A	Salmon Arm B	Revelstoke C	Qualicum Q	Squamish S
Latitude	50°37.44'	50°47.62'	50°57.31'	49°23.6'	49°54.4'
Longitude	120°39.30'	119°24.47'	118°08.32'	124°37.5'	123°09.6'
Elevation (m)	1400	500	1000	20	335
BGC [1] unit	MSxk	IDFmw2	ICHmw	CDFmm	CWHds1
MAP [2] (mm)	394	487	947	1293	1846
MSP [2] (mm)	178	186	275	206	350
MAT [3] (°C)	2.6	7.4	1.1	9.2	5.4
MTWM [3] (°C)	13.1	18.7	12.9	16.6	13.3
ADI [4]	1.89	2.14	0.71	0.91	0.49
SDI [4]	0.85	1.17	0.55	0.93	0.44
Weather station [5]	Highland Valley [6]	Tappen	Revelstoke [6]	Qualicum	Garibaldi [7] & Squamish [6]

[1] BGC : biogeoclimatic zone (Meidinger and Pojar 1991).
[2] MAP and MSP: mean annual and summer precipitation (May to September inclusive).
[3] MAT and MTWM: mean annual temperature and mean temperature of the warmest month.
[4] SDI and ADI: summer dryness index and annual dryness index (Guy and Holowachuk, submitted).
[5] data: Atmospheric Environment Service (1982).
[6] temperatures were adjusted for elevation: 1 °C per 100 m (Barry and Chorley 1976, p.94).
[7] Garibaldi was the most representative station, but lacked temperature data.

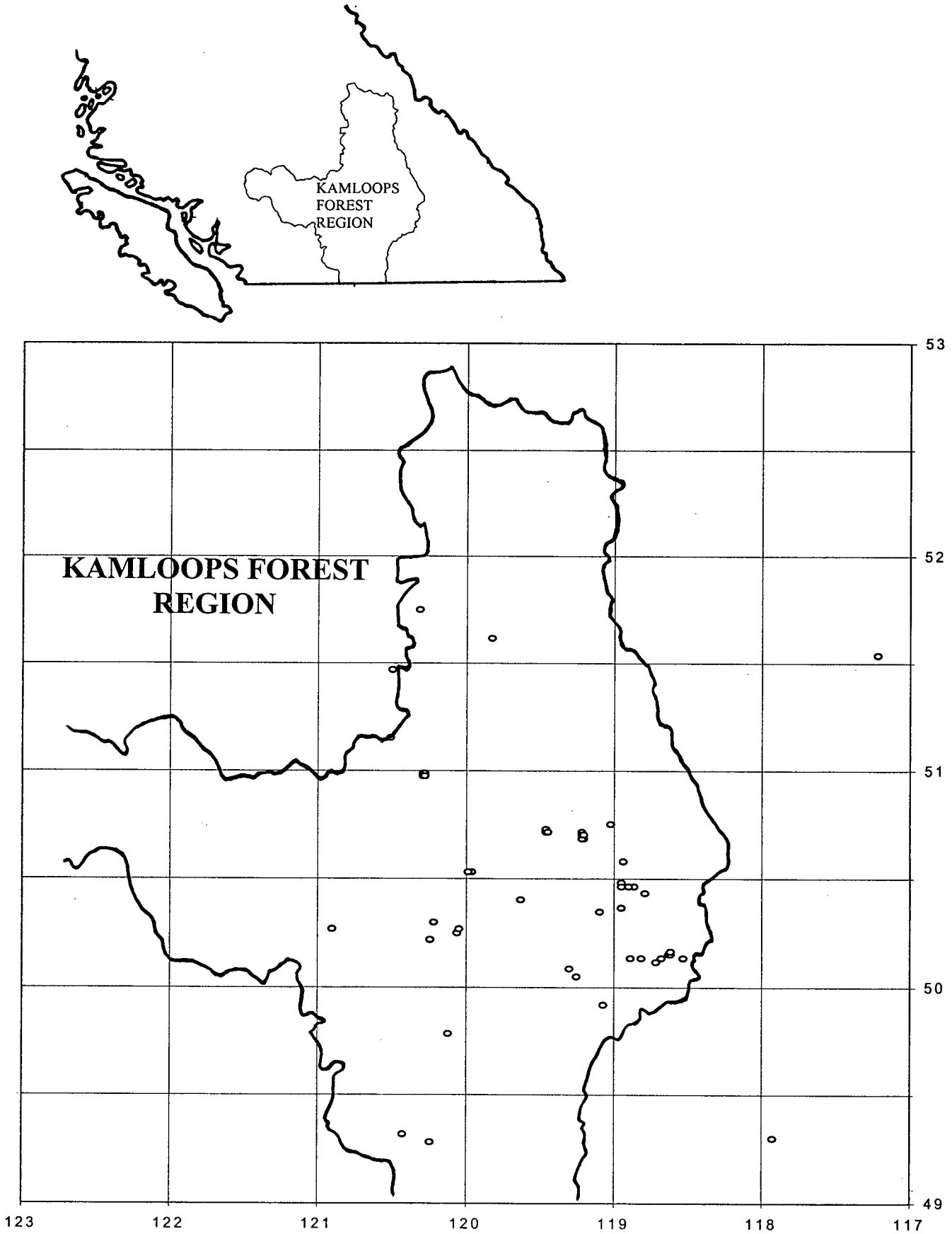

Figure 2. Locations of the 49 families used in the 1997 experiment.

Table 2. Seed sources for the 1997 experiment.

Family	Breeding Zone [1]	Elevation	Breeding value TO sites	Breeding value SA sites	Mean [2]	Difference [2]	Var(i) [3]
1	SA	1000	27.6	-2.2	12.7	29.8	269.8
2	TOD	1650	-21.5	-6.0	-13.8	15.5	251.4
3	SA	1130	13.6	-15.0	-0.7	28.6	242.4
4	SA	1210	14.6	-10.8	1.9	25.4	176.5
5	TOA	1295	-21.6	-10.2	-15.9	11.4	167.2
6	SA	930	7.7	18.4	13.1	10.7	154.5
7	TOD	1075	18.7	-4.9	6.9	23.6	144.0
8	TOA	1390	-21.2	-11.5	-16.4	9.7	137.3
9	TOD	1075	14.2	-8.0	3.1	22.2	121.0
10	SA	1100	14.1	-7.9	3.1	22.0	117.9
11	TOD	1075	6.3	-15.2	-4.5	21.5	110.3
12	SA	650	35.2	14.2	24.7	21.0	102.9
13	TOA	1260	-18.6	-12.2	-15.4	6.4	87.6
14	SA	1040	14.1	-5.1	4.5	19.2	78.4
15	SA	884	16.2	21.6	18.9	5.4	74.7
16	SA	1300	5.3	-13.5	-4.1	18.8	73.5
17	TOA	1190	-0.5	-19.1	-9.8	18.6	71.0
18	SA	1290	11.1	-7.1	2.0	18.2	66.3
19	SA	620	5.6	10.0	7.8	4.4	62.9
20	SA	1196	-6.3	-24.2	-15.3	17.9	62.8
21	SA	1550	-10.7	-6.5	-8.6	4.2	60.6
22	SA	1340	-7.1	-3.0	-5.1	4.1	59.5
Mean for families 1-22			4.4	-5.4	-0.5	16.3	122.4
23	SA	960	26.3	14.5	20.4	11.8	12.6
24	SA	960	25.6	15.8	20.7	9.8	4.4
25	SA	650	19.2	15.4	17.3	3.8	4.1
26	WK	600	15.6		-1.6		
27	BSH	925	17.5		2.6		
28	TOD	1650	-3.2	-7.2	-5.2	4.0	3.5
29	SA	800	13.2	3.7	8.5	9.5	3.5
30	SA	1430	-10.6	-14.8	-12.7	4.2	2.9
31	TOA	1280	-7.7	-12.0	-9.9	4.3	2.7
32	TOD	1650	-20.9	-25.3	-23.1	4.4	2.5
33	SA	900	9.0	-0.1	4.5	9.1	2.4
34	SA	1183	6.3	-2.8	1.8	9.1	2.4
35	SA	1189	13.7	8.9	11.3	4.8	1.6
36	SA	1220	7.8	-0.9	3.5	8.7	1.6
37	TOD	1500	-5.5	-14.0	-9.8	8.5	1.2
38	SA	1122	6.9	-1.5	2.7	8.4	1.0
39	SA	650	13.3	8.1	10.7	5.2	0.9
40	SA	1490	-7.3	-15.6	-11.5	8.3	0.9
41	SA	1645	-12.2	-17.6	-14.9	5.4	0.6
42	TOD	1075	0.7	-4.8	-2.1	5.5	0.4
43	SA	1250	-6.6	-14.6	-10.6	8.0	0.4
44	TOD	1530	2.2	-3.5	-0.7	5.7	0.2
45	TOD	1372	-12.7	-18.5	-15.6	5.8	0.1
46	TOD	1387	-6.2	-13.7	-10.0	7.5	-0.1
47	SA	1165	10.0	2.8	6.4	7.2	-0.3
48	TOA	1432	-18.7	-25.1	-21.9	6.4	-0.3
49	SA	1370	4.7	-1.7	1.5	6.4	-0.3
Mean for families 28-49			-1.1	-7.7	-4.4	6.7	1.3

[1] see text page 17 for abbreviations of breeding zones
[2] of columns 4 and 5
[3] Var(i) = stability variance as calculated by Shukla (1972)

1.1.3 Production of seedlings

After the 1995 harvest, cones were stored until they could be processed. They were then dipped in 70 ^{0}C water to break the resin bonds. After the cones were air-dried, the seeds were shaken out and de-winged. The seeds were then cold-stored at 2 ^{0}C. The seedlots for the 1997 experiments had been cold-stored at below-freezing temperatures by the Ministry of Forests for three decades.

One month before sowing, seeds were soaked in sterile water for 48 hours, followed by cold storage at 2 ^{0}C for four weeks. Seeds were then sown in Ray Leach single-cell 'cone-tainers' (Stuewe & Sons Inc., Corvallis, OR). Each tray (RL-98 tray) contains 98 cells of 164 ml volume (SC-10 super cells, diameter 3.8 cm, and height 21 cm). The individual cells of the cone-tainers allowed the seedlings to be moved around individually at any time. One tray or cone-tainer contained 98 cells. This unit is called a treatment unit for ease of reference.

The cells were filled with a mixture of peatmoss, vermiculite, dolomite lime and 'nutritrace' slow-release secondary plant nutrient mix (WestGro, Calgary, Canada). For each two bales (one bale is four cubic feet or about 0.11 m^3) of peatmoss, one bale of vermiculite (0.11 m^3), 1.2 kg dolomite lime and 225g nutritrace elements were added. Nutritrace contains the micronutrients Mg, Mn, Cu, Zn, Fe, S, Ca, B and Mo. The top of each cell was covered with grit. After sowing, the cells were watered every two days for 2-3 weeks. During the following weeks, before the start of the differential treatments, they were fertilised three times per week with 20-8-20 Forest Seedling Special (Plant Products Ltd., Brampton, Canada) at a concentration of 0.33 g/l.

In 1996, seed was stratified on March 15th and single-sown on April 15th in an unheated greenhouse. Uniform fertilisation started on May 6th, and differential treatments started on June 11th, when the plants were approximately 1-2 cm high. Despite seed stratification, the cool spring temperatures in the unheated greenhouse resulted in uneven rate of germination. Kamloops and Qualicum germinated first. Squamish germinated last.

In 1997, seed was stratified on March 1st and double-sown on April 1st in a heated greenhouse. Uniform fertilisation started on April 15th and seedlings were moved to an unheated greenhouse on May 30th. Differential treatments started on June 5th, when plants were approximately 4.5 cm high. The greenhouse had plenty of ventilation through open side-walls to ensure isotopic uniformity of the source air. Light, temperature and isotopic composition of the air in the greenhouse were similar to those in the open air.

1.2 Experimental design

Just before the start of the differential treatments, the seedlings were rearranged in treatment units and replications. As much as possible - insofar as there were no missing plants - this was done in a balanced manner. Missing plants were replaced to fill gaps but the replacements were not analysed. The design is a split-plot, with families completely randomised within a treatment unit and treatment units randomised in space as much as practically feasible. At the level of the treatment units, the experiment is a factorial experiment with three levels of nitrogen and three levels of water, or nine treatments in total. There were four replications to allow for the estimation of the experimental error.

The main treatment units were not completely randomised. In 1996, the flats were grouped in three blocks according to the water treatments to ensure uniformity of water applications. Strictly speaking, this means that water treatments are confounded with positioning in the greenhouse, even though these 'blocks' were rotated and treatment units were also rotated within 'blocks'. Though this is an unfortunate set-up from a statistical point of view, I believe that the water effects are 'true effects' and have very little to do with positioning of treatment units in space. In the model they are therefore called 'water effects' rather than 'blocking effects'.

In 1997, the flats were grouped into four blocks, which were true blocks, i.e. not confounded with other factors: each block contained one replication. These blocks were again rotated in the greenhouse, and treatment units rotated within blocks.

One of the four replications of the 1997 experiment is shown in figure 3. In this figure, treatment units have been rearranged systematically for the purpose of demonstration, but that was not how they were positioned in the greenhouse during the experiment. The plants in the greenhouse were rotated to mitigate the effect of variability in the local climate. Since plants grew in individual cones there was no root competition. Undesirable effects of light competition were avoided by regularly repositioning plants relative to each other.

Using this factorial combination of treatments, and keeping all other factors constant by randomisation as well as by periodically moving plants and flats around, response curves to both water and nitrogen can be obtained and interactions between these factors can be investigated.

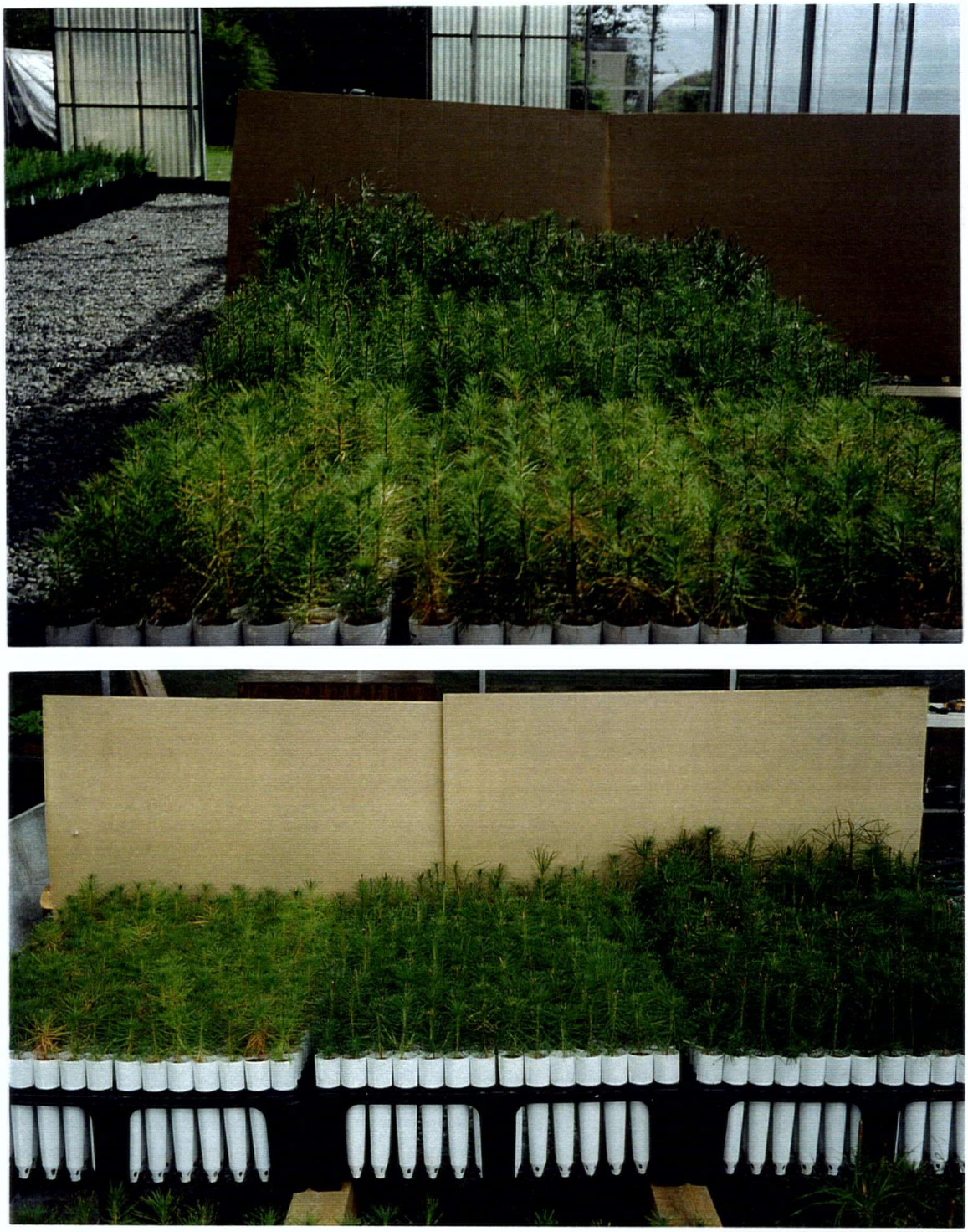

Figure 3. Overview of one replication of the 1997 experiment. Treatments were arranged systematically for the sake of the pictures, which were taken in September 1997. In the top picture the effect of water levels on height growth can clearly be seen. In the bottom picture the effect of nitrogen on height growth can be seen. The colour of the plants is also much lighter in the low-N treatments.

1.3 Treatments

Three levels of each of two environmental factors were chosen, resulting in a factorial combination of nine treatments in total. These treatments or 'environments' are referred to using the notation of table 3, where the subscripts H, M and L stand for high, medium and low, respectively.

Table 3. Symbolic notation of the nine experimental environments

environment	notation [1]	water level (W)	nitrogen level (N)
1	W_HN_H	≥ -0.1 MPa	200 ppm
2	W_HN_M	≥ -0.1 MPa	50 ppm
3	W_HN_L	≥ -0.1 MPa	10 ppm
4	W_MN_H	≥ -0.25 MPa	200 ppm
5	W_MN_M	≥ -0.25 MPa	50 ppm
6	W_MN_L	≥ -0.25 MPa	10 ppm
7	W_LN_H	≥ -1 MPa	200 ppm
8	W_LN_M	≥ -1 MPa	50 ppm
9	W_LN_L	≥ -1 MPa	10 ppm

[1] H=high, M=medium, L=low levels of water (W) and nitrogen (N)

1.3.1 Water treatments

Drought was applied using drought cycles, in which the soil in cone-tainers was saturated with water after they had reached a predetermined minimum soil water potential. Patterson (1994) followed the same approach, using drought cycles to a minimum of -1MPa. This level of drought stress is enough to considerably impact growth without risking cavitation. Variation within treatments results in some plants being stressed more than others, and I didn't want to loose any plants due to the treatments. In a study of Douglas-fir seedlings, initial cavitation occurred at a predawn xylem water potential of –1.0 MPa (Kavanagh et al. 1999).

In practice, soil weight was used to estimate soil water potential. The relationship between soil water content and soil water potential was determined using a Wescor C52 thermocouple psychrometer chamber hooked up to a HR33T microvoltmeter using the dewpoint method. The resulting calibration curve is shown in figure 4. From this curve, the practical relationship between soil weight and soil water potential was derived (table 4).

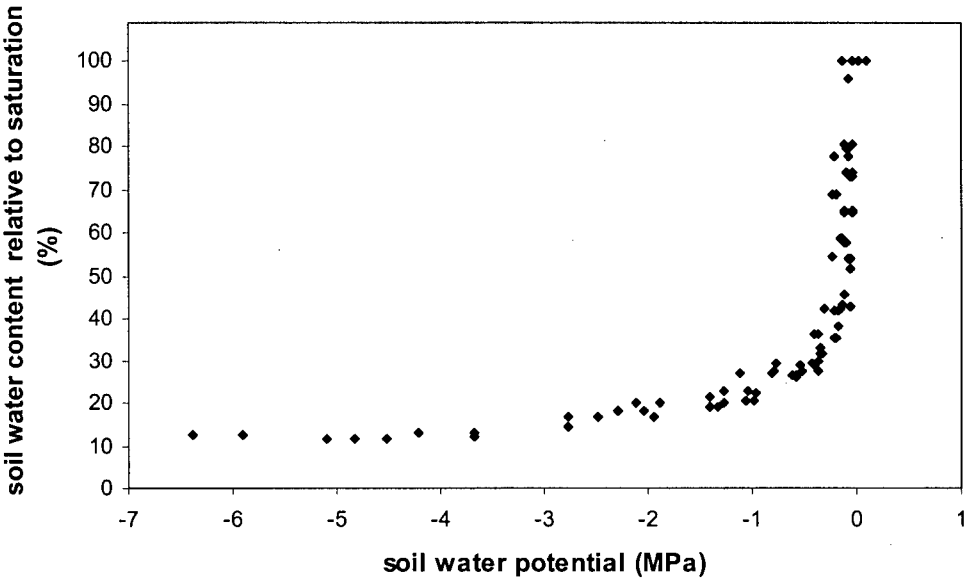

Figure 4. Calibration curve used to derive the soil moisture content for a given soil water potential

Table 4. Soil water potential, soil water content and tray weight at saturation and at the end of a drought cycle for the different water treatments.

Water level	Soil water potential	Soil water content	tray weight
saturation	- 0.1 MPa	100 %	13.5 kg
wet (high)	- 0.1 MPa	> 65 %	>10.5 kg
medium	- 0.25 MPa	38 %	8.4 kg
dry (low)	- 1 MPa	25 %	7.1 kg

Using tray weight implies that an average weight of all 98 cones is used to determine the end of each drought cycle rather than the values for individual cones. Efforts were made to keep drought levels within a treatment unit uniform by regularly repositioning the plants within the tray. The high water treatment was deliberately kept under saturation point (<13.5 kg), since otherwise the nutrients would be leached out. Some leaching is believed to have taken place in the 1996 experiment regardless. Ten drought cycles were achieved between the beginning of June and the end of September in 1996. Eleven drought cycles were achieved in 1997.

1.3.2 Nitrogen treatments

The planting medium already contained lime and microelements. Hence, the elements to be added on a regular basis were N, P and K, and a small amount of S. Since the N-level had to be varied, no commercial fertiliser could be used. The following chemicals were chosen as sources of these macronutrients: NH_4NO_3, KH_2PO_4 and K_2SO_4 (after van den Driessche 1989). The N-levels used in the experiments of van den Driessche (1989) and Patterson (1994) were used as guidance. High nitrogen treatments received 200 ppm N, medium treatments received 50 ppm. For the low-N level 10 ppm was chosen rather than 20 ppm, to better cover the lower range of nitrogen availability. Patterson (1994) found that reducing N-availability to 20 ppm reduced spruce biomass by about 20-34% relative to the control treatments, which had high water and nitrogen levels. His low N and W levels reduced spruce biomass to about half of the control treatment. A more drastic decrease in growth rate and hence more elevated stress levels were aimed at. In Patterson's experiment, drought by itself had a larger influence than nitrogen by itself. Therefore it was decided to lower minimum N-levels to 10 ppm. In table 5 the composition of the fertiliser solution is displayed for the different treatments.

Table 5. Nutrient concentrations in the fertiliser solution

treatment	N concentration	P concentration	K concentration
high N	200 ppm	50 ppm	100 ppm
medium N	50 ppm	50 ppm	100 ppm
low N	10 ppm	50 ppm	100 ppm

The small size of the plants relative to the cone volume and the resulting long drought cycles did not permit me to fertilise as frequently as either van den Driessche (1989) or Patterson (1994). I fertilised at the end of a 10 to 12 day drought cycle, whereas they fertilised two or three times per week. This decreased frequency of fertilisation resulted in a very low N-availability to the plants indeed. The general results show that the low N level was actually more limiting to plant growth than the low water availability. Neither water- nor nitrogen stress caused any mortality in either of the two experiments : all missing values were due to the poor germination rate of certain families and were missing before the start of treatments.

1.4 Traits measured

At the end of the first growing season, height and diameter were measured. All seedlings were harvested. Roots and shoots were separated at the root collar and were washed to remove the growth medium. Immediately after washing they were oven-dried at 70 ^{0}C for approximately 48 hours. After harvesting the whole experiment, plants were briefly (24 h) re-dried before weighing to ensure that they were indeed oven-dry and hadn't re-absorbed any moisture. Shoot and root dry weight were determined and plant parts were recombined to grind. Individual whole plants were ground with a 40-mesh Wiley Mill, followed by pulverisation with a planetary ball mill (Pulverisette-7 Planetary Micro Mill from Fritsch, Germany).

Samples were prepared from this very fine and homogeneous powder by taking 1.0 ± 0.1 mg in a tin capsule (5 x 3.5 mm tin capsules from Europa Scientific). The capsule was compacted and placed into a 96-well Elisa plate (Fisher) together with the appropriate standard samples at regular intervals. Samples were then sent to the carbon isotope lab in Saskatoon, Saskatchewan. Lodgepole pine needles from a grown tree were used as a standard reference sample, which serves to regularly re-calibrate the machine, but this standard was ultimately calibrated against Vienna PD-belemnite, the internationally accepted standard.

$$\delta^{13}C = (^{13}CO_2 / {}^{12}CO_2)_{sample} / (^{13}CO_2 / {}^{12}CO_2)_{standard}$$

Water-use efficiency (WUE) and nitrogen-use efficiency (NUE) were determined on whole-plant tissue. Values for $\delta^{13}C$ and C/N vary significantly among tissues (Leavitt and Long 1986). Whole plant sampling is feasible for small plants provides the best representation of all carbon fixed. Some authors have argued in favour of analysing the carbon isotopic composition of the cellulose fraction only. However, that may be influenced by other fractionation processes downstream of the carboxylation event.

Carbon isotope composition and C/N ratio are determined simultaneously during sample analysis. C/N is determined in the process of sample combustion on an elemental analyser. $\delta^{13}C$ is determined after the CO_2 passes to the mass spectrometer. The equipment used to process the samples was a RoboPrep Biological Sample Converter interfaced to a TracerMass Mass Spec (PDZ Europa Scientific Inc., Crewe, UK).

2. DATA ANALYSIS

Results were analysed using a mixed model typical for split-plot experiments. The procedure Proc Mixed from the SAS system (SAS Institute, 1997) was used, which uses REML (restricted maximum likelihood) to estimate the random effects. Significance of fixed effects was tested using F-tests. For random effects, the variance components were tested for being non-zero. Least squares means are used for all further analyses. Despite the fact that BLUP (Best Linear Unbiased Predictors) are more suitable when families are in reality random, it makes more sense to limit further conclusions to the specific families mentioned. These means are used to draw response functions for the different genotypes. Significance of differences and cross-over interactions are tested using the standard error of the estimated Least Squares Means.

2.1 Linear model

2.1.1 Model for the 1996 experiment

The 1996 experiment has the following factors: water, at three levels; nitrogen, at three levels; provenances, five levels; and families nested in provenances, 20 levels. Subspecies effects were not explicitly included as a factor. Therefore they are confounded with the provenance effects. The model for analysing this experiment is as follows:

$$Y_{ijklq} = m + w_i + n_j + w*n_{ij} + e1_{(ij)q} + p_k + w*p_{ik} + n*p_{jk} + w*n*p_{ijk} + f(p)_{(k)l} + w*f(p)_{i(k)l} + n*f(p)_{j(k)l} + w*n*f(p)_{ij(k)l} + e2_{(ijkl)q}$$

with :

m	overall mean
w_i	effect of water level i
n_j	effect of nitrogen level j
$w*n_{ij}$	interaction between water level i and nitrogen level j
$e1_{(ij)q}$	mainplot error (= first experimental error)
p_k	effect of provenance k
$w*p_{ik}$	interaction of water level i with provenance k
$n*p_{jk}$	interaction of nitrogen level j with provenance k

$w*n*p_{ijk}$	interaction of water level i with nitrogen level j and provenance k
$f(p)_{(k)l}$	effect of family l nested in provenance k
$w*f(p)_{i(k)l}$	interaction of water level i with family l within provenance k
$n*f(p)_{j(k)l}$	interaction of nitrogen level j with family l within provenance k
$w*n*f(p)_{ij(k)l}$	interaction of water level i with nitrogen level j and family l within provenance k
$e2_{(ijkl)q}$	subplot error (= second experimental error)

2.1.2 Model for the 1997 experiment

The analysis of the 1997 experiment differed slightly from the one for 1996, because there was no provenance effect and because of the covariate 'initial height'. Heights of all plants were measured before the start of the treatments in an attempt to compensate for the effect of differences in initial height in the analysis. Although initial height is an ideal covariate for height at the end of the growing season, it is less ideal for the other traits. Nevertheless, measurement of other covariates (like dry weight) before start of treatments is not feasible since such measurements are destructive. Since height is an indication of plant size, it is also a sensible covariate for the other morphological variables. Even for the physiological traits it makes sense to include the covariate in the model. Since larger plants are more likely to have a larger proportion of secondary needles, which have been found to be more efficient for WUE at the leaf level (Kubien 1994), taking initial size into account results in a better model. Also, larger plants can achieve higher C/N ratios (Raven and Farquhar 1990) through internal nutrient recycling.

There were 49 families and two (non-contiguous) seedlings of each family within each treatment unit (repetitions). The 1997 experiment had the following factors: water, at three levels; nitrogen, at three levels; and families, at 49 levels. This results in the following model:

$$Y_{ijklqr} = m + w_i + n_j + w*n_{ij} + r_q + e1_{ijq} + f_l + w*f_{il} + n*f_{jl} + w*n*f_{ijl} + e2_{(ijl)q} + hi_{(ijlq)r} + e3_{(ijlq)r}$$

with :

m	overall mean
w_i	effect of water level i
n_j	effect of nitrogen level j

$w*n_{ij}$	interaction between water level i and nitrogen level j
r_q	replication (blocking term)
$e1_{ijq}$	first experimental error (mainplot error)
f_l	effect of family l
$w*f_{il}$	interaction of water level i with family l
$n*f_{jl}$	interaction of nitrogen level j with family l
$w*n*f_{ijl}$	interaction of water level i with nitrogen level j and family l
$e2_{(ijl)q}$	second experimental error (subplot error)
$hi_{(ijlq)r}$	covariate initial height
$e3_{(ijlq)r}$	sampling error, r repetitions

2.2 Data set, analysis of residuals and data transformations

Four growth traits were measured at the end of the first growing season: height, diameter and root weight and shoot weight of oven-dried plants. Derived from those were: total plant dry weight, referred to as 'biomass' (even though, strictly speaking, biomass refers to living matter and therefore excludes wood), shoot-root ratio (ratio of shoot dry weight over root dry weight), slenderness ratio (ratio of height over diameter), and stem volume (half the diameter squared times height, without adjustment for stem form, as a relative measure only). For these growth traits a 'complete' data set of 3528 observations is available, except for missing values - almost all of these were missing plants before the start of treatments - which did not exceed 5 % in 1996 and 10 % in 1997. For carbon isotope composition and C/N ratio, which are expensive measurements, subsampling was carried out.

For the 1996 experiment, two families of the Qualicum provenance were dropped from all analyses due to their low germination rate, leaving a total of 98 families. Subsampling for carbon isotope composition ($\delta^{13}C$) and C/N ratio was limited to 455 plants. Only four families of each provenance were sampled. Subsampling was unbalanced: the most contrasting treatments were sampled more extensively than the others. Four seedlings per family were sampled for treatments W_HN_H , W_HN_L ,W_LN_H and W_LN_L, as opposed to two seedlings per family for the other treatments.

For the 1997 experiment, half of the samples (i.e. one of the two repetitions or 1700 plants) were processed for carbon isotope composition ($\delta^{13}C$) and C/N ratio. Subsampling was balanced over families and treatments. Four seedlings were sampled for each family.

The validity of the conclusions from an analysis based on a linear model are dependent on the assumptions of normality and homogeneity of variances. Though the F-tests in an analysis of variance are fairly robust to these assumptions, relationships between the variance and the mean are undesirable because they result in the standard error of the estimated means being under- or over-estimated. This must be taken into consideration when deciding whether to try and improve the distribution of residuals by means of a transformation of the data and weighing it against the fact that the interpretation of the transformed traits becomes much more difficult.

Residuals were plotted against predicted values to detect any patterns. Tests are available in SAS (univariate procedure, SAS Institute 1982) to test for the normality of the residuals. However, when the data set is large, this test is very sensitive to non-normality for distributions that appear to be perfectly normally distributed. For this reason, three factors were considered when comparing different transformations:

- the presence of any patterns in the plot of residuals against predicted values that would indicate non-normality
- skewness and kurtosis of the distribution, with a visual comparison of the distributions
- measure of deviation from normality (D or W statistic as given by SAS Proc Univariate).

The logarithmic (natural logarithm) and square root transformations were satisfactory for most growth traits. NUE needed a reciprocal transformation. For volume in the 1996 experiment, the power transformation with exponent 0.25 gave good results. Although these transformations (shown in table 6) greatly improved the distribution of residuals, they did not necessarily result in perfect normality.

Table 6. Abbreviation of the traits measured and transformations.

trait	abbreviation	1996	1997
height	h	square root	-
diameter	d	-	-
volume	vol	power (0.25)	logarithmic
slenderness ratio (=h/d)	hd	-	-
root dry weight	rt	square root	-
shoot dry weight	sht	square root	logarithmic
biomass (total dry weight)	bm	square root	logarithmic
shoot-root ratio	srr	logarithmic	logarithmic
water-use efficiency (=$\delta^{13}C$)	wue	-	-
nitrogen-use efficiency (=C/N)	nue	reciprocal	reciprocal

Although slightly better power transformations might be available, their interpretation would be much more difficult, so they were not used.

Data were then back-transformed for interpretation and graphing. Kung (1988) proposed correction factors to avoid bias. However, for my data these correction factors were not satisfactory: they yielded means outside the range of observations for small plants. Since rank order is preserved by these adjustments and it is mainly the relative performance of genotypes which is of interest, backtransformed and unadjusted estimates were used in plotting and tables. For all statistical testing procedures, transformed estimates were used.

2.3 Random versus fixed effects

Treatment effects and provenances were considered fixed factors, while mainplot error was random. The family effects of both the 1996 and 1997 experiments were considered random in the analysis of variance and for the estimation of genetic parameters. However, to compare specific family means, they were considered fixed.

The significance of fixed factors was tested with F-tests. F-tests are not available for random factors in Proc Mixed. Three options are available (Littell et al. 1996):

1. Consider the result of an F-test from a Proc GLM-analysis. Since the results of Proc GLM are not all that different from those of Proc Mixed for moderately unbalanced designs, this is an acceptable approach.
2. Use the Wald-Z test produced by the Proc Mixed output. This is not too bad an approximation if the degrees of freedom for the random factor are large. This Z-test produces a symmetric confidence interval, which should more realistically be asymmetric.
3. Run Proc Mixed with the random factor and without it. Make the difference between both log likelihood statistics. This difference follows a χ^2 distribution with 1 df.

The Wald-Z statistic has low power, but if it detects significant differences, these will also be significant according to the other criteria. Hence it was used, and where it failed to detect significance of the random factor, the third option was used.

2.4 Multiple range tests

Multiple comparisons of provenance means were made within treatments. Making ten possible comparisons among five different provenance means requires an adjustment of the type I error

in order to avoid an inflated experiment-wise error. As suggested by Neter et al. (1990, p.589) the t- and q- values for Bonferroni, Tukey and Sheffé were calculated and compared and the smallest was selected, which turned out to be the Tukey value (q=3.86 or T=2.2729).

Differences between pairs of provenance means were compared with $q * \sqrt{(s^2_{\bar{X}_1} + s^2_{\bar{X}_2})/2}$.

Individual standard errors on estimated means were used rather than an average to compensate for unequal sample sizes. Transformed values and their standard errors were used for these tests. In order to compare differences between treatments for the means of all genotypes, single comparisons were carried out. Since they were not pre-planned, this results in an inflated overall error rate.

2.5 Testing for significance of rank order interactions.

The parametric test by Azzalini and Cox (1984) investigates the presence of what they call 'qualitative interactions', i.e. interactions that cannot be removed by transformations. Truberg (1996) calls them rank order interactions. The test is asymmetric. Specifically for genotype-environment interaction, this means that there is a separate test for genotype rank change, Gx(E), and for environment rank change, Ex(G). Significance of either of these tests results in a significant rank order interaction in general. This conforms to the concept of separability of genotypic and environmental effects proposed by Gregorius and Namkoong (1986, 1987), where inseparability of either effect results in overall inseparability of the two factors.

Each quadruple of two genotypes and two environments is tested for a significant cross-over, adjusting the overall level of significance for making many such tests simultaneously. The resulting number of quadruples quickly grows enormously, but Azzalini and Cox (1984) provide proof for an adjustment that is less conservative than a Bonferroni-type adjustment.

To test whether genotypes change rank, i.e. a Gx(E) rank interaction test, all pairs of genotypes are compared in all pairs of environments. The difference d_{ij} between two genotypes (g_i-g_j) has to differ in sign between the two environments (E_p and E_q) and it has to exceed a critical difference in each environment. This critical difference is $t_\alpha \, \sigma\sqrt{2}$, where $\sigma\sqrt{2}$ is the standard error of a simple contrast, and the critical t_α is determined according to Azzalini and Cox (1984):

$$t_\alpha = -\Phi^{-1} \sqrt{-2\log(1-\alpha) \;/\; m_1(m_1-1)m_2(m_2-1)} \quad ,$$

where m_1 is the number of genotypes, m_2 the number of environments, α the experiment-wise type I error, and Φ the standard normal integral. The degrees of freedom of t_α are determined by the df of σ, or the df error. The 'TINV' function (SAS) was applied to the square root term to obtain this t-value (table 7). The formula indicates that, as the number of genotypes and of environments increases, t_α increases and the power of detecting cross-over interactions (COI) will decrease (Crossa et al. 1993). However, from a breeder's perspective, a type II error (accepting the false null hypothesis of no COI) is more serious than a type I error (rejecting the true null hypothesis of no COI). Therefore, Cornelius et al. (1992) proposed using a comparison-wise error rate (for d_{ij}) or an interaction-wise error rate (for a pair of G's and E's) of α. Thus, to test H_0 : 'there is no rank order interaction' against H_1: 'there is rank order interaction', the appropriate t-value would be the one proposed by Azzalini and Cox. To test: H_0 : 'there is rank order interaction' against H_1: 'there is no rank order interaction', it can be argued that a comparison-wise t or even an interaction-wise t is more appropriate. In this study, all three tests were carried out.

Table 7. Critical t-values for testing rank order change: Azzalini-Cox t (t_{azzcox}), comparison-wise t (t_{comp}) and interaction-wise t (t_{inter}) for morphological and physiological traits. The number of environments is always nine. The SAS function TINV(x,df) was used to obtain t_{azzcox}.

experiment	data-set	df_{error}	# genotypes	t_{azzcox}	t_{comp}	t_{inter}
1996 exp.	morph.traits	2500	5 provenances	2.39090	1.64546	1.00244
	phys.traits	272	5 provenances	2.40411	1.65047	1.00409
	morph.traits	2500	20 families	2.89105	1.64546	1.00244
	phys.traits	272	20 families	2.91335	1.65047	1.00409
1997 exp.	morph.traits	2749	49 families	3.16709	1.64541	1.00242
	phys.traits	1259	49 families	3.17085	1.64607	1.00264

A significant Gx(E) interaction implies that the G effects are not separable from the E effects. As such, no genotypic effects that are consistent across environments can be defined. Only for a subset of environments can separability be achieved and can genotype effects be defined.

Gx(E) rank interactions are dealt with by grouping environments, such that consistency of G effects is achieved within that group of environments For this clustering procedure, the absolute values of the differences d_{ij} are used to create a distance measure (Truberg 1996). There is one such difference for each of the environments of a comparison, that is one for E_p and another for E_q. The smallest of these two absolute values, $\min[abs(d_{ij})_p, abs(d_{ij})_q]$, is retained.

This minimum times a multiplier - which is one in case of a sign change (i.e. for $(d_{ij})_p * (d_{ij})_q < 0$) and zero in all other cases (i.e. for $(d_{ij})_p * (d_{ij})_q \geq 0$) - yields a series of values, one value for each quadruple of genotypes and environments. The maximum of these values across pairs of genotypes is the distance measure for a given comparison or pair of environments. This distance can be standardised by division by $\sigma\sqrt{2}$, so that it can easily be compared to the critical t-value, which serves as a cut-off value for the clusters.

To test whether environments change rank, i.e. a Ex(G) rank interaction test, all pairs of environments are compared for all pairs of genotypes. The difference d_{pq} between two environments (E_p- E_q) has to differ in sign for the two genotypes (G_i and G_j) and it has to exceed a critical difference in each environment. This critical difference is $t_\alpha\ \sigma\sqrt{2}$, as before. A significant Ex(G) interaction implies that the E effects are not separable from the G effects. As such, no environmental effects that are consistent across genotypes can be defined. Only for a subset of genotypes can separability be achieved and can environmental effects be defined.

Ex(G) rank interactions are dealt with by grouping genotypes, such that consistency of environmental effects is achieved within that group of genotypes. For this clustering procedure, the differences d_{pq} are used to derive a distance measure. The minimum of $abs(d_{pq})_i$ and $abs(d_{pq})_j$ times the multiplier (1 for sign change, 0 otherwise) yields a series of values, one for each quadruple. The maximum of these values over environments gives a distance measure for each pair of genotypes. The distance can be standardised by division by $\sigma\sqrt{2}$, so that it can easily be compared to the critical t-value, which serves as a cut-off value for the clusters.

If rank order interaction exists for either genotypes or environments, then it is said that 'rank order interaction exists', and 'G and E effects are not separable'. For selection, however, we are mainly interested in rank change of genotypes. Because the treatments of this study are based on explicit environmental variables, response surfaces for genotypes can be drawn in space. Rank order changes can then be visualised as intersecting response surfaces. Testing reveals which of these rank changes are significant and which ones may be due to estimation errors of the family or provenance means.

2.6 Calculation of genetic parameters

Genetic parameters were estimated based on the covariance of half-sibs (Falconer 1989), using the following definitions for phenotypic (V_P) and genetic (V_G) variance:

$V_P = V_G + V_E + V_{GE}$, with V_E = environmental variance, and

$V_G = V_A + V_{NA}$, with V_A = additive variance and V_{NA} = non-additive variance,

σ^2_f = cov (half-sibs) = 1/4 V_{add} , where 4 is the coefficient of relationship (r) of half-sibs.

The non-additive variance cannot be estimated unless an explicit full-sib family structure is present. Thus, $V_G = V_A =$ 4 $V_{Family(Provenance)}$. In the absence of a provenance structure, as for the 1997 data, $V_A = 4\ V_{Family}$. However, open-pollinated seedlots may contain a proportion of full-sibs (r=2) and selfed seed (r=1), so using r=4 would result in overestimating V_A. The selfing rate of lodgepole pine is low (<10%, Sorensen 1987). More likely, however, are full-sib family clusters as a result of the serotinous habit of lodgepole pine. Using a realistic value for the coefficient of relationship is important when gain is calculated, but less so when several estimates within one experiment are compared. Therefore, r was not adjusted in this study.

The Proc Mixed procedure (SAS) was used to derive these variance components because it also provides the covariance structure that is used to calculate the errors on the heritability.

2.6.1 Heritabilities

Heritability indicates the proportion of the phenotypic variance that can be translated into genetic gain. This will depend on the breeding and selection methods used. Narrow sense heritability is appropriate because the selected units would be mated randomly to achieve the next generation of the breeding population, so that only additive variation would be transferred and most of the non-additive variation would get lost. The mixed model was run with families and their interactions as random terms to obtain the necessary variance components. The factors water and nitrogen were collapsed into the factor "treatments" to facilitate calculations.

The individual narrow sense heritability was calculated as $h^2 = V_{add} / V_P$, with the phenotypic variance[1] $V_P = \sigma^2_f + \sigma^2_{tr*f} + \sigma^2_{subplot\ error}$. Heritabilities can also be calculated within each of the nine treatments, though the smaller data-set results in larger estimation errors. In that case, the model is simplified to:

$$Y_{ijklq} = m + e1_q + p_k + f(p)_{(k)l} + e2_{(kl)q} \quad \text{(1996 data), or}$$

$$Y_{ijklqr} = m + r_q + e1_q + f_l + e2_{(l)q} + hi_{(lq)r} + e3_{(lq)r} \quad \text{(1997 data).}$$

[1] see pages 28-30 for model term abbreviations

The 1996 model assumes that allele frequencies are uniform across provenances, which is a simplification rather than reality. However, the number of families within provenances was rather small for the calculation of separate genetic parameters for each provenance, especially for resource-use efficiencies. In the 1997 model, also, implicit provenance effects are confounded with family effects and will result in inflated heritability estimates.

Running these models with families as a random term yields variance components for family and error, and heritabilities can be calculated:

For the individual narrow sense heritability, $h^2 = 4\,\sigma^2_f / (\sigma^2_f + \sigma^2_{\text{subplot error}})$.

Standard errors of heritabilities, $SE(h^2)$, were estimated using the delta-method (Lynch and Walsh 1998): $Var(f) = \sum_i \sum_j \left[\left(\frac{\partial f}{\partial x_i} \right) \left(\frac{\partial f}{\partial x_j} \right) \sigma(x_i, x_j) \right]$. The heritability is a function of variances: $h^2 = \frac{4 * V_{fam}}{V_{fam} + V_{fam*tr} + V_{err}} = 4 \frac{x_1}{x_1 + x_2 + x_3} = f$. The first order partial derivatives are $\frac{\partial f}{\partial x_1} = \frac{4}{V_{phen}^2} \left[V_{phen} - V_{fam} \right]$ and $\frac{\partial f}{\partial x_2} = \frac{\partial f}{\partial x_3} = -\frac{4}{V_{phen}^2} \left[V_{fam} \right]$, and the mixed model procedure generates the matrix of variances and covariances $\sigma(x_i, x_j)$. The formula can easily be adapted for within treatment h^2 estimates by dropping x_2.

The errors on the two physiological traits are larger than the errors on morphometric traits because they were determined on a smaller data set.

2.6.2 Genetic correlations

The genetic correlation is calculated as $r_A = \frac{Cov(A,B)}{\sqrt{\text{var}(A)\ \text{var}(B)}}$.

Because the Proc Mixed procedure does not have a multivariate extension like the GLM procedure has MANOVA, the components of covariance are obtained using the MANOVA type III SS/CP matrix for family effects from the GLM procedure.

Formulas for approximate errors of genetic correlations are only available for fairly simple designs. Roff and Preziosi (1994) proposed the use of a jack-knife method for use with any design, but it is intensive in its use of computer time. The simplified, though imprecise formula in Falconer (1989) is used as an approximation. It does not permit rigorous testing since the distributions of genetic correlations are not known.

$$SE(r_A) = (1-r_A)\, SE(h^2_x)\, SE(h^2_y)\, (h^2_x\, h^2_y)^{-1/2}$$

Thus, an error estimate on r_A is not available when either heritability is estimated to be zero.

For correlations within treatments, the number of observations decreases even more and hence the error increases. As a means of comparison, correlations among family means, which carry a far smaller error, were also calculated. The error on simple Pearson correlation coefficients is easy to calculate. For family mean correlations and phenotypic correlations, the errors are dependent on the sample size n and are equal to 1 / (n-3) (Neter et al. 1990).
Since correlations are not normally distributed, these errors must be interpreted with caution. For Pearson correlations, the z-distribution can be used to construct confidence intervals. For genetic correlations, non-parametric statistical tests should be made. Of greater interest, however, are the order of magnitude of the errors, and a qualitative evaluation of the differences.

2.7 Multivariate techniques

Least Squares Means for provenances and families within treatments were used as input for different multivariate methods. Cluster analysis (Cluster procedure) and canonical correlation (Cancor procedure) were used on a set (or subset) of traits across all environments or within environments, depending on the need. Performing separate analyses for each environment provides a means of maintaining a reasonable number of degrees of freedom (Gittins 1985).

RESULTS AND INTERPRETATION

1. GENERAL RESULTS

The Least Squares Means of different treatments are connected to form response functions that show visually how trait expression varies over environments. These response functions may therefore have odd, irregular (non-smooth) forms. Since responses may well be non-linear over such a large range of source variables (Knight 1970), a linear regression would hide rather than reveal information. If more levels of each environmental variable had been available, non-linear regression techniques could have been used.

1. 1 General patterns of response to environmental variables

The general patterns of response are illustrated with data of the 1997 experiment. The results of the 1996 experiment are very similar - except in absolute value, since the plants of the 1997 experiment were larger - and are shown in appendix 2. Pair-wise comparisons of treatment means without adjustment of the overall α are shown in appendix 4.

Figure 5 shows how height, diameter, biomass, shoot-root ratio, water-use efficiency (WUE) and nitrogen-use efficiency (NUE) responded to water and nitrogen levels in the environment, without considering the genetic differences among plants. General trends include:

- increased availability of water and nutrients resulted in increased growth (height, diameter, biomass) and shoot-root ratio
- drought induced increased WUE
- nitrogen shortage induced increased NUE and decreased WUE

Morphological traits (i.e. height, diameter, biomass and shoot-root ratio) responded little to increased water levels, except when the plants had high levels of nitrogen available. The growth limitations imposed on the plants by the nitrogen shortage were harsher than those imposed by drought. These same traits responded vigorously and positively to added nitrogen. At low water levels the response to N levelled off at higher N levels. At high water levels the response to N was approximately linear. This implies that below a certain level, water was a limiting factor in growth, but that above that level, nitrogen became the limiting factor.

The two resource-use efficiencies, WUE and NUE, likewise responded more strongly to increased nitrogen than to increased water. WUE responded to increased water regardless of the nitrogen level. Less negative values for carbon isotope composition ($\delta^{13}C$) are indicative of higher WUE. Thus, WUE increased with increasing drought. WUE decreased with decreasing N-levels, regardless of the available water. A nitrogen shortage, leading to a protein shortage in general and to a shortage in carbon-fixing enzymes specifically, will result in a decreased carboxylation capacity. A reduced photosynthetic rate will automatically result in a reduced WUE. To maintain the rate of C uptake would require an increased amount of substrate (i.e., an increased CO_2 concentration), for which the stomates would have to open more, resulting in more water transpired for the same amount of carbon fixed, which would also reduce WUE.

NUE was not very responsive to water availability. There was a slight decrease of NUE as drought increased. However, the response of NUE to nitrogen was considerable. As nitrogen shortage became more acute, the NUE of the plants increased steeply.

In this study the effect of available nitrogen (N) on trait expression was larger than the effect of available water (W). This may be due to the specific environmental conditions of the experiment, as it is easier to achieve extremes in N-levels (while keeping W-levels constant) than it is to vary W-levels (while keeping N-levels constant). This is because fertilisation using a liquid solution of fertilisers is only possible at the end of each drought cycle. It is unknown to what extent the water and nitrogen shortages in my experiment were representative of those in nature for seedlings in British Columbia.

As a result of the water-by-nitrogen interaction effect the response functions to water at different nitrogen levels were not parallel. The biologically more interesting question, though, is whether they intersect and whether the rank order change that this causes is significant. This was not the case for any single trait: for the 1997 data there were no intersections of the response curves. For the 1996 data there were a few, but a significant rank order change requires at least a significant difference on both sides and the comparisons in appendix 4 show that this was not the case. Therefore, the effects of water and nitrogen can be termed 'separable' at this level and can be defined individually.

In the 1996 experiment the differences among water levels were smaller. The larger size of the plants in 1997 made it easier to differentiate the water treatments in the nursery.

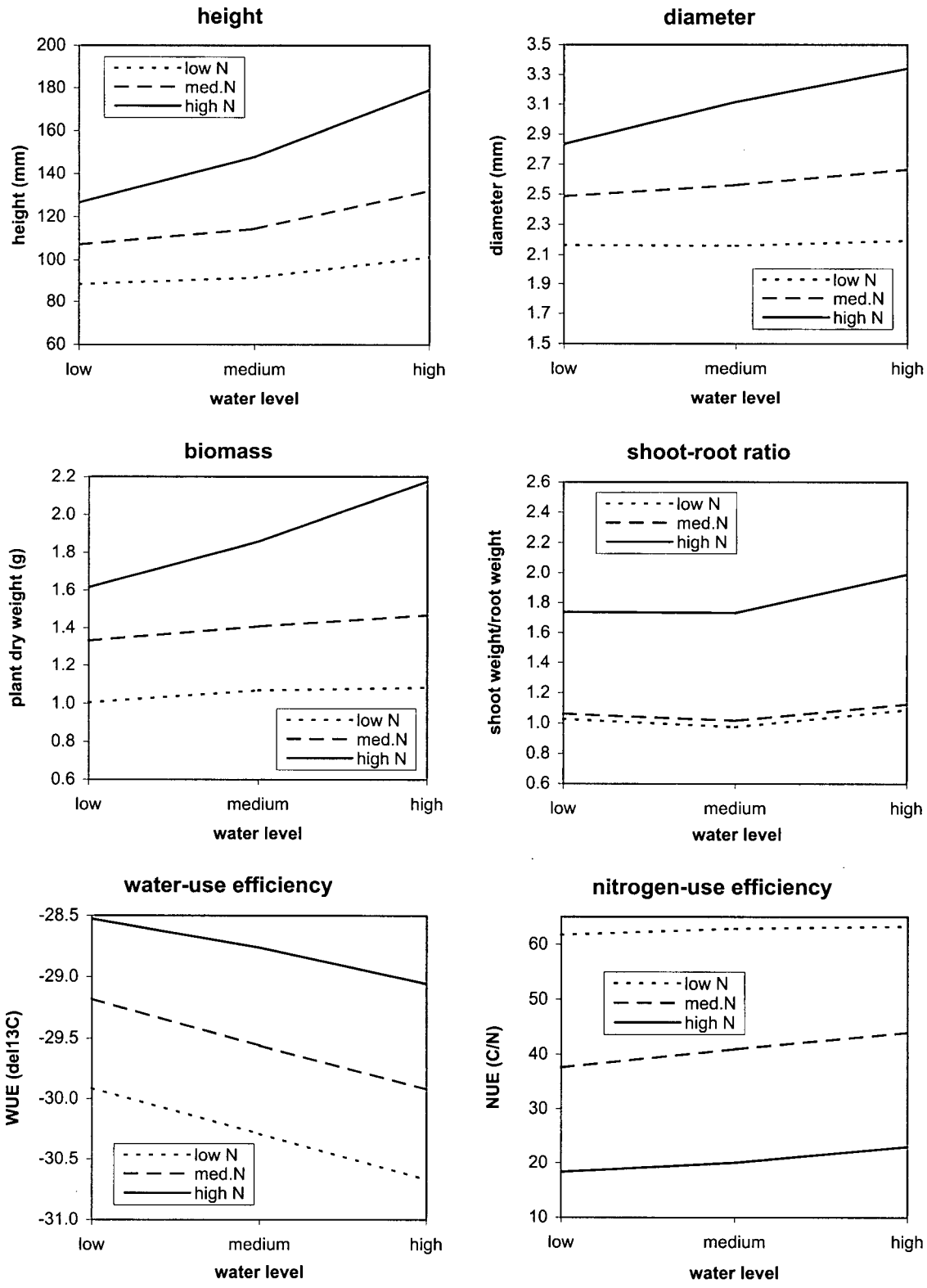

Figure 5. General patterns of response to water and nitrogen for the traits height, diameter, biomass, shoot-root ratio, water-use efficiency and nitrogen-use efficiency, based on data of the 1997 experiment.

1. 2 Environmental niches.

Nine distinct environments were created in the experiment, yet the average plant in the 1996 experiment did not show differences in growth for all nine of them. At low and medium nitrogen levels, the shortage of nutrients was so acute that, effectively, the plants were unable to make use of the additional amounts of water. As a result, the progeny of an average parent could distinguish less than nine environmental niches as far as growth was concerned. Considering the significant differences for individual comparisons of (1996) treatment means as in appendix 4, the following interpretations were made.

For height and diameter, five niches can be distinguished: one at low N (W_HN_L + W_MN_L + W_LN_L), a second at medium N (W_HN_M + W_MN_M + W_LN_M), and three at high N (W_HN_H, W_MN_H and W_LN_H separately). For biomass, four niches can be distinguished: one at low N (W_HN_L + W_MN_L + W_LN_L), a second at high N (W_HN_H + W_MN_H + W_LN_H), and two at medium N (W_HN_M separately and W_MN_M + W_LN_M combined). For shoot-root ratio, four niches can be distinguished: one at low N (W_HN_L + W_MN_L + W_LN_L), a second at medium N (W_HN_M + W_MN_M + W_LN_M), and two at high N (W_HN_H separately and W_MN_H + W_LN_H combined). Thus, considering all growth and allocation traits simultaneously, the following environments were perceived as separate niches: W_HN_H, W_HN_M, W_MN_H, W_LN_H, W_MN_M + W_LN_M, and W_HN_L + W_MN_L + W_LN_L. This yields a total of six niches.

Considering resource-use efficiencies, additional environments could be separated. WUE clearly distinguished the low water level from medium and high water levels at low N, i.e., it separated W_LN_L from W_HN_L + W_MN_L. Likely, it does the same at medium N, i.e. it separates W_LN_M from W_HN_M + W_MN_M, but since these treatments were less intensively sampled, the statistical power to differentiate these treatment means is lacking. Thus, as far as WUE is concerned, the plants perceive five niches: W_LN_L, W_HN_L + W_MN_L, (W_HN_M + W_MN_M + W_LN_M), W_HN_L, and W_HN_M + W_HN_H. NUE distinguished W_HN_L from W_MN_L + W_LN_L. As far as NUE is concerned, the plants perceive six niches: W_HN_L, W_MN_L + W_LN_L, W_HN_M + W_MN_M + W_LN_M, W_HN_H, W_MN_H and W_LN_H.

For all traits simultaneously then, the progeny of an average parent effectively perceived all 9 niches as different, assuming that all that is lacking to distinguish W_LN_M from W_MN_M is power. This is more than for each trait separately.

For the 1997 data, more pairwise comparisons are significant, but the addition of extra traits still results in the separation of more niches.

1.3 Sources of variation in the 1996 experiment

The results of univariate analyses of variance are shown in table 8. Tests of the fixed effects are given: water and nitrogen levels in the environment, provenance effects, family effects and their interactions. See pages 28-30 for the abbreviations of model terms.

Table 8. Tests of fixed effects for ten traits in the 1996 experiment. Effects that are significant at $\alpha = 0.05$ are in bold print. The first column of df is valid for the first eight traits, the second column for the last two traits (see p.30 for details about subsampling). The first three effects are tested against the mainplot error (df=27), the remainder against the subplot error (df=2476 for the first eight traits and df=255 for the last two traits).

Source	df_1	df_2	Pr > F									
			h	d	vol	hd	rt	sht	bm	srr	wue	nue
W	2	2	**0.0001**	**0.0108**	**0.0000**	**0.0000**	0.0799	0.0624	0.2905	**0.0148**	**0.0001**	**0.0001**
N	2	2	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0000**	**0.0000**	**0.0001**	**0.0001**
W*N	4	4	**0.0001**	**0.0000**	**0.0000**	**0.0021**	**0.0000**	**0.0001**	**0.0000**	**0.0248**	0.0884	**0.0001**
P	4	4	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0000**	**0.0000**	**0.0001**	**0.0001**
W*P	8	8	**0.0001**	**0.0000**	**0.0000**	0.1510	**0.0114**	**0.0001**	**0.0001**	**0.0317**	0.2035	**0.0103**
N*P	8	8	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0000**	**0.0000**	**0.0001**	**0.0001**
W*N*P	16	16	**0.0003**	**0.0003**	**0.0001**	0.2250	**0.0364**	**0.0018**	**0.0048**	0.0778	**0.0266**	0.2614
F(P)	93	15	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0000**	**0.0000**	**0.0001**	**0.0001**
W*F(P)	186	30	0.1517	0.0670	**0.0454**	0.9199	0.1442	0.1212	0.1448	0.6329	0.3289	0.1477
N*F(P)	186	30	**0.0001**	**0.0335**	**0.0031**	**0.0072**	0.0835	**0.0004**	**0.0157**	**0.0000**	0.4820	**0.0222**
W*N*F(P)	372	60	0.0613	0.2991	0.1971	0.0772	0.4001	0.2096	0.2818	**0.0236**	0.0791	0.1231

The environment in general had a significant influence on trait expression. The water by nitrogen interaction was significant for all traits except WUE. Both environmental factors (W and N levels) must thus be considered simultaneously and in the proper combinations in order to understand the changes in trait levels. For WUE, only the main effects of water and nitrogen were significant, but the interaction was not.

Provenances differed significantly for all traits, not only in overall mean (significance of P) but also in plasticity (significance of any of W*P, N*P, or W*N*P interactions). For height, diameter, volume, shoot weight, root weight, biomass and WUE, both environmental factors have to be considered simultaneously in order to understand the provenance differences in plasticity (the W*N*P effect is significant). For slenderness ratio only the plasticity with regard to N levels differed significantly between provenances. For shoot-root ratio and NUE the provenances differed in plasticity to both W levels and N levels, but response of a provenance to one factor did not vary significantly depending on the level of the other factor.

There was considerable variation among families for all traits even after provenance effects were accounted for. This pertains to family differences in overall mean (significance of F(P) for all traits) as well as family differences in plasticity (significance of any of W*F(P), N*F(P) or W*N*F(P) for all traits except root weight and WUE). For shoot-root ratio it is necessary to consider each combination of W and N levels before family response to either variable can be predicted. For height, diameter, slenderness ratio, shoot weight, biomass and NUE and within provenances, family performance depended only on available nitrogen. For volume, it depended on both available water and nitrogen but both factors acted independently of each other.

Whereas most of the variation is caused by the environment (table 9), genetic variation was significantly different from zero for all traits except NUE. The largest amount of variation is caused by the nitrogen treatments. Water availability has a relatively large effect on water-use efficiency, but not on other traits. The only other trait on which the overall effect of water is still fairly large is the slenderness ratio, i.e. the allocation of photosynthate to height growth versus diameter growth. Most of the GxE interaction variance is at the nitrogen*provenance level, except for WUE where there is an equal amount of variance for the interaction among water, nitrogen and families.

Table 9. Variance components as a percentage of total variance for ten traits in the 1996 experiment

Variance comp.	h	d	vol	hd	rt	sht	bm	srr	wue	nue
W	0.8	0.0	0.0	5.7	0.0	0.0	0.0	0.2	11.4	0.1
N	78.1	74.5	78.8	44.5	57.7	82.6	77.0	79.3	43.8	84.4
W*N	5.1	3.5	4.5	2.7	0.6	0.4	0.5	0.3	0.9	1.8
P	2.4	2.7	2.3	6.3	2.5	1.4	1.5	3.3	3.7	1.0
W*P	0.9	1.6	1.1	3.0	3.7	1.0	1.6	1.3	3.2	0.8
N*P	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	0.4
W*N*P	0.8	1.1	0.9	0.9	1.1	1.4	1.2	1.5	4.3	1.8
F(P)	0.2	0.3	0.3	0.1	0.3	0.2	0.3	0.1	1.2	0.1
W*F(P)	0.0	0.1	0.1	0.0	0.3	0.1	0.1	0.0	0.0	0.0
N*F(P)	0.4	0.2	0.3	0.7	0.4	0.4	0.3	0.6	0.0	0.5
W*N*F(P)	0.3	0.2	0.2	0.0	0.1	0.2	0.2	0.3	3.6	0.7
e_1	0.3	1.1	0.5	2.7	0.2	0.1	0.1	0.6	1.9	0.0
e_2	10.7	14.5	11.0	33.4	33.0	12.1	17.2	12.6	26.2	8.4

Figure 6 shows the relative amounts of V_P and $V_{F(P)}$ for several traits. In other words, it shows how genetic variance for mean performance was divided into among- and within- population variance. Slightly more than half of the genetic variation was among-population variance.

However, substantial amounts of variation were left within populations, even for WUE and NUE, which should, by definition, be strongly fitness-related. Genetic variation for plasticity was present at both the provenance and family levels, though it was somewhat smaller (roughly about half as large, data not shown) at the family level.

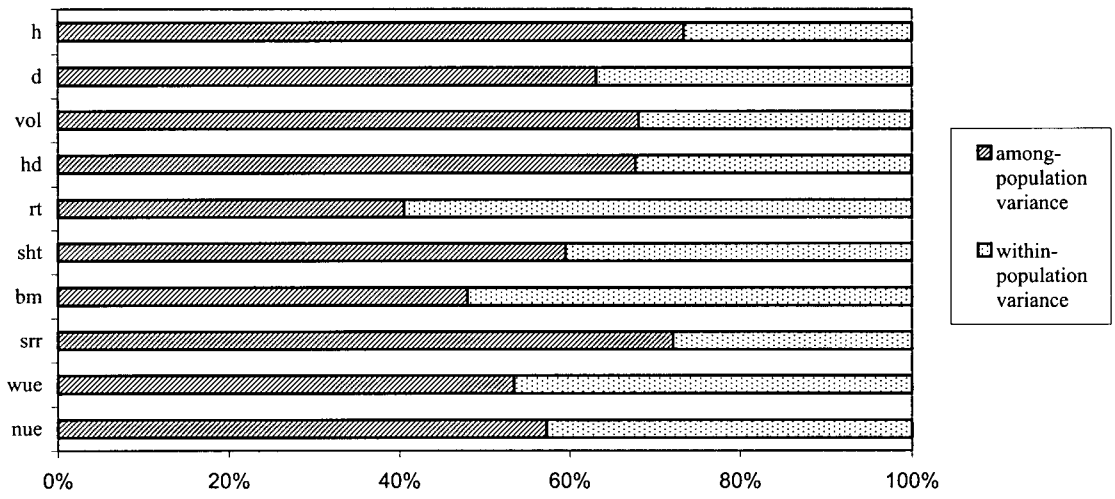

Figure 6. Relative amounts of among- and within- population variance

This was also the case for WUE, though for this trait the genetic differences in plasticity were not significant (table 8) due to the large error variation. This relatively larger error variation for WUE may indicate that water stress was not all that uniform for the four plants of the same family-by-treatment combination. This may be related to the limited success of applying the water treatments uniformly, as well as to differences in individual plant size: small plants would last longer with the same amount of water and presumably be less stressed than large plants. The latter would be true also at the family, provenance and subspecies level: larger families or provenances would be more water stressed and develop higher levels of WUE.

1.4 Sources of variation in the 1997 experiment

Whereas genetic variation in the 1996 experiment was divided over provenance and family variation, for 1997 genotypes no provenance component could be extracted, since there was no explicit provenance structure. When comparing the two experiments, the genetic effects of

provenance (P) and family within provenance (F(P)) are separated in 1996 and lumped together into the genetic effects of family (F) in 1997. Inclusion of the covariate initial height resulted in a better model with less influence of source elevation on growth traits. Table 10 shows the results of the univariate analyses of variance with tests of the fixed effects.

Table 10. Tests of fixed effects for ten traits, 1997 experiment. Effects that are significant at $\alpha = 0.05$ are in bold print. The first three effects are tested against the mainplot error (df=24), the remainder against the subplot error (df=2720 for the first eight traits and df=1231 for the last two traits).

Source	df	Pr > F									
		h	d	vol	hd	rt	sht	bm	srr	wue	nue
W	2	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
N	2	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
W*N	4	**0.0001**	**0.0001**	**0.0001**	0.2427	**0.0001**	**0.0001**	**0.0001**	0.2007	0.0972	**0.0001**
F	48	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
W*F	96	**0.0071**	**0.0021**	**0.0012**	0.0630	0.1426	0.1066	0.3466	**0.0121**	**0.0185**	0.4649
N*F	96	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
W*N*F	192	0.1534	0.5879	0.5780	**0.0405**	0.3212	0.1048	0.5491	**0.0035**	0.4475	0.3456
hi	1	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0015**	**0.0001**	**0.0001**

These results differed from those of the 1996 experiment in several ways. The main effects of water levels were significant for all traits. The differences between water levels emerged more clearly because the treatments were carried out with more precision. Due to a better start in a heated greenhouse the plants germinated more evenly. As a consequence, water treatments were probably more homogeneous among different cones of the same flat. Also, plants were larger in 1997, resulting in slightly shorter drought cycles (one day less on a total of 11 days per cycle on average) and a better separation of especially the medium and high water levels.

The W*N interaction effect was not significant for allocation ratios (srr and hd). Interaction for WUE was not significant in either experiment. In the first experiment, genotypes did not differ in their response to water for slenderness ratio and WUE. In the second experiment, genotypes differed in their response to water for WUE, but not for root weight, shoot weight, biomass and NUE. A three-way interaction effect W*N*genotypes was present only for the allocation traits (hd and srr). In the first experiment, it was found (though mainly at provenance level) for all traits except slenderness ratio and NUE.

As in the first experiment, the choice of genotypes revealed genetic differences in two important traits of interest: WUE and NUE. The range of water and nitrogen levels was large

enough for the expression of genetic differences in mean performance as well as in plasticity. Genotype-environment interaction was detected for all traits. Variance components are given as a percentage of total variance in table 11.

Table 11. Variance components as a percentage of total variance for ten traits in the 1997 experiment.

Variance comp.	h	d	vol	hd	rt	sht	bm	srr	wue	nue
W	13.2	3.1	7.1	14.9	5.2	3.7	4.3	2.2	10.8	1.2
N	49.3	67	67.7	8.3	29.1	72.9	64.3	68	53.3	93.5
W*N	5.3	4.1	2.8	0	2.8	1.4	2.2	0.1	0.1	1.7
F	8.3	3.2	5.1	15.4	6	4.1	3.9	5.3	5.4	0
W*F	0.3	0.4	0.3	0.1	0.2	0	0	0.1	0.7	0
N*F	1	0.8	0.7	2.1	3.7	1.3	2.2	0.7	3.8	0.5
W*N*F	0.1	0	0	1.1	0.1	0	0	0.6	0	0
replication	0.3	1	0.6	0.2	0.1	0.1	0	0.8	0.8	0.3
e_1	0.4	1.5	0.6	2.3	1.9	0.3	0.5	0.6	0.3	0.2
e_2	21.8	18.9	15	55.6	50.8	16.3	22.7	21.7	24.7	2.6

Compared with the first (1996) experiment, several differences are present. The variance component for the nitrogen main effect (N) is relatively smaller in the second experiment for all traits except WUE and NUE, where it is relatively larger. In the second experiment, the variance component for the water main effect (W) is relatively larger for all traits except WUE, where it is approximately the same. Genotypic variation for the mean (F) as well as for plasticity (W*F, N*F or W*N*F) is larger in the second experiment for all traits except for WUE and NUE, where it is smaller. Error variations are slightly larger (relatively speaking) in the second experiment for all traits, except WUE and NUE, where they are the same or a little smaller. This error variation, which was higher for root weight and slenderness ratio than for other traits in the first experiment, is high for these same traits in the second experiment also.

The former points indicate that a larger range was achieved for the water levels. This is mainly because of the increased plant size. However, the same increased plant size implies that the plants were developmentally at a later stage. This may have influenced the expression of WUE and NUE. Genetic variation for NUE was very limited, even if according to table 10 it was significant when the factor is considered fixed. Plasticity for NUE was considerable, but little genetic variation for plasticity existed. The lack of genetic variation for both the mean and plasticity of NUE may be a combined effect of the larger plant size and the absence of coastal families, which, in the 1996 experiment, proved to be the most nitrogen-use efficient ones.

Families, considered as a random factor, had a variance estimate significantly different from zero for all traits except NUE.

A factor for breeding zone was introduced into the model in an attempt to obtain a similar division of genetic variation into within- and among- provenance variation as for the first experiment. The two families from zones other than the TOA, TOD and SA were dropped for this analysis. The relative amounts of within and among- zone variation are shown in appendix 3. These zones are very different in moisture level, but moisture level is not the only factor responsible for genetic variation, as among-zone differences constitute only a small part of the variation. The factor 'breeding zone' was dropped from further analyses, since I considered it artificial rather than indicative of any population structure.

1.5 Heritability in the 1996 experiment

Heritability estimates the proportion of the phenotypic variance that can be translated into gain. Depending on the environment where selection takes place, the heritability can be calculated across all treatments or within treatments. Both calculations were made. The traits were transformed as shown in table 6. The model was run on the complete data set with families and their interactions as random terms. For heritability estimates within each of the nine treatments, the same transformations were used to remove any scale effects and to ensure that the estimates are comparable with the across-treatment estimates. The resulting estimates and their standard errors are shown in table 12. Since the provenances were widely different, it is assumed that restrictions with regard to climatic adaptation would apply and the genetic gain from provenance selection was not considered.

The REML method of the Mixed (SAS) procedure does not allow estimates of variance components to take negative values: such estimates are set to zero. When the family variance component is estimated as zero, the heritability estimate is also zero, and no error estimate is available. A few estimates of h^2 are larger than one. This is due to the large sampling error, which is especially high for WUE and NUE, as there were only an average of 50 observations within each treatment. For morphological traits, there were approximately 370 observations within each treatment, resulting in a smaller error.

The estimates of individual heritability across all treatments were rather low due to the effect of GxE interaction variance (in this case, family* treatment) in the denominator. This

estimate can only be considered suitable for predicting gain if the same set of genotypes will be used for all field environments and if nursery environments are representative of these field environments and contribute in exactly the same proportions, 1/9 each. This is not likely to be a realistic scenario.

Table 12: Across-treatment and within-treatment individual heritability estimates (h^2) for ten traits, 1996 experiment. A standard error on the across-treatment estimate (s.e.), an average of the nine within-treatment estimates (avg.) and an average standard error of the within-treatment heritability estimates (avg.s.e.) are also given.

	h	d	vol	hd	rt	sht	bm	srr	wue	nue
across-treatment	0.32	0.40	0.37	0.35	0.42	0.31	0.36	0.39	0.38	0.33
s.e.	0.06	0.07	0.07	0.06	0.07	0.06	0.07	0.07	0.20	0.19
$1 = W_HN_H$	0.56	0.61	0.59	0.58	0.74	0.51	0.58	0.79	0.00	0.94
$2 = W_HN_M$	0.50	0.40	0.46	0.38	0.35	0.18	0.27	0.20	0.82	0.03
$3 = W_HN_L$	0.22	0.20	0.21	0.07	0.00	0.00	0.00	0.28	1.05	0.73
$4 = W_MN_H$	0.68	0.86	0.91	0.44	1.02	0.98	1.01	0.80	0.46	2.06
$5 = W_MN_M$	0.92	0.60	0.73	0.87	0.44	0.60	0.52	0.54	1.84	0.34
$6 = W_MN_L$	0.07	0.00	0.01	0.10	0.09	0.03	0.05	0.31	0.79	0.18
$7 = W_LN_H$	0.58	0.58	0.49	0.80	0.82	0.87	0.87	0.75	0.71	1.01
$8 = W_LN_M$	0.77	0.82	1.10	0.00	0.85	0.57	0.70	0.85	0.00	0.00
$9 = W_LN_L$	0.40	0.47	0.46	0.15	0.41	0.38	0.38	0.98	1.68	1.21
avg. (1-9)	0.52	0.51	0.55	0.38	0.52	0.46	0.49	0.61	0.82	0.72
avg.s.e. (1-9)	0.21	0.19	0.21	0.18	0.19	0.19	0.19	0.21	0.59	0.68

The most stressed treatment, W_LN_L, always had a low heritability except for shoot-root ratio, WUE and NUE. Furthermore, nitrogen shortage had a detrimental effect on the expression of genetic variance of growth traits. Regardless of the water level, a decrease in N-level resulted in a lower heritability. For both resource-use efficiencies, genetic differences were expressed best in the most stressed environment (W_LN_L). The largest estimate for height occurred in W_MN_M. The largest estimate for biomass occurred in W_MN_H. Considering all traits jointly, no simple rule could be made that identifies one environment as being ideally suited for selection.

1.6 Heritability in the 1997 experiment

Compared those of 1996, the heritability estimates for 1997 were more precise for resource-use efficiencies (larger sample size) but less precise for growth traits (fewer families). They were, however, biased upwardly. Firstly, they were based on a group of genotypes that did not

constitute a random-mating population (they lacked an explicit provenance component whereas this component was implicitly present due to the large area sampled). Secondly, the coefficient of relationship was not adjusted downwards (p.36). Thirdly, effects of seed weight (not measured) and other maternal effects beyond seed weight (Dormling and Johnsen 1992; Johnsen and Skroppa 1996) would have resulted in larger differences between families and smaller differences within families than expected based on genetic similarity alone. As such, the estimates were artificially high and should not be used to calculate genetic gains. Within-treatment estimates were still calculated in order to evaluate the pattern of change over environments (table 13), but the results were very different from those of 1996. This may be partly due to leaching of nitrogen in the high-W treatment. There was no common pattern of change across treatments for heritabilities of growth traits. The heritability in the average treatment (W_MN_M) was about average (as opposed to large in the 1996 experiment), while the estimates for the treatment with maximum stress (W_LN_L) were relatively high (as opposed to very low in 1996). Stress did seem to bring out genetic differences in this experiment, in contrast to the first experiment. For height and volume, nitrogen stress resulted in higher heritability estimates. For diameter, an average environment (W_MN_M) resulted in the strongest expression of genetic differences. Differences in shoot weight and biomass were revealed best in the dry but N-rich environments (W_MN_H and W_LN_H).

Table 13. Across-treatment and within-treatment individual heritability estimates (h^2) for ten traits, 1997 experiment. A standard error on the across-treatment estimate (s.e.), an average of the nine within-treatment estimates (avg.) and an average standard error of the within-treatment heritability estimates (avg. s.e.) are also given.

	h	d	vol	hd	rt	sht	bm	srr	wue	nue
across-treatment	1.08	0.60	1.02	0.83	0.50	0.71	0.57	0.74	0.71	0.18
s.e.	0.17	0.12	0.17	0.15	0.11	0.13	0.12	0.14	0.14	0.07
1 = W_HN_H	1.09	0.69	0.76	1.24	0.75	0.95	0.94	0.64	1.21	0.36
2 = W_HN_M	1.2	0.68	1.16	0.63	0.65	0.94	0.77	0.8	0.96	0.3
3 = W_HN_L	1.51	0.96	1.44	1.25	0.89	0.91	0.77	1.21	0.71	0.4
4 = W_MN_H	0.93	0.81	1.01	0.51	0.7	1.26	1.12	0.87	1.01	0.89
5 = W_MN_M	1.23	1.15	1.35	0.95	0.73	0.94	0.84	0.66	1.31	0.6
6 = W_MN_L	1.59	0.68	1.24	1.29	0.78	0.9	0.74	1.1	1.27	0.43
7 = W_LN_H	1.19	0.56	1.09	0.61	0.96	1.15	1.08	1.08	1.21	0.68
8 = W_LN_M	1.25	0.41	0.94	0.84	0.38	0.81	0.49	0.88	1.17	0.4
9 = W_LN_L	1.85	0.92	1.44	1.56	0.94	0.9	0.88	1.17	1.41	0.75
Avg. (1-9)	1.31	0.76	1.16	0.99	0.75	0.97	0.85	0.93	1.14	0.53
Avg.s.e. (1-9)	0.25	0.22	0.24	0.23	0.22	0.24	0.22	0.23	0.32	0.30

For root weight, allocation traits (slenderness ratio and shoot-root ratio) and NUE, there was no pattern in the changes across environments. The heritability of WUE was fairly stable across environments, but was relatively low in W_HN_L.

Such profound changes in the pattern of expression of genetic differences from one experiment to another do raise questions. A number of factors differed between the two experiments and could be responsible for these changes. The genotypes differed between experiments. The second experiment had a 'provenance' effect that could not be separated out. Also, there were differences in early test environment and in the weather of the year. Finally, different developmental stages of the plants may have played a role in the expression of genetic variation. However, until the role of these factors is better understood, it is impossible to make any recommendations for the choice of testing environments.

Within-treatment heritabilities for height, biomass, WUE and NUE were plotted against environments (figure 7). For 1996, the errors on the estimates for WUE and NUE are too large and no pattern could be distinguished, so they were not plotted. One estimate for biomass larger than one has been set to one. For 1997 estimates, all four traits were plotted and no estimates larger than one were set to one, since that would obscure any pattern.

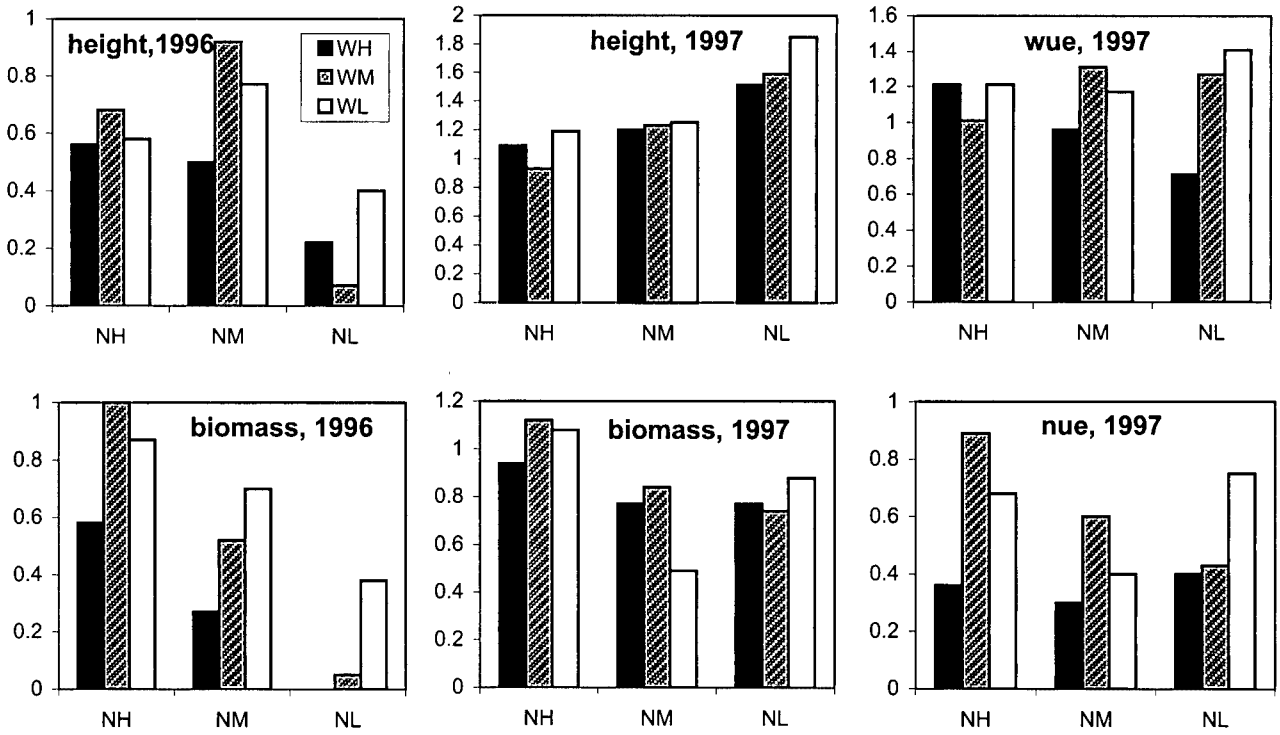

Figure 7. Within-treatment heritability as a function of the environment.

2. GENETIC VARIATION, GENOTYPE-ENVIRONMENT INTERACTION AND RANK ORDER CHANGE

In this section provenance and family responses from the 1996 experiment are evaluated and tested for rank order change. Family responses from the 1997 experiment are evaluated for rank order change and compared to field data.

2.1. Provenance response

First, some caveats to enable a proper interpretation of the results. The five provenances of the 1996 experiment represent two of the four subspecies or geographical races of *Pinus contorta*, namely ssp. *latifolia* (Rocky Mountain lodgepole pine; interior provenances) and ssp. *contorta* (shore pine; coastal provenances). These subspecies have markedly different evolutionary histories and profound differences should be expected. Provenances also differ genetically due to differences in source elevation and continentality. This resulted in large variations in germination speed, which were only partially removed by seed stratification. As a consequence, provenances already differed in size before treatments started. This should be considered at the time of interpretation. The plants were too small to measure their heights before the start of treatments with any reasonable accuracy and then use initial height as a covariate to correct for these unwanted effects. Neither was seed weight available to use as a covariate. The small seed size of the coastal provenances Squamish and Qualicum resulted in smaller plant sizes initially, although this was largely offset by their continued growth into September: as coastal provenances they set bud much later in the season and so were able to accumulate more height growth.

With these limitations in mind, consider the figures in appendix 5, showing response functions for each of the provenances. Appendix 6 shows the provenance means and their rank in each environment, as well as the significant differences according to a multiple range test. For growth traits, differences became smaller in the stressed environments and provenances were harder to distinguish there. For the resource-use efficiencies, there was a lack of power to clearly distinguish the provenances due to the small sample sizes.

The tallest provenances were Salmon Arm and Qualicum. They did not differ significantly. The shortest one was usually Kamloops. This was largely determined by germination speed and bud set. Plants from Salmon Arm, Qualicum and Revelstoke achieved

the largest diameter. The same provenances also had high volumes. However, plant shape differed between high-elevation and low-elevation provenances: the slenderness ratio was highest for the provenances from low elevations, and the high elevation interior sources of Kamloops and Revelstoke produced more sturdy plants with relatively thicker stems.

Biomass was highest for Qualicum and Salmon Arm. Kamloops, Squamish and Revelstoke plants had rather small values for dry weight.

Allocation of photosynthate to roots and shoots differed considerably. Plants from Kamloops, the source receiving the least precipitation, had a relatively larger root system. Plants from Squamish had large shoots relative to the root size. Coastal and interior provenances separated clearly for shoot-root ratio.

For WUE, Kamloops was the most water-use efficient provenance under low and medium nitrogen (except in W_LN_M). Salmon Arm was the most water-use efficient provenance under high nitrogen, regardless of available water. Kamloops responded less to nitrogen, whereas Revelstoke was more plastic with regard to nitrogen. As far as the coastal provenances are concerned, the differences may not be significant but Qualicum was, in most environments, more water-use efficient than Squamish.

As far as nitrogen-use efficiency is concerned, Squamish was most efficient at high nitrogen levels and Qualicum at medium and low nitrogen levels. Here again, response curves differed for the provenances, lines were not parallel, and there was some rank interaction. Apparently, the trade-off for the higher WUE of Kamloops is a lower NUE.

2.2. Provenance rank order change

The response curves in appendix 5 show intersections, yet these may be caused either by estimation errors or by real crossovers. To test this, asymmetric Azzalini-Cox tests were carried out for genotype rank change for each of the ten traits. Azzalini-Cox tests were also carried out for environment rank change. The number of quadruples for which t is exceeded is given in table 14. As soon as there is one quadruple with a significant crossover, the effects are not separable and there is rank change. Numbers larger than one (for genotype rank change) indicate either that more than two genotypes intersect, that their response planes intersect more than once, or simply that the intersection of two planes is significant at multiple points. Thus, this number does not have a clear meaning, but it does indicate that the data underneath contain more

detailed information that can be analysed. Genotype rank order change (the one most relevant to the breeder) is analysed in detail for a few traits in appendix 7. Different genotypes change rank and different environments are responsible for the rank change for each trait. From these detailed results can be read which genotypes change rank, which I did by means of example for biomass, shoot/root ratio, WUE and NUE. The figures in appendix 8 illustrate that the intersection of response surfaces can be complex.

For biomass, two response surfaces intersect according to the Azzalini-Cox criterion: that of Kamloops and that of Squamish. These two genotypes change rank over environments. Other rank order changes for biomass are not significant, that is, they are thought to be a result of estimation errors rather than real rank order changes. For shoot-root ratio, Salmon Arm and Qualicum change rank according to the Azzalini-Cox criterion, and Revelstoke and Qualicum change rank according to the interaction-wise criterion.

Table 14. Tests for provenance and environment rank order change in the 1996 experiment. Number of quadruples (out of a total of 360) with significant rank order change as indicated by three criteria, using the t-values from table 7. Only a value of zero indicates separability. Overall inseparability can be one-way (G or E rank change) or two-way (G and E rank change).

	Genotype rank change (separability of G effects) according to 3 criteria			Environment rank change (separability of E effects) according to 3 criteria			Overall separability (of G and E effects)
	t_{azzcox}	t_{comp}	t_{inter}	t_{azzcox}	t_{comp}	t_{inter}	t_{azzcox}
h	0	9	24	0	4	4	yes
d	0	0	2	0	0	0	yes
vol	1	4	12	0	0	4	no
hd	0	0	0	0	0	3	yes
rt	0	6	13	0	0	5	yes
sht	2	12	16	0	0	2	no
bm	2	3	8	0	2	4	no
srr	3	6	16	0	0	2	no
wue	1	9	15	1	1	4	no (2-way)
nue	4	19	39	0	0	6	no

For water-use efficiency, Kamloops and Salmon Arm change rank according to the Azzalini-Cox criterion. Revelstoke changes rank with both Qualicum and Squamish according to the interaction-wise criterion. For nitrogen-use efficiency, Salmon Arm changes rank with Kamloops and Revelstoke according to the Azzalini-Cox criterion. Furthermore, Kamloops and

Revelstoke change rank according to the comparison-wise criterion. Salmon Arm and Qualicum change rank with Squamish according to the interaction-wise criterion. A statistician will only accept the Azzalini-Cox criterion to be proof of rank order changes. A breeder may choose to avoid rank changes for any of the three criteria.

Most of these rank changes are with regard to both water and nitrogen levels simultaneously, though nitrogen plays a bigger role than water, but then, nitrogen had a larger effect on all traits. To separate environments into groups without rank order change may thus be quite difficult in practice.

There are more rank changes for shoot-root ratio and resource-use efficiencies than for growth traits, despite the fact that the larger error on resource-use efficiency traits reduces the chance of finding significant differences there. The largest number of rank order changes at the provenance level occurs for NUE.

2.3 Family response

Table 8 showed significant genetic differences among families within provenances with regard to overall means (all traits) and plasticity (most traits). A plot of the range of response against the mean response for heights of individual families (not shown) revealed that the best average performers are also more plastic. Since the heights of different families are fairly similar in the stressed treatments, the plants from the W_HN_H treatment would determine both the range and, to a large extent, the mean. As such, a positive relationship between the mean height and the range of heights for a family (an indicator for plasticity or responsiveness) is to be expected. A similar plot for WUE and NUE, however, revealed no relationship between mean and plasticity.

A second indicator was Shukla's stability variance (Shukla 1972). This measure describes deviation from an average response, eliminating the influence of variable size in each environment. Mean and stability variance were plotted against each other for height (figure 8). For Kamloops (A), the most deviant families are the ones with lower means. For Salmon Arm (B), the opposite is true: the more deviant families have higher means.

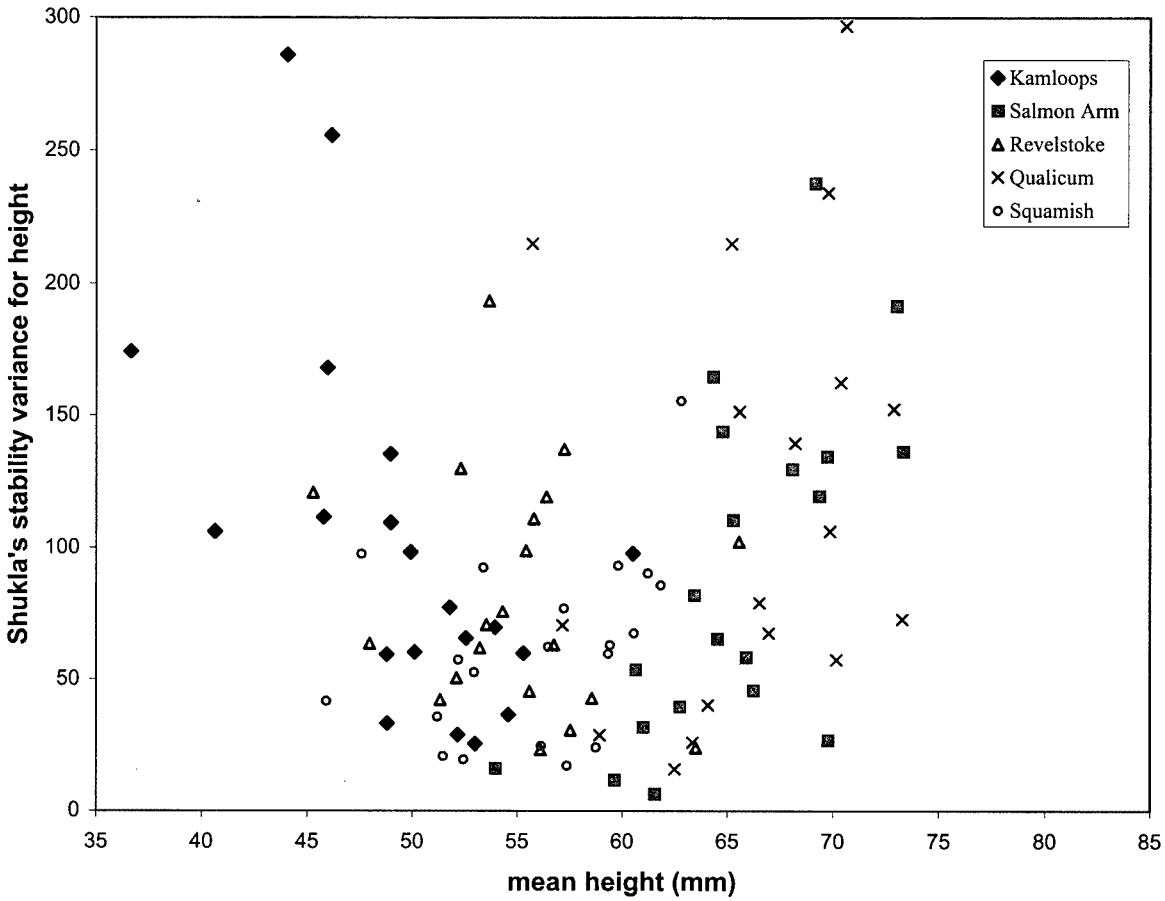

Figure 8. Plot of Shukla's stability variance against mean trait expression for height.

2.4 Family rank order change in the 1996 experiment

Very few rank changes among families were statistically significant according to the Azzalini-Cox criterion. The power of the test decreases with an increase in the number of families and environments. Only those 20 families which were subsampled for WUE and NUE and for which all 10 traits were available, were considered for this analysis, so that the power of the test would be equal for all traits. Those rank changes that are significant are almost invariably rank changes of families across provenances. When less stringent criteria are used (i.e., comparison-wise and interaction-wise t-values, respectively), the preponderance of across-provenance family rank changes gradually disappears, until there are roughly an equal number of across- and within-provenance rank changes. This pattern held for all traits investigated.

Table 15. Testing for family and environment rank order change in the 1996 experiment.

Number of quadruples (out of a total of 6840) with significant rank order change as indicated by three criteria, using the t-values from table 7. Only a value of zero indicates separability. Overall inseparability can be one-way (G or E rank change) or two-way (G and E rank change).

	Genotype rank change (separability of G effects) according to 3 criteria			Environment rank change (separability of E effects) according to 3 criteria			Overall separability (of G and E effects)
	t_{azzcox}	t_{comp}	t_{inter}	t_{azzcox}	t_{comp}	t_{inter}	t_{azzcox}
h	0	122	442	0	16	100	yes
d	2	57	296	0	7	66	no
vol	2	110	420	0	16	77	no
hd	0	8	125	0	9	134	yes
rt	0	64	348	0	9	134	yes
sht	0	123	460	0	12	85	yes
bm	3	111	478	0	11	110	no
srr	0	98	466	0	11	80	yes
wue	3	84	400	0	35	209	no
nue	3	140	523	0	19	113	no

2.5 Family rank order change in the field and in controlled environments

For the 1997 families, Shukla's stability variance was calculated for field as well as controlled environments. Stability variance in the field, whether based on performance in six field sites or on mean performance in two breeding zones, could not be related to stability variance in the controlled environments of the nursery. Genotypes were grouped to minimise rank order change as proposed by Truberg (1996). Again, groupings based on field growth could not be related to groupings based on growth in controlled nursery environments. Either these techniques are not powerful enough or the many more environmental factors operating in the field and their specific levels make it impossible to achieve such a correspondence.

2.6 Cluster analysis: similarity between families of one provenance

Cluster analysis was used to investigate whether families group into their respective populations based on the pattern of response of their growth and physiological traits. Families within the same population are expected to be more similar than families from different populations, because the populations come from very different environments and some degree of differential

adaptation of populations to their respective environments is expected to have taken place. Thus, the predicted tree would have five clusters, one for each population.

The response pattern of each trait is defined by nine points, one point for each treatment. Six traits were considered: height, diameter, root weight, shoot weight, WUE and NUE. These six traits in each of nine environments can be regarded as a set of 54 separate but correlated traits. Since there were only 20 families for which all six traits were available, the data set consists of 20 observations, one for each family, and 54 variables. The clustering procedure (SAS) was used to answer the question: which families are alike, not only in the way they react in one single environment, but in the way they react to a range of environments. Traits were standardised in order to give equal weight to all traits and environments. The distance measure used was the squared Euclidean distance. Different clustering methods were used: nearest neighbour (simple linkage), farthest neighbour (complete linkage), average linkage, centroid linkage, and Ward's minimum variance method.

The cluster methods of the nearest neighbour (simple linkage), average linkage, and the centroid method all resulted in classification trees that don't show clear clusters at all (data not shown). The farthest neighbour (complete linkage) method and Ward's minimum variance method resulted in trees that seemed to have three clusters, but the two trees differed from each other (figures 9 a and b). The families of Kamloops and Revelstoke definitely cluster together and seem to be different from the others. Both of these seed sources are from higher altitudes, resulting in a shorter growing season. The families from Salmon Arm are at the opposite end of the scale, very different in characteristics from the families of Kamloops-Revelstoke. Two of the Qualicum families also belong in this Salmon Arm group. The Squamish families are intermediate in characteristics. The two other Qualicum families group with them.

The families from Squamish and Qualicum are somewhat intermediate, and do not form really distinct groups. Families within populations vary so widely in their response to a range of environments that the cluster groupings are unstable, i.e. dependent on the method and distance measure used, and cannot be considered real groups.

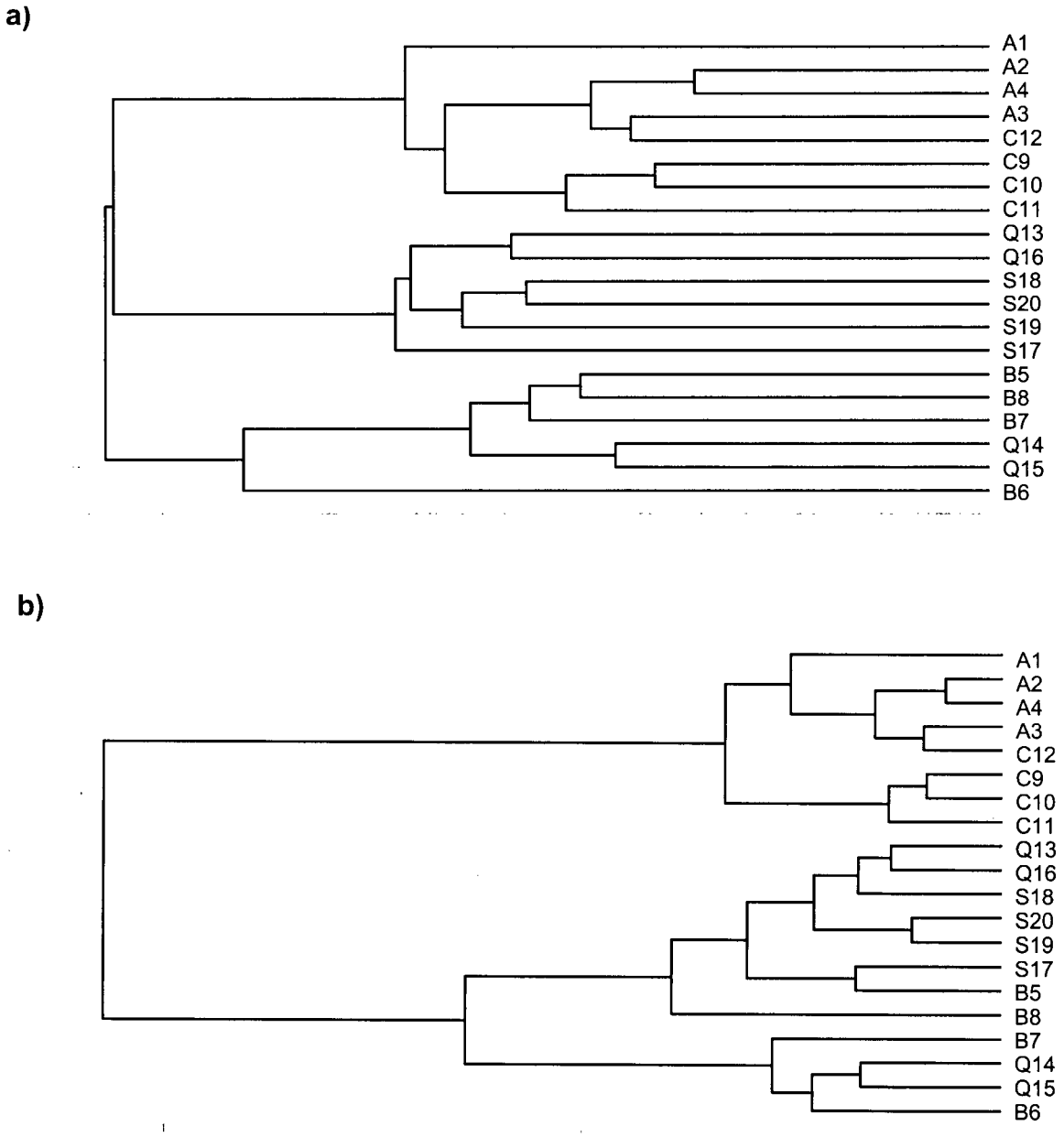

Figure 9. Trees resulting from clustering 20 families based on multi-trait multi-environment performance using the complete linkage method (a) and Ward's Minimum variance method (b).

3. ADAPTATION TO SOURCE ENVIRONMENTAL VARIABLES

The performance of each of the 49 families from the 1997 experiment was related to source environmental variation to detect patterns of correlation, which may reflect adaptation. The relationship between resource-use efficiencies and climate was of primary interest but other growth variables as well as the relationship with elevation were also investigated. A visual inspection of simple plots of trait values against source variables for all families did not suggest any specific relationships, so a simple correlation analysis (i.e. a search for simple linear relationships) was carried out. Though this data set seems ideal for canonical correlation analysis, there are far more traits (10 traits in 9 environments) and climate variables (9) than there are observations (49 families). A suitable selection of traits and variables has to be made beforehand. Results from the correlation analysis help guide this selection.

3.1 Source environmental data

Two types of source environmental data were available. Firstly, there were the estimates derived from the climate model developed in Rehfeldt et al. (1999). Secondly, estimates were available for each of the biogeoclimatic (BGC) subzones in the Kamloops forest region (Lloyd et al. 1990). Variables from the Rehfeldt et al. (1999) model that were used include the mean annual temperature (MAT), the frost-free period (FFP), the number of frost-free days (NFFD) and the difference between the mean temperatures of the hottest and coldest months (TDIFF=MTWM-MTCM). The estimates from their precipitation models were not used, since they are known to have only moderate predictive value. Lloyd et al. (1990) provide temperature and precipitation estimates for each BGC subzone: mean annual temperature (SZMAT), mean annual precipitation (SZMAP), summer temperature (SUMT) and summer precipitation (SUMP). The geographical locations of the seedlots were plotted on a detailed BGC map (British Columbia Ministry of Forests 1998) to determine from which BGC subzone a seedlot originated. Appendix 9 provides, for each of the 49 families, annual and summer temperature and precipitation variables from both Rehfeldt et al. (1999) and Lloyd et al. (1990). New variables include the moisture level (MOIST), calculated as the ratio of SUMP over SUMT, similar to Rehfeldt et al. (1999), as well as a summer and an annual dryness index (SDI, ADI), as proposed

by Guy and Holowachuk (*in press*). See appendix 10 for the derivation of SDI and ADI. Most of the source environmental variables are fairly strongly correlated with each other (table 16), indicating there is a pattern: higher elevation locations have colder and wetter climates.

Table 16. Correlation between various climate variables and elevation (see text for abbreviations) Correlation coefficients[1] in bold print are significant at $\alpha = 0.05$.

	MOIST	SDI	ADI	SZMAP	SUMP	SZMAT	SUMT	MAT	FFP	NFFD	TDIFF	ELEV
MOIST [2]	**1**											
SDI [2]	**-0.78**	**1**										
ADI [3]	**-0.57**	**0.90**	**1**									
SZMAP [3]	**0.30**	**-0.60**	**-0.86**	**1**								
SUMP [2]	**0.60**	**-0.81**	**-0.80**	**0.80**	**1**							
SZMAT [2]	**-0.78**	**0.37**	0.13	0.19	0.01	**1**						
SUMT [2]	**-0.82**	**0.42**	0.16	0.17	-0.04	**0.98**	**1**					
MAT	**-0.68**	**0.82**	**0.62**	-0.15	**-0.34**	**0.62**	**0.63**	**1**				
FFP	**-0.69**	**0.80**	**0.61**	-0.15	**-0.31**	**0.64**	**0.66**	**0.98**	**1**			
NFFD	**-0.66**	**0.80**	**0.64**	-0.20	**-0.32**	**0.60**	**0.62**	**0.96**	**0.99**	**1**		
TDIFF	**-0.72**	**0.55**	0.27	0.01	-0.25	**0.71**	**0.71**	**0.61**	**0.64**	**0.57**	**1**	
ELEV	**0.73**	**-0.84**	**-0.65**	0.21	**0.38**	**-0.65**	**-0.65**	**-0.97**	**-0.98**	**-0.96**	**-0.73**	**1**

[1] Based on 49 data points, except for [2] and [3], which are based on 46 and 45 points respectively (see appendix 9).

3.2 Correlation of single traits with single source environmental variables

A correlation analysis was carried out for the variables from table 16 and the response of plants for seven traits. These traits are water-use efficiency, nitrogen-use efficiency, height, diameter, biomass, root weight and shoot/root ratio. Since it wasn't clear whether average response, treatment-specific response or range of response would reveal a clearer pattern, all three were investigated. The stronger patterns were presumed to be the more meaningful ones. The variables FFP, NFFD and ELEV were so closely correlated that only the relationships between traits and ELEV are represented in tables 17-20. Individual climate variables explained at best 30% of the existing variation in the performance of different families.

3.2.1 Results for WUE

The correlation between WUE and source moisture level (table 17) was significant only for WUE measured in the high nitrogen environments (W_HN_H, W_MN_H and W_LN_H). The best

relationship (r = -0.47) was obtained in a dry environment, W_LN_H. A good relationship (r = -0.45) was also obtained for the range of WUE and source moisture level.

Table 17. Correlation between water-use efficiency, as expressed in various experimental environments, and source environmental variables. Correlation coefficients in bold print are significant at $\alpha = 0.05$. Coefficients that are significant at $\alpha = 0.01$ and $\alpha = 0.001$ are indicated with * and ** respectively.

Trait	Exp.env.	MOIST	SDI	ADI	SZMAP	SUMP	SZMAT	SUMT	TDIFF	ELEV
wue1	W_HN_H	**-0.31**	0.10	0.07	0.02	0.04	**0.40***	**0.42***	0.20	-0.21
wue2	W_HN_M	0.14	-0.23	-0.17	0.04	0.11	-0.15	-0.08	-0.20	**0.29**
wue3	W_HN_L	0.19	**-0.32**	-0.29	0.17	0.16	-0.12	-0.12	-0.09	0.27
wue4	W_MN_H	**-0.30**	0.15	0.11	0.00	0.00	**0.36**	**0.40***	0.07	-0.21
wue5	W_MN_M	-0.24	0.03	-0.02	0.02	-0.08	0.21	0.24	0.03	-0.01
wue6	W_MN_L	0.05	-0.04	-0.05	-0.03	-0.11	-0.14	-0.14	-0.13	0.14
wue7	W_LN_H	**-0.47***	**0.35**	0.20	0.01	-0.11	**0.50****	**0.50****	**0.34**	**-0.48****
wue8	W_LN_M	-0.16	-0.11	-0.16	0.22	0.19	**0.32**	**0.34**	0.11	-0.01
wue9	W_LN_L	0.10	-0.22	-0.21	0.16	0.15	-0.01	-0.03	-0.06	0.13
mean wue	(1-9)	-0.22	0.00	-0.06	0.09	0.05	0.29	**0.32**	0.08	-0.07
range wue	(1-9)	**-0.45***	**0.37**	**0.32**	-0.10	-0.08	**0.51****	**0.51****	0.24	**-0.48****

The patterns were quite weak. The reason for this may be twofold. Moisture level may be an inaccurate indication of the level of drought that the plants at the source are subjected to. Though SDI is, in theory, a better measure, the relationships with SDI are even weaker. All of these variables are only as good as the climate data on which they are based, and the fact that SDI has to use data from two different models (MTWM and SUMP) may obscure the relationships. On the other hand, within-population variation for WUE may be very large, as was indicated in the 1996 experiment. A visual inspection of the plots for the corresponding regressions revealed a slight non-linearity, where the decrease of WUE with moisture regime levelled off at the highest levels of source moisture. However, introduction of additional non-linear terms in the regression equation was non-significant, and was therefore not pursued further.

The correlation coefficient was negative: families from dryer sources were more water-use efficient (as expressed by wue7, in W_LN_H) and had a larger plasticity (as expressed by the range) in response to water and nitrogen levels in the environment.

Temperature variables (mean annual and summer temperature of the subzone) were slightly better than moisture level at describing family variation. Again, the best relationships were obtained for W_LN_H (WUE as expressed in dry but N-rich environments) and the range of

WUE (r = 0.51). Length of growing season and elevation were likewise closely related to WUE. Adding extra source variables and performing multiple regression did not result in a better explanation of variation: subsequent reduction to significant variables resulted in the same simple regressions as before.

WUE showed no relationships with precipitation (MAP or SUMP). The relationships with ADI were weak. Further improvement might be obtained by using a summer moisture deficit variable (cfr. Thornthwaite 1948), as used by Campbell and Sugano (1979), who related flushing date to both temperature and moisture deficit. They chose their seedlots right next to existing climate stations. However, even with improved estimates for source moisture levels, the relationship between seedlot performance and source environment is likely to remain weak due to the large within-population variation for WUE as indicated by the 1996 experiment (figure 6).

WUE in W_LN_H and the range of WUE showed highly significant relationships with temperature variables as well as with elevation. Sources from low elevations with warm temperatures had higher water-use efficiencies and were more plastic, as expressed by the range of WUE. Since the climatic variables themselves are interrelated, it is not clear which variable is the critical factor.

The correlation coefficients between the range of WUE and climatic variables are as high as these for WUE in W_LN_H. Although WUE7 and the range of WUE are themselves related, this correlation was not very high (r = 0.60).

3.2.2 Results for NUE

Table 18. Correlation between nitrogen-use efficiency, as expressed in various experimental environments, and source environmental variables. Correlation coefficients in bold print are significant at α = 0.05. Coefficients that are significant at α = 0.01 and α = 0.001 are indicated with * and ** respectively.

Trait	Exp.env.	MOIST	SDI	ADI	SZMAP	SUMP	SZMAT	SUMT	TDIFF	ELEV
nue1	W_HN_H	0.16	**-0.31**	-0.26	0.28	**0.45***	0.14	0.12	0.09	0.05
nue2	W_HN_M	-0.18	0.11	0.08	0.02	0.02	0.23	0.25	0.26	-0.18
nue3	W_HN_L	0.10	0.08	0.11	-0.14	-0.04	-0.14	-0.11	0.01	-0.05
nue4	W_MN_H	**0.37**	**-0.48****	-0.26	0.07	**0.37**	-0.19	-0.19	**-0.44***	**0.45***
nue5	W_MN_M	-0.19	-0.03	-0.08	0.20	0.21	**0.43***	**0.39***	0.15	-0.14
nue6	W_MN_L	0.14	0.01	-0.03	0.01	0.01	-0.15	-0.15	-0.07	0.02
nue7	W_LN_H	0.22	**-0.50****	**-0.54****	**0.41***	**0.42***	0.02	0.01	-0.12	**0.39***
nue8	W_LN_M	-0.26	0.21	0.12	0.07	0.05	**0.39***	**0.37**	0.28	**-0.37***
nue9	W_LN_L	0.01	0.05	0.01	0.06	0.05	0.01	0.05	0.00	-0.04
mean nue	(1-9)	0.04	0.01	-0.02	0.08	0.16	0.09	0.11	0.07	-0.09
range nue	(1-9)	0.05	0.15	0.10	-0.06	-0.06	-0.09	-0.07	0.01	-0.10

No relationships were found for either the mean or the range of NUE. NUE as expressed in rich or medium rich environments (W_HN_H, W_MN_H, W_LN_H, W_MN_M and W_LN_M) did show some relationships with source variables. Families from moister source environments (indicated by SDI, ADI, MOIST, SZMAP or SUMP) have higher NUE as expressed in rich environments (W_HN_H, W_MN_H and W_LN_H), regardless of drought level. The strongest relationships were obtained for ADI ($r = -0.54$) and SDI ($r = -0.50$). NUE in medium-rich environments under some level of drought (W_MN_M and W_LN_M) was positively related to source temperature and negatively to source elevation.

3.2.3 Results for growth traits

Height, diameter, biomass and root growth were all weakly related to source environmental variables. Few of the correlation coefficients were significant at the 1 % or 0.1 % level. Some of the correlation coefficients that are significant at the 5 % level will be 'false positives', since no adjustment was made for the overall level of significance. The correlation coefficients are shown in table 19 and the results are briefly summarised below.

Height in general (regardless of planting environment) was positively correlated with temperature variables, especially SUMT, and, to a lesser extent, with source precipitation. Mean height across environments and height in any separate environment had similar relationships with source variables. The range of heights showed no significant relationships with source variables.

Diameter was positively correlated with precipitation variables. Families from high rainfall areas had larger diameters. Diameter in N-poor environments shows no relationships with source environmental variables. Diameter is either uncorrelated or positively correlated with source temperature, except as expressed in environment W_LN_M. The range of diameter growth is positively correlated with the length of the growing season.

The general results for biomass are very similar to those for height and diameter: warmer and wetter sources have genotypes with higher growth rates than colder and drier environments. There is a positive correlation between biomass with precipitation and temperature variables.

Root weight was positively correlated with source precipitation and moisture, especially in N-stressed environments. Root weight, especially as expressed in dry and medium- to poor-N environments (W_LN_M and W_LN_L), was negatively related to temperature and length of growing season, and positively to elevation.

Table 19. Correlation between growth traits, as expressed in various experimental environments, and source environmental variables. Correlation coefficients in bold print are significant at $\alpha = 0.05$. Coefficients that are significant at $\alpha = 0.01$ and $\alpha = 0.001$ are indicated with * and ** respectively.

Trait	Exp.env.	MOIST	SDI	ADI	SZMAP	SUMP	SZMAT	SUMT	TDIFF	ELEV
h1	W_HN_H	-0.14	-0.02	-0.15	**0.32**	0.27	**0.33**	**0.37**	0.15	-0.21
h2	W_HN_M	0.04	-0.01	-0.03	0.11	0.13	-0.01	0.06	-0.06	-0.03
h3	W_HN_L	-0.13	0.01	-0.11	**0.30**	0.19	0.25	**0.30**	0.25	-0.19
h4	W_MN_H	-0.14	-0.08	-0.16	**0.32**	0.29	**0.33**	**0.38***	0.03	-0.11
h5	W_MN_M	-0.04	-0.24	**-0.30**	**0.32**	**0.29**	0.19	0.26	0.06	0.12
h6	W_MN_L	-0.12	-0.01	-0.05	0.20	0.21	0.26	**0.33**	0.06	-0.14
h7	W_LN_H	-0.23	0.12	-0.06	0.26	0.10	**0.34**	**0.39***	0.12	-0.26
h8	W_LN_M	-0.21	-0.07	-0.16	0.28	0.25	**0.38***	**0.44***	0.10	-0.07
h9	W_LN_L	-0.20	-0.02	-0.08	0.17	0.14	**0.32**	**0.36**	0.10	-0.10
mean h	(1-9)	-0.15	-0.04	-0.15	**0.30**	0.25	**0.31**	**0.38***	0.11	-0.13
range h	(1-9)	0.01	-0.09	-0.18	0.25	0.24	0.16	0.15	0.09	-0.11
d1	W_HN_H	0.10	-0.06	-0.05	0.19	**0.30**	0.12	0.12	0.06	-0.15
d2	W_HN_M	0.17	-0.16	-0.11	0.14	0.27	-0.03	0.00	-0.10	0.04
d3	W_HN_L	0.11	-0.05	-0.04	0.13	0.22	0.05	0.06	0.10	-0.12
d4	W_MN_H	-0.21	-0.02	-0.14	**0.30**	0.18	**0.36**	**0.41***	0.15	-0.12
d5	W_MN_M	0.22	**-0.31**	**-0.31**	**0.30**	**0.33**	-0.06	-0.04	0.07	0.12
d6	W_MN_L	0.21	-0.22	-0.18	0.19	0.28	-0.03	-0.02	-0.07	0.06
d7	W_LN_H	-0.19	0.04	-0.03	0.21	0.15	0.28	0.36	0.18	-0.15
d8	W_LN_M	0.20	**-0.39***	**-0.32**	0.29	**0.39***	0.01	0.03	-0.27	**0.32**
d9	W_LN_L	0.21	-0.24	-0.17	0.14	0.27	-0.06	-0.05	-0.13	0.15
mean d	(1-9)	0.10	-0.20	-0.20	0.29	**0.36**	0.12	0.16	0.02	0.01
range d	(1-9)	-0.03	0.10	0.09	0.07	0.15	0.17	0.17	0.15	-0.26
bm1	W_HN_H	-0.04	-0.08	-0.12	0.23	**0.29**	**0.30**	0.27	0.13	-0.17
bm2	W_HN_M	-0.01	-0.11	-0.01	0.04	0.19	0.14	0.17	0.09	0.00
bm3	W_HN_L	-0.07	0.07	0.04	0.05	0.06	0.14	0.14	0.23	-0.18
bm4	W_MN_H	-0.10	0.02	0.10	-0.08	0.07	0.18	0.19	-0.02	-0.10
bm5	W_MN_M	-0.07	-0.20	-0.23	0.26	0.23	0.25	0.22	0.18	0.04
bm6	W_MN_L	-0.04	0.08	0.04	0.02	-0.03	0.05	0.05	0.20	-0.11
bm7	W_LN_H	**-0.30***	0.07	0.02	0.06	0.04	**0.41***	**0.42***	0.22	-0.19
bm8	W_LN_M	0.10	**-0.35**	-0.27	0.18	**0.30**	0.09	0.08	-0.02	0.24
bm9	W_LN_L	0.15	**-0.30**	**-0.32**	**0.32**	**0.31**	0.06	0.02	0.05	0.14
mean bm	(1-9)	-0.09	-0.10	-0.10	0.16	0.24	**0.31**	**0.30**	0.16	-0.09
range bm	(1-9)	-0.08	-0.02	-0.03	0.10	0.19	0.27	0.25	0.07	-0.16
rt1	W_HN_H	-0.03	-0.14	-0.24	**0.31**	0.27	0.27	0.22	0.12	-0.05
rt2	W_HN_M	0.07	-0.26	-0.23	0.15	0.25	0.12	0.08	0.12	0.13
rt3	W_HN_L	0.23	-0.21	-0.19	0.09	0.15	-0.14	-0.17	-0.03	0.15
rt4	W_MN_H	-0.15	0.03	0.00	0.05	0.11	0.28	0.27	0.08	-0.15
rt5	W_MN_M	0.00	-0.27	**-0.35**	**0.31**	0.22	0.19	0.12	0.19	0.12
rt6	W_MN_L	0.23	-0.13	-0.12	0.02	0.02	-0.24	-0.26	-0.08	0.16
rt7	W_LN_H	-0.01	-0.25	**-0.31**	0.27	0.26	0.21	0.18	0.02	0.12
rt8	W_LN_M	**0.33**	**-0.47***	**-0.38***	0.20	**0.33**	-0.16	-0.18	-0.16	**0.41***
rt9	W_LN_L	**0.40***	**-0.49****	**-0.46***	**0.34**	**0.38***	-0.21	-0.26	-0.15	**0.39***
mean rt	(1-9)	0.15	**-0.34**	**-0.36**	0.29	**0.33**	0.08	0.03	0.04	0.18
range rt	(1-9)	-0.29	0.12	0.05	0.08	0.05	**0.42***	**0.40***	0.18	-0.25

3.2.4 Results for shoot-root ratio

Shoot-root ratio showed the strongest relationships with source climatic variables of any trait. It was not related to precipitation, but was related to all other variables: moisture level, temperature, length of growing season and elevation.

Table 20. Correlation between shoot-root ratio, as expressed in various experimental environments, and source environmental variables. Correlation coefficients in bold print are significant at $\alpha = 0.05$. Coefficients that are significant at $\alpha = 0.01$ and $\alpha = 0.001$ are indicated with * and ** respectively.

Trait	Exp.env.	MOIST	SDI	ADI	SZMAP	SUMP	SZMAT	SUMT	TDIFF	ELEV
srr1	W_HN_H	-0.09	0.15	0.22	-0.09	0.07	0.18	0.21	0.08	**-0.29**
srr2	W_HN_M	-0.22	**0.33**	**0.35**	-0.14	-0.13	0.13	0.21	0.04	**-0.33**
srr3	W_HN_L	**-0.43***	**0.42***	**0.37**	-0.10	-0.17	**0.39***	**0.43***	**0.32**	**-0.50****
srr4	W_MN_H	-0.02	0.07	0.19	-0.16	-0.05	-0.04	-0.01	-0.08	-0.03
srr5	W_MN_M	-0.16	0.25	**0.35**	-0.18	-0.10	0.10	0.17	-0.03	-0.23
srr6	W_MN_L	**-0.46***	**0.30**	0.24	-0.02	-0.06	**0.51****	**0.54****	**0.41***	**-0.44***
srr7	W_LN_H	**-0.43***	**0.45***	**0.44***	-0.27	-0.28	**0.31**	**0.36**	**0.29**	**-0.44***
srr8	W_LN_M	**-0.40***	0.22	0.22	-0.05	-0.08	**0.42***	**0.44***	0.25	**-0.31**
srr9	W_LN_L	**-0.53****	**0.48****	**0.43***	-0.23	-0.27	**0.47***	**0.50****	**0.37***	**-0.53****
mean srr	(1-9)	**-0.36**	**0.37**	**0.40***	-0.19	-0.15	**0.32**	**0.37**	0.21	**-0.42***
range srr	(1-9)	0.06	-0.05	0.08	-0.06	0.17	0.07	0.07	-0.10	-0.07

The stronger relationships were found for shoot/root ratio as expressed under stress, i.e. either drought or nitrogen-shortage or both. The best results were obtained for shoot/root ratio as expressed in dry, poor (W_LN_L) environments (up to $R^2 = 0.30$).

Shoot-root ratio was negatively correlated with source moisture level. Families from dry sources had relatively higher shoot-root ratios when under W and N stress. There was no relationship when plenty of water and a medium amount of nitrogen were available. Shoot-root ratio was positively correlated with source temperature and length of growing season. Shoot-root ratio was negatively correlated with source elevation, i.e., families from high elevations have low shoot-root ratios. This is likely determined at least partly by the length of the growing season.

3.3 Canonical correlation between trait expression and source environment

Four traits were retained for canonical correlation analysis: WUE, NUE, biomass and shoot-root ratio. The climatic variables retained were annual and summer precipitation and temperature (SZMAP, SUMP, SZMAT and SUMT respectively). Since Holowachuk (1993) found that source temperature and source rainfall interact to produce an adaptive response for populations of lodgepole pine, and because correlations with a moisture index were lower than those with temperature in my study and in others (e.g., Rehfeldt et al. 1999), I kept precipitation and temperature variables separate.

Separate analyses were carried out for each experimental environment in order to maintain sufficient degrees of freedom (Gittins 1985). The source environmental variables were significantly correlated with genotype multi-trait performance in five of the experimental environments (Table 21). Only the first pair of canonical variates was significantly correlated and is presented in this table.

Table 21. Canonical correlation between four traits and climatic variables. Each column corresponds to an analysis for one treatment[1]. Cross-loadings, i.e. the correlations of the first canonical variate with the original variables of the opposite set, the canonical correlation and the test of significance are given.

	W_H			W_M			W_L		
	N_H	N_M	N_L	N_H	N_M	N_L	N_H	N_M	N_L
Traits									
bm	0.39	0.28	0.12	-0.01	0.33	0.03	0.30	0.04	-0.18
srr	0.39	0.41	0.47	0.05	-0.24	0.58	0.52	0.49	0.60
wue	0.41	0.34	-0.19	0.26	-0.04	-0.16	0.42	0.30	-0.13
nue	0.41	0.19	0.05	-0.40	0.39	-0.15	-0.28	0.32	0.07
Climate variables									
SZMAT	0.36	0.14	0.36	0.38	0.28	0.60	0.41	0.52	0.45
SUMT	0.36	0.22	0.41	0.42	0.19	0.62	0.45	0.54	0.49
SZMAP	0.13	-0.07	-0.19	-0.06	0.36	0.00	-0.31	-0.02	-0.24
SUMP	0.29	-0.03	-0.20	-0.30	0.42	-0.05	-0.36	-0.13	-0.27
Statistics									
Corr	0.55	0.52	0.53	0.55	0.61	0.64	0.61	0.55	0.61
$F_{16/126}$	1.82	1.63	1.13	1.77	1.90	1.53	1.92	1.48	1.90
Pr > F	**0.037**	0.071	0.336	**0.043**	**0.027**	0.102	**0.026**	0.121	**0.027**

[1] Treatments are indicated by their water (W) and nitrogen (N) levels: high, medium and low.

In a non-stressed testing environment (W_HN_H), the families from warm and wet sources grew better and used their resources relatively better. They had a high biomass, a high shoot-root

ratio, high WUE and high NUE. In the moderately stressed environment (W_MN_M), the families from warm and wet sources had a high biomass and NUE, but a low shoot-root ratio relative to families from cold and dry sources. WUE was only weakly related to source climate in this treatment.

In the three remaining environments where the canonical correlation was significant (W_MN_H, W_LN_H, W_LN_L) the correlation was between warm and dry (not wet) sources and nursery multi-trait performance. Families from warm and dry sources had a high WUE and a low NUE in high-N treatments. Their biomass and shoot-root ratio was high at low water levels and not related at medium water levels. In the most stressed treatment (W_LN_L), the families from warm and dry source environments had a large shoot-root ratio, but slightly lower biomass and WUE scores than families from cold, wet source environments.

The cross-loadings change substantially in size and sign from one treatment to another. Although these canonical correlation coefficients are larger than the simple correlation coefficients, some increase in R^2 is expected automatically by the inclusion of extra variables. Despite the fact that climate variables are correlated, it does not seem that the co-ordinated response of genotypes to climate follows a clear pattern. Thus, the dimensions of this response can not easily be reduced for practical use by the breeder.

3.4 Canonical correlation between resource use efficiencies and field performance

To investigate whether resource-use efficiencies can be used to predict field performance on stressed sites a canonical correlation analysis was carried out (table 22). Separate analyses were carried out for each experimental environment. Only the first pair of canonical variates was in some cases significantly correlated and is listed in the table. Cross-loadings between variables and canonical variates of the opposite set indicate the relative contributions of variables to the overall canonical correlation. Cross-loadings are considered more conservative and less inflated than within-set loadings and are more reliable for interpretation (LeMay 1997).

In three testing environments (W_MN_H, W_LN_H and W_LN_M) resource-use efficiencies were significantly correlated with genotype field performance. When nitrogen levels in the testing environment were high, a high WUE and a low NUE in the test environment resulted in a good field performance. Under medium nitrogen levels, a low WUE and a high NUE in the test environment were correlated with field performance. The trade-off between WUE and NUE (see

section 4 of the results) seems to play a role here: WUE can only be optimised when enough nitrogen is available.

When nitrogen levels were high, high WUE and low NUE were associated with better field performance at all sites, although for the Thompson-Okanagan sites (TO 1-3) the loadings were not as high as for the Shuswap-Adams sites (SA 1-3). Thus, while WUE was the main predictor, it worked best not for the very dry sites of the TO but for the slightly moister sites of the SA. When the nitrogen level was medium, the combination of a low WUE and a high NUE resulted in a good field performance of these families on some sites (SA1, SA3 and TO2) and a poor performance on other sites (TO1). The performance on sites TO3 and SA2 contributed little to the relationship between the two sets of variables.

When the water level was low, the cross-loading of WUE was larger in absolute value. When the water level was medium, the cross-loading of NUE was larger in absolute value.

The signs and values of these cross-loadings illustrate again how intricate the relationships between WUE, NUE, water levels and nitrogen levels are.

Table 22. Canonical correlation between resource use efficiencies and field performance at six planting sites. Each column corresponds to an analysis for one treatment[1]. Cross-loadings, i.e. the correlations of the first canonical variate with the original variables of the opposite set, the canonical correlation and the test of significance for a canonical correlation are given.

	W_H			W_M			W_L		
	N_H	N_M	N_L	N_H	N_M	N_L	N_H	N_M	N_L
Resource use efficiencies									
wue	0.40	-0.35	-0.44	0.28	0.19	0.42	0.43	-0.35	-0.17
nue	0.21	0.19	0.15	-0.44	0.42	0.02	-0.35	0.26	0.48
Field performance									
TO 1	0.18	0.11	-0.20	0.17	0.30	0.02	0.09	-0.18	-0.07
TO 2	0.33	0.33	0.03	0.25	0.22	-0.03	0.28	0.15	-0.06
TO 3	0.29	0.20	0.02	0.18	0.22	0.12	0.14	-0.09	0.04
SA 1	0.37	0.31	0.23	0.46	0.23	-0.12	0.42	0.22	0.09
SA 2	0.35	0.26	0.21	0.32	0.20	-0.23	0.39	0.09	0.05
SA 3	0.20	0.38	0.17	0.53	0.06	-0.09	0.49	0.20	-0.14
Statistics									
Corr	0.42	0.47	0.45	0.60	0.42	0.43	0.60	0.55	0.48
$F_{12/82}$	0.89	1.25	0.91	2.18	1.28	0.94	2.36	2.08	1.21
Pr > F	0.559	0.262	0.541	**0.020**	0.244	0.517	**0.012**	**0.028**	0.293

[1] Treatments are indicated by their water (W) and nitrogen (N) levels: high (H), medium (M) and low (L).

4. PHENOTYPIC INTEGRATION

The concept of phenotypic integration means different things to different authors, but it is used here in the sense of Schlichting and Pigliucci (1998, p.192): "(it) encompasses the genetic, structural and physiological bases of the correlation and co-ordination of traits." As such, it refers to both phenotypic correlations of one or more genotypes over a series of environments and to genetic correlations of a population (a group of genotypes) within an environment.

Phenotypic correlation coefficients contain a genetic as well as an environmental component. To separate the genetic effect, it is better to calculate a genetic correlation (genetic trade-off). To separate the effect of the environment, it is better to study the effect of treatments on a pair of traits point-by-point in the treatment space for a single or an 'average' genotype (plastic trade-off).

4.1 Trait correlations

Phenotypic (r_P) and genetic (r_A) correlations across environments were calculated for both experiments (tables 23-25) based on transformed[1] traits (in the case of r_P for the sake of conformity). Phenotypic as well as genetic correlations among size-related traits are fairly high and positive. Correlations between size traits and shoot/root ratio are somewhat smaller. Correlations with the resource-use efficiency traits are smaller still.

The phenotypic correlations are all positive except for those involving NUE. These are all negative, which seems counterintuitive, but it is a consequence of the huge influence of available nitrogen on both growth and NUE in opposite directions. The smaller plants are all growing in the more nitrogen-stressed treatments, where NUE is high. In the estimation of genetic correlation coefficients this influence of the environment is corrected for, and the resulting sign is therefore positive: the more efficient families do indeed grow better, as we would expect intuitively.

Phenotypic correlations tend to be higher than genetic correlations, except for WUE in the second experiment. The genetic correlations among size-related traits are positive.

[1] As a consequence, NUE, having been transformed to 1/NUE, effectively reflects "nitrogen use inefficiency" (NUiE). Correlations with NUiE have exactly the opposite sign as would be expected for NUE. To avoid confusion, a sign change was applied to all correlation coefficients involving NUiE, such that NUE in the table effectively indicates NUE and not NUiE. The same was done for the tables in appendix 11.

Table 23. Across-treatment phenotypic correlations among ten traits. Significant correlations (α =0.05) are in bold print. (1996 estimates top right, 1997 estimates bottom left).

	h	d	vol	hd	rt	sht	bm	srr	wue	nue
h		**0.91**	**0.97**	**0.79**	**0.80**	**0.93**	**0.91**	**0.79**	**0.59**	**-0.70**
d	**0.75**		**0.98**	**0.47**	**0.88**	**0.94**	**0.94**	**0.70**	**0.70**	**-0.71**
vol	**0.91**	**0.94**		**0.62**	**0.86**	**0.95**	**0.95**	**0.76**	**0.67**	**-0.72**
hd	**0.76**	**0.17**	**0.47**		**0.41**	**0.60**	**0.56**	**0.65**	**0.21**	**-0.43**
rt	**0.40**	**0.51**	**0.50**	**0.10**		**0.88**	**0.94**	**0.47**	**0.77**	**-0.60**
sht	**0.77**	**0.83**	**0.86**	**0.36**	**0.57**		**0.99**	**0.80**	**0.69**	**-0.75**
bm	**0.72**	**0.81**	**0.83**	**0.30**	**0.77**	**0.96**		**0.72**	**0.73**	**-0.72**
srr	**0.66**	**0.65**	**0.69**	**0.37**	-0.01	**0.81**	**0.62**		**0.43**	**-0.75**
wue	**0.56**	**0.61**	**0.64**	**0.27**	**0.44**	**0.65**	**0.64**	**0.48**		**-0.68**
nue	**-0.51**	**-0.66**	**-0.64**	**-0.15**	**-0.21**	**-0.68**	**-0.59**	**-0.68**	**-0.63**	

Table 24. Across-treatment genetic correlations among ten traits. (1996 estimates top right, 1997 estimates bottom left). Significant correlations are in bold print (α =0.05 using Chebychev's rule and the error estimates of table 25).

	h	d	vol	hd	rt	sht	bm	srr	wue	nue
h		**0.69**	**0.88**	**0.62**	**0.66**	**0.86**	**0.83**	0.23	0.18	0.55
d	**0.63**		**0.95**	-0.13	**0.88**	**0.70**	**0.82**	-0.30	0.65	0.16
vol	**0.91**	**0.89**		0.18	**0.86**	**0.83**	**0.89**	-0.10	0.50	0.37
hd	**0.92**	**0.29**	**0.69**		-0.06	0.42	0.24	**0.64**	-0.33	0.55
rt	0.08	**0.44**	**0.29**	-0.12		**0.77**	**0.91**	-0.42	0.71	0.05
sht	**0.59**	**0.73**	**0.73**	**0.37**	**0.52**		**0.96**	0.24	0.27	0.64
bm	**0.46**	**0.72**	**0.65**	0.22	**0.77**	**0.94**		-0.03	0.46	0.46
srr	**0.61**	**0.48**	**0.60**	**0.53**	-0.18	**0.74**	**0.48**		-0.47	0.68
wue	**0.72**	**0.63**	**0.76**	**0.59**	**0.42**	**0.74**	**0.70**	**0.57**		-0.45
nue	**0.38**	**0.49**	**0.50**	**0.23**	**0.45**	**0.46**	**0.51**	**0.20**	**0.33**	

Table 25. Standard errors on the estimates of genetic correlation coefficients. (1996 estimates top right, 1997 estimates bottom left). Two n/a estimates: see p.37.

	h	d	vol	hd	rt	sht	bm	srr	wue	nue
h		0.07	0.03	0.08	0.07	0.03	0.04	0.12	0.39	0.29
d	0.05		0.01	0.12	0.03	0.07	0.04	0.11	0.20	0.34
vol	0.02	0.02		0.12	0.03	0.04	0.03	0.13	0.26	0.31
hd	0.02	0.06	0.05		0.12	0.11	0.12	0.08	0.34	0.28
rt	0.07	0.03	0.06	0.06		0.05	0.02	0.10	0.18	0.37
sht	0.06	0.03	0.04	0.07	0.04		0.01	0.12	0.35	0.23
bm	0.06	0.02	0.04	0.06	0.02	0.01		0.13	0.29	0.30
srr	0.06	0.05	0.06	0.06	0.05	0.03	0.04		n/a	n/a
wue	0.05	0.04	0.04	0.05	0.05	0.03	0.03	0.05		0.30
nue	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	

For correlations involving shoot/root ratio, some are positive, others negative or not significantly different from zero. WUE and NUE are both positively correlated with growth. The genetic correlation between WUE and NUE is negative in the first experiment and positive in the second.

Correlation coefficients were also estimated within treatments (appendix 11). Within treatments, environments were apparently very uniform, such that phenotypic and genetic correlation coefficients were almost identical. Phenotypic correlations were estimated with more precision (smaller errors) and so can be used to back up our speculations about the less reliable (less precise) estimates of the genetic correlations.

Most across- and within-treatment estimates carried the same sign. Most of the time the within-environment phenotypic correlations also did not change sign from one environment to another, although in some environments they did become non-significant. Exceptions to this rule were two correlations involving NUE: NUE-shoot/root ratio and NUE-WUE. For these estimates, considerably lower and even negative values were found in the nitrogen-stressed environments.

Whereas in 1996, genetic correlations with WUE and with NUE were of a similar magnitude, the 1997 data showed much lower correlations with NUE, which is due to the limited genetic variation available for this trait for the 1997 genotypes.

In general, phenotypic correlations changed much less from one environment to the other in 1997 than in 1996 and the patterns of change are much less clear. The same is true for genetic correlations, though they change slightly more from one environment to another.

No attempt was made to estimate r_A within provenances. The data set was too small for this purpose. In the second experiment, a possible provenance effect could not be separated. Thus, it remains possible that r_A varies among provenances, since it is a result of both genetic linkage and pleiotropic effects. Pleiotropy would be similar for different provenances. It would also be similar for the 1996 and 1997 genetic materials. However, more linkage disequilibrium is expected in the 1996 genotypes because the five populations (covering two subspecies) are more widely apart than the 49 families of the interior. As a consequence, genetic correlations would be larger in 1996. Also, since r_A depends on variances and covariances, two traits can be intimately genetically related while their genetic correlation is zero because there is no variance or no covariance.

4.2 Phenotypic integration

To get a better grasp of how the whole set of pair-wise correlations changes across environments, the within-treatment correlation matrices were considered in their entirety and compared across environments. Phenotypic correlations were chosen for this purpose because of their smaller estimation errors. Phenotypic correlation matrices for each of the nine environments were clustered based on the Mahalanobis distance using Ward's minimum variance method. Using the data of the first experiment, two groups can be distinguished: the severely N-stressed environments (W_HN_L, W_MN_L and W_LN_L) and all other environments (W_HN_H, W_MN_H, W_LN_H, W_HN_M, W_MN_M and W_LN_M). These groupings appear, though somewhat less clearly, for complete, single and average linking methods. They also appear when Euclidean distances are used, though these are less desirable because the correlations are not independent,. In other words, the groupings were fairly stable.

When data of the second experiment are used, two groups can be distinguished when using Ward's minimum variance method: the high-N environments (W_HN_H, W_MN_H, W_LN_H, W_LN_M) and the low- and medium-N environments (W_HN_M, W_MN_M,W_HN_L, W_MN_L and W_LN_L). The complete and average linkage methods yield the same results. The centroid and single linkage methods yield no clusters at all, though the sequence in which environments are grouped is largely the same. Interestingly, use of the Euclidean distance with Ward's minimum variance method yields a grouping similar to the one of the first experiment, where the low-N environments (W_HN_L, W_MN_L and W_LN_L) were separated from all others.

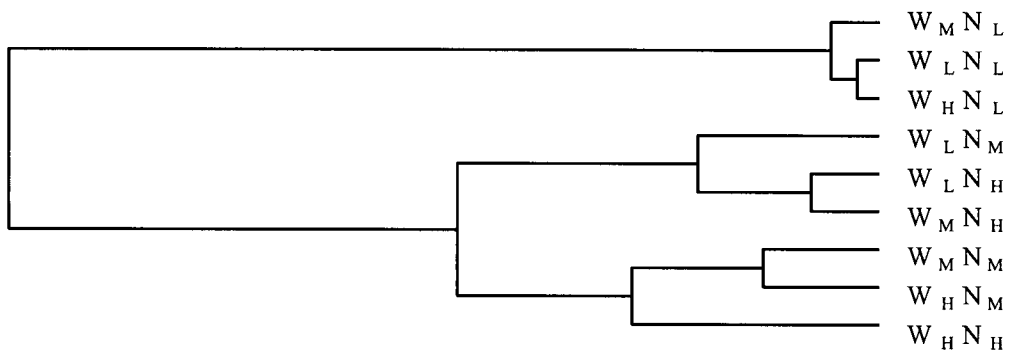

Figure 10. Environments clustered based on phenotypic correlation matrices of the 1996 experiment, using Mahalanobis distances and Ward's minimum variance method.

Thus, inducing water stress did not change trait integration much, but inducing nitrogen stress did result in changes of the trait correlation structure with certain traits becoming de-coupled and others more closely linked. Moreover, it seems that in the first experiment, the medium N treatment is closer (in the physiological results it produces) to the high-N treatment, and in the second experiment it is closer to the low-N treatment.

4.3 Plastic trade-off between pairs of traits across environmental variables

To investigate a plastic trade-off, a phenotypic correlation is not suitable. It can be confusing, like the negative phenotypic correlations with NUE, or it can hide rather than reveal information, because it only explores simple linear relationships. The term trade-off is usually reserved for trait combinations where an increase in one trait results in a decrease of another. However, interesting patterns of joint change were found for various trait combinations, including some that do not represent trade-offs in the strict sense (figure 11).

Figure 11 is based on the treatment means of the 'average' genotype in the 1997 experiment. It illustrates how relationships between traits need not be linear and how they can change across environments. These patterns vary for individual genotypes due to genotypic differences and estimation error, but possibly also due to experimental anomalies, so only the general results are shown. NUE really does trade off, in the strict sense, with other traits. For WUE and growth, the relationships are positive, but not necessarily linear. As an example of how joint change and trade-off need not be linear, consider the relationships with shoot-root ratio. Shoot-root ratio declines sharply when water level is lowered from high to medium, but hardly changes (may even increase slightly) when water is further reduced to low. WUE, on the contrary, continues to increase. Similarly, shoot/root ratio declines drastically when nitrogen is lowered from high to medium, but a subsequent reduction from medium to low has little influence. WUE, on the other hand, continues to decline. The trade-off between WUE and NUE is linear for the average genotype.

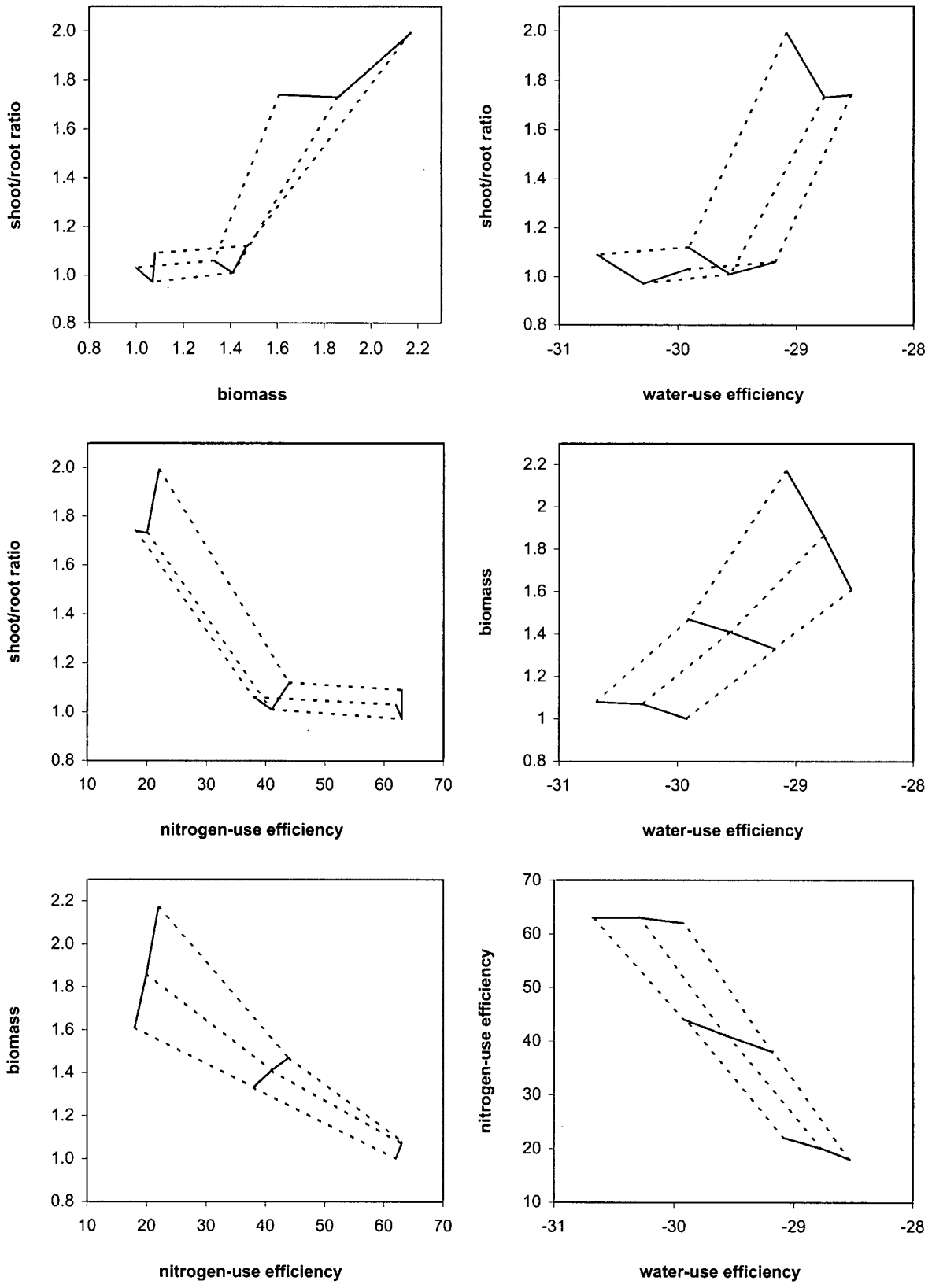

Figure 11. Response of pairs of traits to nitrogen and water treatments (1997 experiment).

DISCUSSION

1. Existence of genetic variation for resource use efficiencies and other component traits of growth, either for mean genotype performance or for plasticity or for both.

In both experiments of this study, evidence exists for genetic variation for water-use efficiency and growth traits. Genetic variation for nitrogen-use efficiency was evident in the first experiment, but was very small (not significant as a variance component) in the second experiment. This may be due to the exclusion of coastal genotypes in the second experiment.

Genetic differences were found in mean performance, as well as in plasticity. Genotype-environment interaction or genetic variation in plasticity was detected at both the provenance level and the family level in the first experiment. Genetic variation for plasticity at the provenance level was significant for all traits and roughly 1.5 to 3 times larger than genetic variation for plasticity at the family level. Genetic variation for plasticity at the family level was significant for all traits except root weight and WUE. Genetic variation for mean performance is distributed roughly 60-40 % between among-population and within-population (or family) sources of variation. For root weight and biomass, the relative amount of family variation is slightly larger, for height and shoot-root ratio slightly smaller.

The existence of among-provenance variation for traits of adaptive significance indicates that differential selection is likely to have taken place. However, interpreting the existence of within-provenance variation is not straightforward. The existence of genetic variation for overall means within a random-mating population is, at first glance, a bit surprising, especially for resource-use efficiencies, because they are presumably strongly related to fitness. Variation in the microenvironment combined with strong selection may lead to different genotypes being optimally adapted to each micro-site. However, exchange of genes among plants from different micro-sites within the population prevents these advantages from being fixed in the population. Random mating mixes the gene pool and, over many generations, results in the selection of those genotypes that are best on average (Hedrick 1986). Variation remaining within populations for single traits is therefore expected to be for plasticity rather than for overall means.

Genotypes may have sub-optimal trait values for several reasons. (1) The population may not have reached equilibrium with regard to selection. (2) The traits considered may not be as closely related to fitness as previously thought. (3) The environment may not be as stable or

uniform as thought, either at the univariate or the multivariate level, so that the erosion of genetic variation by natural selection is much less complete. (4) Multiple correlated traits may be involved in such a way that several combinations of these trait values could result in equal overall fitness of the different genotypes.

For a variety of growth, developmental, morphological and physiological traits, as well as for isozymes, a similar pattern has been found: genetic variability within populations tends to be high despite pronounced differentiation of populations (reviewed by Rehfeldt et al. 1999). Thus, although it is often not well understood how this within-population variability is maintained, the phenomenon seems to be common for many populations and traits.

Known to result in the maintenance of variation in a random mating population are (1) overdominance, (2) epistasis and (3) environmental fluctuation. Practical examples of overdominance in nature are rare. Epistasis, i.e. the interaction between alleles at different loci, is definitely pervasive. However, variation resulting from overdominance and epistasis is nonadditive.The contribution of environmental fluctuation is hard to evaluate. Climate variables do vary substantially from year to year, and these fluctuations may play a large role, especially if they influence the adaptive characteristics of the offspring from the seed which was set in that year (Dormling and Johnsen 1992).

The existence of additive genetic variation within provenances for resource-use efficiencies may be also indicate that multiple traits need to be considered simultaneously. The value of a gene or trait depends on the genetic context, be it another locus or another trait. The observed trait correlations imply pervasive epistasis at the multiple trait level. If a composite trait is under strong selection as opposed to separate single traits, differences in mean values for single component traits could continue to exist as long as they would result in the same composite fitness value. Genotypes could then survive and thrive by means of different strategies, based on their own individual 'forte', compensating for less desirable characteristics by better scores for other relevant traits. It is also possible that other environmental factors, not considered in this study, play a role in maintaining genetic variation in natural stands.

2. The role of multiple environmental variables and multiple traits in creating additional environmental niches for the maintenance of genetic variation

The range of water and nitrogen levels applied in this study was deliberately chosen to be wide,

because large amounts of environmental variation are also expected to exist in nature and genotypic differences may be most clearly expressed in extreme environments. In 1996, at low and medium nitrogen levels, the shortage of nutrients was so acute that, effectively, the plants were unable to make use of the additional amounts of water to achieve more growth. As a result, fewer than nine environmental niches existed for growth traits. However, resource-use efficiencies differed significantly in environments that were similar in terms of total growth. Thus, the addition of resource-use efficiency traits resulted in the plants responding differently to all nine environments.

If fitness can be assumed to be a composite of all of these traits (ignoring others), there would effectively be nine niches where fitness differs, and natural selection could act differently in each of these nine niches (i.e. selection coefficients would differ). All of the traits investigated were genetically correlated into a network of reactions, though not all pair-wise correlation coefficients were significant. This implies pervasive epistasis, which, in combination with the existence of a large amount of micro-environmental variation, can result in the maintenance of large amounts of genetic variation.

3. Multivariate clustering of families

Clustering of twenty families of the first experiment based on their multi-trait multi-environment response showed that families did not group into the clusters that corresponded to their provenances. Families within populations vary so widely in their response to a range of environments that the cluster groupings were unstable, i.e. dependent on the method and distance measure used, and cannot be considered real groups. This means that populations are not defined by a narrow range of response curves.

4. Patterns of adaptation to source environmental variables

Detailed investigations into adaptation of lodgepole pine provenances in British Columbia to climatic source environmental variables were made by Rehfeldt et al. (1999). Multiple regression of population response (for height and survival over a range of field sites) on climatic

variables was carried out. Certain climatic variables were consistently effective at describing population differentiation. However, causal mechanisms were obscured by estimation errors on climatic variables and imprecise knowledge of the interaction between climate and physiology. Survival was linked to (1) moisture (summer precipitation / summer temperature) (2) the temperature differential (3) summer precipitation and (4) mean temperature of the coldest month. This would imply that drought as well as frost damage are important factors influencing survival. The height growth of a population is most effectively predicted from the mean annual temperature and the mean temperature in the coldest month of the source. To avoid maladaptation (frost damage, susceptibility to diseases), seed transfer guidelines restrict the transfer of seed. For lodgepole pine in the southern interior of B.C., seed transfer is restricted to 2°N, 1°S, 3°W, 2°E, 300 m upwards and 100 m downwards (British Columbia Ministry of Forests, 2000). Drought is not explicitly included in these guidelines, though by restricting the altitudinal transfer, the drought factor is included implicitly.

Is it necessary to take WUE and NUE into account during seed transfer, provenance selection and breeding in order to ensure the survival and growth of lodgepole pine on marginal sites? The study I conducted is suitable for an investigation of the relationships among resource-use efficiencies, growth (but not survival) and source drought or source climate (but not source soil richness). The questions that need to be addressed are whether WUE might provide adaptation to drought, and whether NUE might provide adaptation to poor sites.

4.1 Trends in the 1996 experiment

Considering that Rehfeldt et al. (1999) found that the patterns of response to source environmental variables differed fundamentally between coastal and interior populations, these two groups should not be lumped. Since there are only two coastal populations and three interior populations, it is hard to distinguish trends with certainty. A confounding effect of source elevation on plant size was expected, so no attempts were made to relate growth traits to source moisture levels. The relationship between WUE and growth of populations was also obscured by the influence of these elevation differences on growth and is hard to interpret.

In the first experiment, within each subspecies, the provenances from drier sources had a higher WUE. Kamloops and Salmon Arm were more efficient at using water than Revelstoke and Qualicum was more efficient than Squamish. However, the interior provenances were not always (under all treatments) more efficient in their use of water than the coastal ones. For some treatments, Qualicum had a higher WUE than expected. Salmon Arm was, for some treatments,

more efficient than Kamloops. The summer dryness index and annual dryness index (table 1) indicate that the Salmon Arm site is a slightly drier environment than the Kamloops site. On the other hand, the higher WUE of Salmon Arm at high water and nitrogen levels may be an indirect effect of a higher nitrogen uptake and/or better growth and the resulting (sink effect) higher photosynthetic rates. It also seems that Kamloops is less responsive than average to nitrogen, and Revelstoke more responsive than average with regard to nitrogen. Nitrogen may be more limiting on colder sites, since nitrogen-cycling is slowed down by low temperatures (Miller 1984). Alternatively, this responsiveness of WUE may also evolve as a correlated trait with the responsiveness of growth to nutrient availability, i.e., as a consequence of the sink effect (plants grow faster and therefore photosynthesise at a higher rate).

It is noteworthy that based on a conventional nursery experiment, where plants receive high W and N levels, Salmon Arm would be selected as the most water-use efficient provenance. Planting the seedlots in field trials simultaneously subjected to both drought and nitrogen-shortage would result in different conclusions with regard to adaptability, though. Thus, the approach of this experiment to use varying testing environments is not only appropriate but also necessary.

In nitrogen-use efficiency, the two coastal provenances (ssp. *contorta*) had higher nitrogen-use efficiencies than the interior provenances (ssp. *latifolia*). Qualicum had the highest values in most treatments, followed by Squamish. This is not entirely surprising, since the sites where seeds were harvested were very sandy or rocky and poor, which was reflected in poor growth of the seed trees. Nitrogen-use efficiency may have played a large role in natural selection on these sites as well as on similar sites in the coastal area. Squamish was most efficient at using nitrogen when nitrogen was abundant in the environment, which may be an artefact of its prolonged growth in the autumn. Qualicum was most efficient at medium and low nitrogen levels. Kamloops' NUE was more responsive to water under severe N-stress. The trade-off for the higher WUE of Kamloops at low N may be a lower NUE.

4.2 Trends in the 1997 experiment

When relating the trait expression of the 49 interior families of the 1997 experiment to single source environmental variables, only certain combinations of traits and test environments yielded significant correlations with source variables. The correlations were often quite weak, confirming the importance of (1) having the best estimates possible for climatic variables themselves and (2) choosing the right climatic variables and traits in order to detect the

underlying causal relationships. Climate variables themselves are interrelated, and it is not clear whether one of them is the critical factor, or whether they really interact. Relationships with temperature were the strongest, and this was true for all traits.

Relationships with source moisture level were also investigated. An index of moisture deficit as proposed by Thornthwaite (1948) was not available. The challenge consists of finding reliable monthly temperature and rainfall data. Climate stations are often located in urban or agricultural areas and many forest areas are poorly represented. A moisture index, summer dryness index and annual dryness index were used. These indices gave good results, but less so than temperature. The lack of any relationship between precipitation (mean annual as well as summer precipitation) and water-use efficiency was initially somewhat surprising. Precipitation was apparently an inaccurate indication of drought. Alternatively, there may be no natural selection for high water-use efficiency in dry environments.

NUE was correlated with source moisture level as well as source temperature. Since NUE as expressed in high-N environments may reflect luxury N consumption rather than the efficiency to grow well with a limited amount of nitrogen, the correlations with SDI and ADI may be an indirect effect. It is not clear, however, of what they would be an indirect effect, since plants from wet sources develop larger root systems (which would result in higher N-uptake) only in the very dry environments (table 19). NUE in medium-rich environments under some level of drought (W_MN_M and W_LN_M) was positively related to source temperature and negatively to source elevation. Root weight itself (partly reflecting uptake capacity) is largely unrelated to temperature variables (table 19), but biomass in W_LN_M is strongly and positively related to temperature (table 19). The increased NUE may therefore well be a side effect, resulting from the increased growth and photosynthetic rate of the families from warm sources, while the amount of N taken up remains constant (sink effect). Considering all these relationships together, correlations between NUE and source variables did not provide insight into possible patterns of adaptation to source variables.

4.3 Could a high WUE indicate adaptation to dry sites ?

There is indeed a cline where the values of WUE change with source drought. However, Endler (1977, p.95) emphasised that "it is impossible to interpret a natural cline without knowing the geography of absolute survival values (the shape of the fitness curves) or the extent of gene flow". In the absence of this knowledge (i.e., in most real-life cases), one can only speculate. Yet, since lodgepole pine regenerates in dense monospecific stands and the water saved by a

high WUE would not simply be lost to other vegetation, an adaptive advantage of high WUE in dry environments would be expected intuitively. Water saved could still be lost to other genotypes, but their more wasteful use of water should lead to a disadvantage for the population as a whole. Considering the relatedness among individuals of the same population, group selection for increased WUE is likely to take place.

Although in my experiments WUE was higher for populations from dry sources, most other studies to date, on lodgepole pine as well as other species, show exactly the opposite trend, or no trend at all.

Holowachuk (1993) found that of ten interior lodgepole pine populations, covering a large part of B.C., those with a higher WUE came from wetter source environments. The single coastal population included in her study had the highest WUE of all. Her populations covered a larger geographical area than the set of 49 populations of the southern interior that I used. Possibly different patterns may be predominate at different geographical scales.

Zhang et al. (1993) found a lower WUE for the drier sources of the interior compared to the wetter coastal sources. They studied 25 populations (bulked seedlots) of Douglas-fir, growing in a 15-year old plantation in the Interior Cedar Hemlock biogeoclimatic zone (Pojar et al. 1987). Still, their coastal populations were biased towards drier sources since the wetter coastal sources would not have survived the winter on the planting site in the interior. Their indication of source moisture level was therefore rather limited. However, they did find that carbon isotope discrimination increased (WUE decreased) with elevation ($r = 0.76$).

Aitken et al. (1995) found a negative phenotypic correlation between WUE and source drought for populations of Douglas-fir. Since their common garden field testing environment was intermediate in moisture level among the four sources evaluated, this negative relationship may have been caused by a rank order change of populations over water levels. Alternatively, it is possible that drought tolerance and WUE are poorly related or unrelated in Douglas-fir, or that elevation or temperature plays a larger role than drought in the evolution of WUE.

Read and Farquhar (1991) found that for several species of mountain beech (*Nothofagus* spp.) the wetter sources had a higher WUE. Lauteri et al. (1997) found that the wetter sources of European chestnut (*Castanea sativa* Mill.) had a higher WUE than drier sources.

Zhang et al. (1997) found that ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) populations did not differ significantly in WUE (measured as $\delta^{13}C$) as was expected based on their greatly varying source moisture levels. Natural selection may not favour a high WUE in dry environments for ponderosa pine because other vegetation may simply use the water saved.

The possibility of such a phenomenon was illustrated, among others, by DeLucia and Schlesinger (1991), who found that the trees and shrubs in their study developed different strategies in that regard. The shrubs had a high drought tolerance but a low WUE. The evergreen trees, with their deep root systems, more conservative use of water (high WUE) and high growth-based NUE and N retranslocation efficiency, were more adapted to nutrient-poor sites or to the lower layers of the soil where the trees don't have to compete with the shrubs for nutrients.

Comstock and Ehleringer (1992) integrated monthly temperature and precipitation data into an 'effective seasonal leaf-to-air water vapour gradient'. They studied a desert shrub, for which the environmental factors influencing WUE are only relevant when sufficient soil moisture is available to permit photosynthetic activity. The ecotypes with the highest WUE came from the sites with the highest 'effective seasonal leaf-to-air water vapour gradient'. This site ranking, however, was quite different from the one based on available moisture. The plants in their study with a high WUE were indeed ecotypes with a more conservative water-use behaviour.

Genetic changes in $\delta^{13}C$ with elevation are consistently observed, but the precise mechanisms are unknown. Hultine and Marshall (2000) investigated the influence of physiological traits, reflecting the shifts between demand and supply for CO_2, on $\delta^{13}C$. Abiotic factors, such as vapour pressure, change with elevation and influence $\delta^{13}C$. Guy and Holowachuk (*in press*) developed the summer- and annual dryness indices, SDI and ADI, which combine the effects of vapour pressure, determined primarily by temperature, and rainfall. These indices resulted in a clarification of the relationships for the first set of five provenances, but were not better than the moisture index for the second set of 49 families. The results of several studies jointly imply that multiple environmental factors may interactively be driving adaptation in terms of WUE. Using a multivariate approach for the 1997 data, however, I did not manage to integrate the climate variables in a functionally meaningful way and failed to highlight the causal relationships. According to Comstock and Ehleringer (1992), this was to be expected, since "complex environmental gradients can be interpreted meaningfully only if explicit functional mechanisms linking organismal traits and environmental parameters can be defined".

In the case of lodgepole pine, a high WUE may be related to the inherent growth rate of populations, which is strongly related to temperature (Rehfeldt et al. 1999), or may be the result of increased N-availability in low elevation warmer sites, so trees may simply be able to afford better growth. In other words, WUE may well evolve in response to factors other than drought,

such as temperature, which would explain why the strongest relationships are found there. The relative importance of several factors may also vary over the distribution range of the species, depending on the scale considered.

4.4 Might a high plasticity in WUE indicate adaptation to dry sites ?

From the detailed analyses of the 1997 experiment, a relationship was found between the range of WUE across environments, a measure of plasticity, and source environmental variables. Though mean and range are weakly correlated, this correlation is not very high, so the range of response may be a trait under selection by itself. A study on spruce from the introgression zone of white and Sitka spruce (Silim et al. 2000) found that WUE was more plastic for genotypes from dry, interior sources. These environments would have larger year-to-year variations in available moisture. Thus, plasticity for WUE may well be selected for in these dry, interior source environments.

Although Via (1993) has argued against plasticity being a selectable trait, controversy in the scientific community remains (Via et al. 1995; Scheiner 1993a; Schlichting and Pigliucci 1993). My own opinion is that, considering continuous variation in the environment, the interactions among multiple correlated traits, the pervasiveness of epistatic effects, and the likelihood of costs for plasticity, selection on plasticity is not merely possible but even likely. More realistically the reaction norm should be considered as a meta-character that can be moulded by selection (Gomulkiewicz and Kirkpatrick 1992).

5. Provenance selection to ensure adaptation for resource-use efficiencies

Provenances differed in growth traits, water-use efficiency and nitrogen-use efficiency. Some degree of differential adaptation of sources to sites may exist in lodgepole pine for WUE and NUE. If so, it is rather small.

NUE was larger for coastal populations. This was not expected, but neither is it surprising given that shore pine naturally grows on poor sites. In the interior, lodgepole pine often regenerates following fire, when nitrogen is, for a short while, more available. However, to what extent population differences represent adaptation, or are a side-effect or the consequence of developmental constraints, remains unclear.

In my study, WUE was slightly larger for populations from dry sites. However, this

relationship was weak. It was also contradictory to what was found for other species and studies. WUE may not be advantageous by itself but may be a side-effect of the increased growth potential or increased nitrogen availability for sources with higher temperatures.

Is selection of dry sources or testing for WUE a useful step in provenance selection to ensure adaptation to dry sites? Even if WUE is of some adaptive significance, given that only provenances with suitable growth phenology can be considered, it is not necessary to severely restrict the choice of provenances. In part, since temperature variables and drought are correlated, and since WUE is more closely correlated with temperature, adaptation to drought is sufficiently assured by ensuring phenological adaptation. Also, the variation for WUE within populations is so large that survival of trees is evidently possible even when WUE is less than maximal or even less than average. Presumably, trees can compensate by scoring higher for other traits that affect their overall fitness.

Relying on phenological adaptation to ensure drought adaptation, however, may not be well-advised for coastal populations. Based on the fact that coastal populations have been found to be growing on wetter sites than where their growth is optimal due to interspecific competitive exclusion (Rehfeldt et al. 1999), lack of drought adaptation might not be a problem for coastal populations. However, not enough is known about coastal populations to say this with any degree of certainty.

Testing for NUE may also not be a useful step in provenance selection to ensure adaptation to poor sites. The changing relationship of NUE with WUE over varying N-levels indicates that, over the large range of nitrogen levels considered, NUE, measured as C/N, may reflect developmental constraints rather than adaptation (see p.92 and further). Population differences for NUE are small relative to plasticity for NUE. But even plasticity for NUE seems likely to be a by-product of environmental variation rather than an adaptive mechanism.

Other measures of nitrogen-use efficiency may yield more 'meaningful' results in terms of adaptation. Leaf nitrogen content has been investigated in that context. Yet, problems have een encountered also with leaf-N content on a per weight basis. Johnsen et al. (1999) noted that the absence of a strong correlation between leaf-N and photosynthetic capacity may result because a significant amount of leaf nitrogen is used for other functions (e.g. herbivore defence). This possibility indicates that leaf-N content on a per area basis is unlikely to solve the problem.

6. Individual selection for single traits

The phenotypic expression of genetic variance varies with the environment, so care must be taken when interpreting the estimates of genetic parameters. Across-treatment heritability estimates have limited practical value because the study, with its nine environments in equal proportions, is not representative for conditions found in the field. Comparing them with the average of within treatment estimates indicates that more gain may be achieved if selections are made in and for specific environments, especially for resource-use efficiencies (though for the first experiment this remains speculative due to the large estimation errors).

Nitrogen shortage had a large detrimental effect on the expression of genetic variance of growth traits, especially in the first experiment. The same pattern held, though to lesser extent, for the allocation traits: in N-poor but moist environments few genetic differences were revealed. For both resource-use efficiencies genetic differences were expressed best in the most stressed environment. Profoundly different patterns, however, were found in the second experiment. There, depending on the trait, either drought or nitrogen shortage can increase or decrease the expression of genetic variance.

Other studies have found variable results for the relationship between the amount of genetic variation expressed and the stress level of the environment considered. In some cases, more genetic variation was expressed under stress (e.g. Pigliucci and Schlichting 1995b), in others, less. Several arguments have been made as to the reasons for one relationship or the other. A possible reason for finding more variation expressed in stressful environments would be that these environments are less common (Pigliucci and Schlichting 1995b). As such, latent variation that has not been selected upon in the common environment could be present. Thus, the frequency of occurrence of environments would play a larger role than their degree of stress. However, it seems likely that the relationship between the amount of genetic variation and the environment will depend on the trait considered, and that no general rule should be expected. Apart from that, the different results for the second experiment indicate that environmental variation may be very fine-tuned. After all, the treatments were virtually the same in both experiments, though the smaller size of the plants in the first experiment may have resulted in a different perception of these treatments by the plants.

7. Individual selection for multiple traits.

Because of the existence of genetic correlations between traits, every single trait selection process results in a correlated response for other traits. Effectively, even if inadvertently, it becomes a multiple trait selection process. Thus, while a breeder may merely want to increase yield (in terms of height growth or biomass), it is still worthwhile to consider the effects that selection for increased height might have on, say, resource-use efficiency, to avoid maladaptation. Especially negative genetic correlations among yield components cause conflicts. Such negative correlations are notably absent from this study. Selection for growth does not compromise an individual's resource-use efficiency and vice versa. The possibility remains that for different populations, correlations differ, since it is unknown to what extent they are caused by pleiotropy and to what extent by linkage disequilibrium. Also, the genetic correlation is dependent on the relative values of genetic variances and covariances, and can be zero for traits that are very closely related physiologically, or change where the physiological relationship between the traits does not change.

Can indirect selection for growth be carried out using $\delta^{13}C$? Johnsen et al. (1999) argued that the strong positive correlation between growth and $\delta^{13}C$, high heritabilities for $\delta^{13}C$, and a lack of rank order change for $\delta^{13}C$ in black spruce families made indirect selection using $\delta^{13}C$ promising. Direct gas exchange measurements (Johnsen and Major 1995) had shown that the main cause of variation for $\delta^{13}C$ was the variation in photosynthetic capacity of populations. However, heritabilities are always specific for the populations considered, and their controlled crossings between widely divergent individuals may have resulted in artificially high heritability estimates. Although WUE and growth were positively correlated genetically (Johnsen et al. 1999), photosynthetic capacity is only one aspect of growth (Johnsen et al. 1999), such that incorporating WUE assessment into established tree breeding programs may prove difficult until the physiology is better understood. However, the high cost of testing may be more than compensated for by the fact that WUE can be assessed at the juvenile stage, especially if genotype-environment interaction would be absent or minimal not only for water availability but also for nitrogen availability. Sun et al. (1996) also found no rank change in WUE over water levels. Cregg et al. (2000) found no rank change in WUE across years, which were thought to differ mainly in moisture level, but did find rank order changes across widely different sites. In my study, genotype rank change was clearly present for WUE over varying water and nitrogen levels.

There may be a cost incurred by selecting for high WUE. Where the high WUE is the result of an increased photosynthetic capacity, the cost may be in terms of the amount of nitrogen needed, and where high WUE is the result of differences in stomatal conductance, the cost may be in terms of growth. On the other hand, where a high WUE is the indirect result of a stronger sink effect, no cost need be present at all.

8. Genotype-environment interaction and rank order change

The provenances from the first experiment exhibited significant rank order changes over the environmental range considered according to the Azzalini-Cox criterion. A breeder may want to avoid rank changes for any of the three criteria. Which provenances changed rank, and where, varied for each of the different traits. Most rank changes were with regard to both water and nitrogen levels simultaneously. There were more rank changes for shoot-root ratio and resource-use efficiencies than for growth traits, despite the fact that the larger error in estimating resource-use efficiency traits reduced the chance of finding significant differences there.

Thus it becomes very important to define accurately which traits one wants to select on and what the target environment is. Otherwise, it is possible that non-optimal genotypes will be selected because the observations have not been made in the right environment. Creating a selection index for multiple traits may result in different rank changes depending on how the index is constructed.

Very few rank changes among families were found to be statistically significant, according to the Azzalini-Cox criterion, because the power of the tests decreases with the number of genotypes and environments. Those rank changes that were significant were almost invariably rank changes of families from different provenances. When less stringent criteria were used for rank order change, the preponderance of across-provenance family rank changes gradually disappeared, until there was roughly an equal amount of across- and within-provenance rank change. This pattern held for all traits investigated.

The choice of a testing environment (or a set of testing environments) for the selection of genotypes will depend on (1) the relative importance of traits, (2) the range of planting environments targeted, (3) the expression of genetic variation in testing environments, (4) the existence of genetic correlations and their expression in both testing and planting environments, and (5) rank order change of genotypes between test environments and planting environments.

To evaluate GxE in the field, type B genetic correlations between traits as they are expressed in two environments, are often used (Burdon 1977; Falconer 1989; reviewed by Lynch and Walsch 1998 chpt.22). They are called 'type B' because the traits are measured not on the same individual but on two different, related individuals. Type B correlations address the role of environments, as opposed to genotypes, in generating interactions. They allow the researcher to combine information from varous trials with different experimental layouts. They are useful to predict genetic gain resulting from the correlated selection response when one cannot test genotypes in all environments or in the environment which is 'best' for a certain trait. However, my experiments are not well set up to calculate gain (p.48-50) and the field data of the 1997 families were only considered with regard to how they relate to the nursery results. Shukla's stability variance was compared for families in the field and in the nursery. This measure evaluates the contribution of genotypes to the total GxE interaction variance. A similar contribution of environments to the total GxE interaction variance could have been calculated, and would be directly related to the type B genetic correlation coefficient. However, since no detailed environmental data about the field sites were available, the interpretation of type B genetic correlation coefficients would not have clarified the importance of various environmental factors in causing GxE.

The choice of a generalist or specialist breeding strategy depends on the biological understanding of rank changes, whether rank changes can be characterised by environmental variables in a reasonably consistent way, and on the economic feasibility of breeding for several target areas (Nissilä 1996). In practice, matching populations to specific sites is not done for lodgepole pine in British Columbia, and a generalist genotype would be preferred to a specialist genotype for ease of management. What makes genotypes better than average on one site and worse than average on another site may well be their combination of traits rather than any single trait. Different combinations of traits may be equivalent on one site but their ranking may change over sites. It may be prudent to preserve a variety of trait combinations.

9. Plastic trade-off patterns and genetic variation in that trade-off

Patterns of plastic trade-offs between traits across environmental variables provide a more detailed kind of information than phenotypic correlation coefficients. The trade-offs investigated in this study were not always linear and the relationship between the two traits often changed

across environments.

The trade-off of WUE and NUE over water and nitrogen levels in the environment is expected from theory and has also been found in other experiments (e.g. Patterson et al. 1997). As long as water is freely available, stomata will be open to allow rapid CO_2 exchange even at the expense of large losses of water per CO_2 fixed. If water is in short supply, stomata will close and WUE will increase, but growth and NUE will decrease. As nitrogen becomes a limiting resource, less carbon-fixing enzyme is available. Rubisco alone contains up to ¼ of nitrogen in leaves, and together with other proteins of the photosynthetic apparatus accounts for a large proportion of plant nitrogen. With less enzyme available, photosynthetic capacity and thus WUE will decrease.

Individual genotypes can and did differ in their pattern of trade-off, but a systematic investigation of individual genotypes was not carried out. Just like species have different strategies to deal with drought (e.g. Lechowicz and Ives 1989), individual genotypes may also deal in varying ways with low water levels, low nitrogen levels, or both. Increasing WUE could compensate for low water levels. This could be accompanied by an increased nitrogen uptake to produce more photosynthetic enzymes, which would result in decreased NUE, or by growing larger roots, which would result in a decreased shoot-root ratio. Similarly, limited nitrogen levels may be dealt with by decreasing the shoot-root ratio for better nitrogen uptake, or by using relatively more N for structural growth, resulting in a decreased WUE. Given the various source environments and evolutionary backgrounds of the provenances tested, a variety of combinations could be made for different genotypes to deal with the imposed experimental environments.

Plastic trade-offs, as well as genetic trade-offs, are important for evolution: they imply restrictions in the ability of plants to respond to their environment, either now (plastic trade-off) or in future generations (genetic trade-off). When there is genetic variation in two traits that are associated in a physiological trade-off within an individual, one expects a negative genetic correlation in the population (Stearns et al. 1991). Indeed, such a relationship was found for WUE-NUE, but not for WUE-growth.

10. Trait correlations and phenotypic integration

Few empirical studies have attempted to analyse plasticity (change in trait expression over

environments) at a multi-trait level (change in trait relationships over environments). Yet the importance of doing so is widely recognised (Cheverud 1982, Pigliucci and Schlichting 1998). Ultimately, it is the integrated phenotype which is subjected to evolutionary forces. Several approaches have been proposed.

Studying correlation networks, i.e. looking at pair-wise phenotypic and genetic correlations (Hebert et al. 1994), requires studying one network for each environment. Visual interpretation of such networks is daunting, especially for many traits or many environments.

Reducing the number of traits by collapsing them into fewer dimensions, e.g. using Principal Component Analysis, Canonical Correlation, or Canonical Discriminant Analysis (Pigliucci and Schlichting 1995 a,b) leads to problems in interpreting the new variables.

Path analysis has been suggested. Its main advantage would be that all correlations are estimated simultaneously in a causal and hierarchical framework (Pigliucci et al. 1995a and 1998). However, interpreting the sensitivity of path coefficients to environmental change or interpreting differences among genotypes based on their set of path coefficients basically poses the same problems as interpreting the changes in phenotypic or genetic correlations. Therefore, this approach was not pursued further.

Pair-wise phenotypic and genetic correlations were analysed and provided interesting information about trait integration. Phenotypic correlation coefficients, containing a genetic as well as an environmental component, were sometimes confusing. To separate the effect of the environment, the effect of treatments on a pair of traits was studied point-by-point in the treatment space for a single or an 'average' genotype (plastic trade-off, though in the strict sense the term trade-off is reserved for a negative relationship). To separate the genetic effect, a genetic correlation was calculated. It may be called a genetic trade-off if it is negative. The genetic correlation, based on genetic variances and covariances so as to correct for (i.e., exclude) any effects of the environment, is a better indicator of the genetic control of traits but has much higher estimation errors. The environment was very uniform within treatments of the study, such that phenotypic and genetic correlation coefficients within treatments were nearly identical and interchangeable.

Correlations were not found to be consistently higher or lower in certain environments. However, changes in environmental conditions resulted in a restructuring of character correlations. The patterns of change of correlations over treatments differed for trait groups. Correlations involving size-related traits changed little across environments. They appear to constitute a single group of functionally related traits. Correlations involving allocation ratios

and resource-use efficiencies varied much more from one environment to another. Their patterns of change were also much more varied. Growth, shoot/root ratio, water-use efficiency and nitrogen-use efficiency should likely be considered as four separate traits, even if they are not necessarily evolving independently.

Clustering of environments based on Mahalanobis distances of phenotypic correlation matrices revealed that especially by inducing severe nitrogen-stress certain traits become de-coupled and other traits more closely linked. Water stress did not have a large influence on the relationships among traits in this study, but then water stress was not as severe as nitrogen stress.

11. Genetic trade-off between WUE and NUE

Evidence in the literature for a genetic trade-off between WUE and NUE is contradictory. In this study, the genetic correlation between WUE and NUE was found to change with environments. According to Stearns (1991), this is to be expected when there is plasticity for both traits and it can even lead to sign changes.

The data of the first experiment seemed to indicate a genetic trade-off for WUE and NUE: a negative genetic correlation implies pleiotropic and linkage effects that result in genotypes having either a high water-use efficiency or a high nitrogen-use efficiency, but not both. However, the genetic correlation is not negative in every environment. Instead, it varies with the environment according to a pattern, which is shown in figure 12. This pattern seems to indicate that, depending on the environment, different physiological processes are taking on a varying importance.

Photosynthetic capacity is affected by two types of factors: (1) those which affect the amount of photosynthetic "machinery"; and (2) those which regulate the activity of that machinery. For example, increased uptake of N or increased allocation of N to photosynthesis will result in an increase of the amount of photosynthetic machinery. This machinery, if active, will draw down the intercellular partial pressure of CO_2 (c_i) and, as a result, WUE will increase but NUE will decrease. Alternatively, if the activity of a given amount of machinery were to increase, that would also result in a higher WUE, this time accompanied by an increase in NUE. Thus, it is possible that the following scenario was operating in this study (R.Guy, pers. comm.):

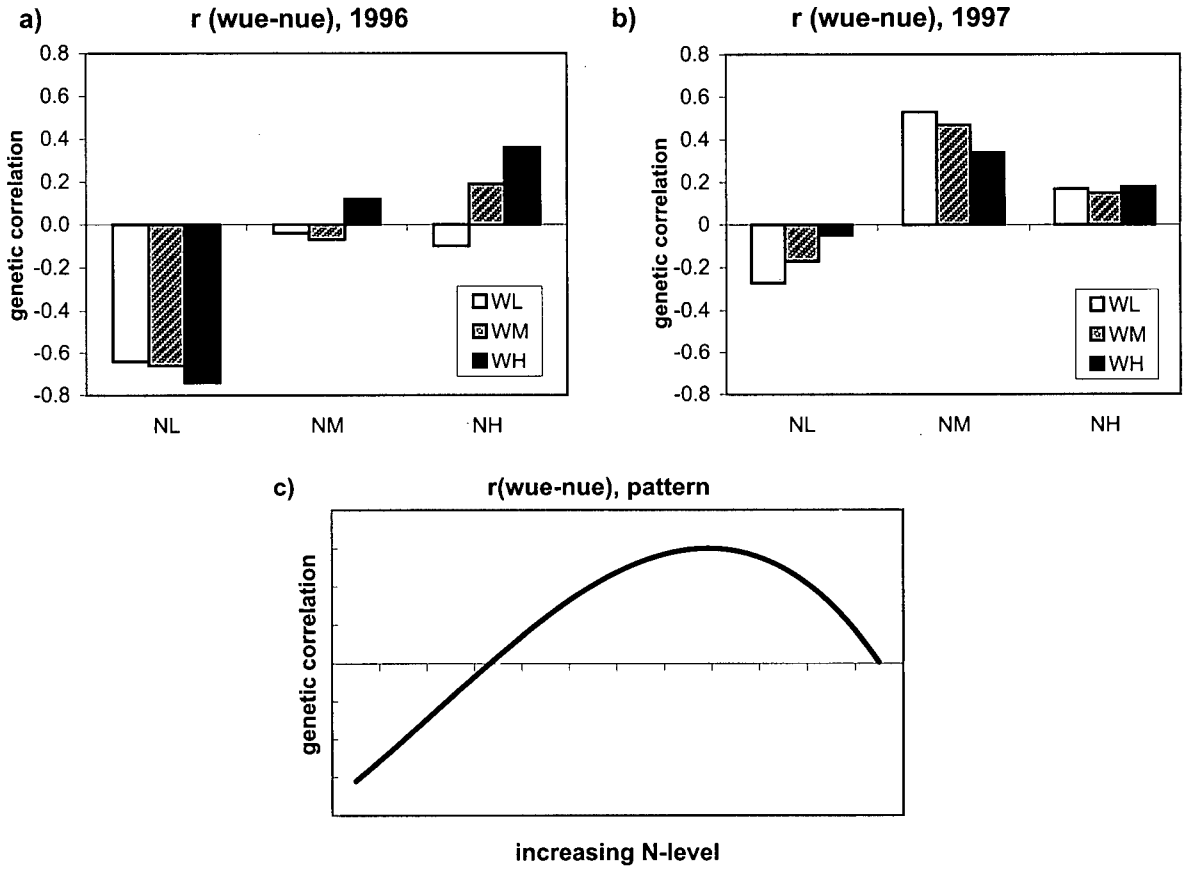

Figure 12. Genetic correlations between WUE and NUE as a function of N-level in the environment: a) results from 1996 experiment, b) 1997 experiment and c) inferred pattern.

(1) When the amount of available N is very low, the correlation between WUE and NUE may be driven by the ability of the plant to acquire and/or retain nitrogen. Genotypes with more nitrogen and therefore more photosynthetic machinery will have a higher WUE but a lower NUE. The resulting correlation is negative.

(2) When N is plentiful, luxury consumption of N results in a large amount of N being stored in the plant, unrelated (in the near term) to photosynthesis or structural growth. Differences in NUE would then reflect differences of the genotype in nitrogen-storage capacity, rather than the efficiency to grow with the available N. At high N levels, the genetic correlation between WUE and NUE then becomes obscured by the 'noise' caused by excess uptake of N. The resulting correlation would be low or zero.

(3) Somewhere in the middle, there is presumably a sufficient N concentration for optimum growth, where all nitrogen is used. Genetic differences in NUE might then simply reflect meristematic activity. Some genotypes would grow fast, others slow, with the same amount of

nitrogen. Photosynthesis is known to increase or decrease in response to sink strength (Taiz and Zeiger 1998): rapid growth of new plant organs locally depletes assimilate and phosphate levels, a signal for photosynthesis to synthesise more assimilates. Genotypes may differ in their relative growth of different plant organs. To the extent that leaves need relatively more water and more protein, and stems and roots need relatively less protein and loose less water, this would result in WUE, NUE and growth all being positively correlated.

Even the minor differences in r_A between water levels can be understood in this light: low water levels may make the small amount of nitrogen in the soil (which is water-soluble) even less available. An exception is the low nitrogen treatment with high water levels in the 1996 experiment. Some leaching of nutrients has probably taken place in this treatment, such that this treatment would have a lower instead of a higher nitrogen level than the dry, poor treatment of the same year. If so, this treatment also follows the general trend.

This variation of the genetic correlation over environmental variables complicates the situation considerably for a breeder. Clearly, if the speculations above hold, an estimate across treatments is of little use: though correcting for treatment effects, it is still mixing up the effect of these different processes in different environments. For selection purposes then, it may be necessary to redefine the traits of interest and either choose an appropriate environment or another trait to measure.

This may also explain why the across-treatment estimate for the first experiment is negative, whereas the one for the second experiment is positive. When considering the 1996 genetic correlations within environments, they do not appear to be contradictory to the 1997 estimates, considering that the 1996 plants were effectively more nitrogen-stressed than the 1997 plants because N-shortage hit them earlier (at a smaller size), often before they could develop a good root system. They are simply located more to the right of the curve in the inferred pattern.

12. Genetic trade-off between WUE and growth

Photosynthetic WUE is a function of the ratio of photosynthetic capacity over stomatal conductance. If a genotype attains a high WUE by lowering its stomatal conductance, all other things remaining equal, its photosynthetic rate and thus its growth will be reduced. However, when variation in carbon isotope discrimination is the result of changes in photosynthetic capacity, theory (Farquhar et al. 1982) predicts that discrimination values will be negatively

correlated with plant growth (Farquhar et al. 1989). In other words, $\delta^{13}C$ and WUE will be positively correlated with growth.

Genetic correlations between WUE and growth traits were positive. Thus, there is no genetic trade-off between WUE and growth. Holowachuk (1993) found the same and concluded that for lodgepole pine, genetic variation for WUE is mainly caused by genetic differences in photosynthetic capacity. Of course, stomatal conductance may still differ somewhat among genotypes. However, it is not the major cause of genotypic differences, for if it were, growth and WUE would be negatively correlated.

Gas exchange measurements, being momentary rather than time-integrated in nature, have deviated from what was expected based on the correlation between growth and $\delta^{13}C$. Cregg et al. (2000) found that for black spruce, $\delta^{13}C$ of families was related to stomatal conductance and not to photosynthetic capacity, while $\delta^{13}C$ was still positively correlated with growth. Also Aitken et al. (1995) found that their positive correlation between $\delta^{13}C$ and growth was not supported by gas exchange measurements in another study using similar Douglas-fir seed sources, where genetic variation was found for stomatal conductance but not for photosynthetic capacity. Apart from the momentary nature of gas exchange measurements, the choice of testing environments in the studies of Cregg et al. (2000) and Aitken et al. (1995) may also have influenced the value of the genetic correlation between WUE and growth traits. However, if a pattern for these correlations exists (figure 13), I have not been able to find an explanation for it yet. Lastly, net photosynthesis alone does not control tree growth (Johnsen et al. 1999).

Other studies have found variable results for the sign of the correlation between WUE and growth. This is true for agricultural crops as well as for forest trees, and only the latter will be mentioned here in more detail. Positive, negative and non-significant correlations have been found. There are differences among species (e.g. Zhang et al. 1996) and within the same species, the relationship depends on the environment (Aitken et al. 1995, Flanagan and Johnsen 1995, Donovan and Ehleringer 1994, Zhang et al. 1996). However, the changes could not be attributed to specific environmental factors. These authors calculated phenotypic correlations among individuals or family means within environments. Phenotypic and genetic correlations should therefore be relatively close and of the same sign. It is not clear to what extent the change (from positive or negative to non-significant) was the result from a lack of power in these studies.

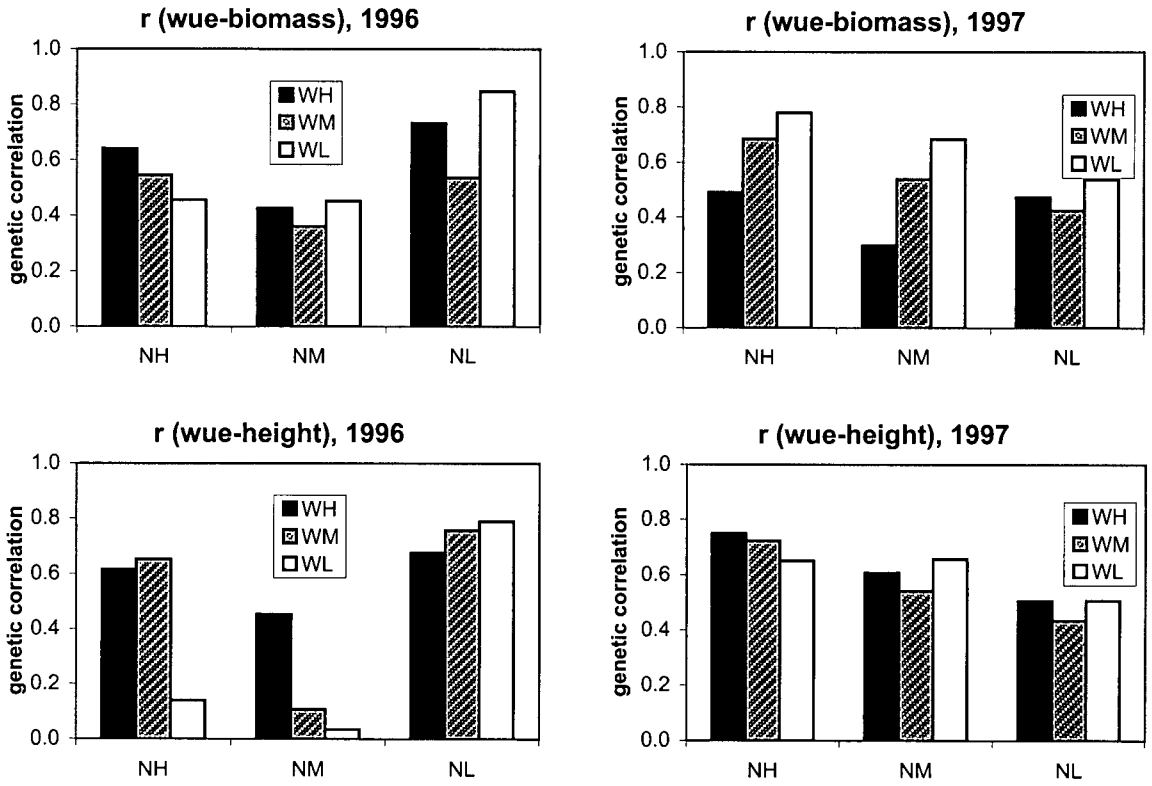

Figure 13. Genetic correlations between biomass and WUE or height and WUE as a function of the nitrogen level in the environment for the 1996 and 1997 experiment.

It has often been thought that water availability is a major factor in genotype-environment interaction for growth in the field (reviewed by Cregg et al. 2000) and that variation for WUE might explain the rank order changes. Yet, WUE, measured as $\delta^{13}C$, did not show rank change for genotypes across water levels for ponderosa pine (Cregg et al. 2000). They found no rank change for seed sources over years. Years varied mainly in temperature and rainfall levels, in other words, in available water. Geographic effects did result in rank change of genotypes, and may have included either phenologically important factors or site fertility factors or both.

Stomatal conductance is expected to vary with available water in the environment. The rate of responsiveness may vary among genotypes, possibly resulting in genotype rank change for WUE across water levels. Photosynthetic capacity is not expected to vary as much with water levels, but large changes with varying nitrogen availability in the environment could possibly result in genotype rank order changes for WUE over environments that vary in available nitrogen. It is possible that here, just like for the WUE-NUE correlation, N availability plays a role. No clear and repeatable pattern appears in figure 13, but then, any pattern may be

obscured by the change of genetic variances over environments, which does influence the genetic correlation, even after traits have been properly transformed. For growth, there is less genetic variation in stressed environments, while for WUE, there is an equal amount of genetic variation in stressed as in non-stressed environments. Also, genetic variation changes across environments more for biomass than it does for height, so different patterns may emerge for these correlations.

It is conceivable that a pattern might be found for the change of the correlation between WUE and growth if a better measure for NUE was found, i.e., a measure expressing plant nitrogen use in a way that is directly related to plant growth, such as total leaf nitrogen concentration. Unfortunately, Johnsen et al. (1999) found that leaf total nitrogen concentration in black spruce was uncorrelated (r_A zero and r_P very low) with any of the other traits. It may be a coincidence that there was no genetic variation for leaf-N in his study. Also, leaf-N expressed per area unit rather than per weight unit might have been better.

Nitrogen may well play a role in the adaptation and evolution of WUE, especially since, for lodgepole pine, genetic variation in WUE is mainly caused by variation in photosynthetic capacity, which is expected to change little across water levels. As a consequence, genotype-environment interaction for growth in the field may not have been caused by water availability for lodgepole pine for the genotypes studied. However, trees in stands may behave differently from individual trees in containers in controlled environments.

13. Resource-use efficiencies and field performance

Traditional approaches in agriculture have generally considered GxE as 'noise' and have avoided the use of very plastic genotypes (Finlay and Wilkinson 1963; Eberhardt and Russell 1966; Lin 1982; Lin et al. 1986). However, this strategy is not always optimal. Nissilä (1996) recommended, for barley germplasm in Finland, a generalist strategy for low-yielding sites and a specialist strategy for high yielding sites. The issue is therefore to distinguish pattern from noise and to associate that pattern of GxE interaction with environmental variables. Nissilä (1996) did this using multiple regression of yield factors on explicit environmental factors using a large number of field sites. The six field sites for which I have data are far fewer in number, may not cover a large enough environmental range, and a larger within-site variability than is commonly found for agricultural sites also makes characterisation of forested sites more difficult. Therefore

I did not follow a similar approach. All other applied analyses of field data have assumed implicit (unknown) environmental factors wherever clear patterns were discernible. The problem with an approach based on implicit rather than explicit factors is that the repeatability of such a pattern is unknown. The range of sites would have to be very large indeed to be sure that all the relevant environmental factors were somehow incorporated.

The nursery experiments of this study take a different approach: they take into account important environmental factors in a controlled manner. Though the choice of environmental factors is well founded, the way plants experience 'field environments' may involve yet other environmental factors, or attach a different relative importance to them. To test the hypothesis that the most relevant environmental effects have indeed been investigated in the nursery trial, the results of height growth in the nursery experiment and in the field trials were compared.

Two criteria revealed that stability or plasticity of a genotype in the experiment does not indicate stability or plasticity for its field performance. Rank order change in the field could not be predicted from or even related to rank order change in the nursery trial. There are several possible reasons. Firstly, there may be other environmental factors operating in the field, which have not been investigated here. Secondly, the range of environmental factors may not be well chosen in the nursery experiment or their relative importance may differ. Thirdly, one-year heights and ten-year heights are not perfectly correlated or even well-correlated, even in the same environment. Wide field-testing remains important to properly select the best growing genotypes.

Resource-use efficiencies measured at high nitrogen levels were moderately useful to predict field height growth on field sites. A high water-use efficiency was correlated with better height growth in the field, especially so on the wetter sites of the Shuswap Adams. This was also connected to low nitrogen use efficiency, but given that at high N levels NUE may be misleading due to luxury N consumption, the importance of NUE may be low. The Shuswap Adams sites may have had other factors in common than moisture level, though.

CONCLUSIONS

This study was set up to investigate genetic variation for resource-use efficiencies in lodgepole pine, which are presumed to be important traits with regard to adaptation. Specifically, water-use efficiency and nitrogen-use efficiency were considered, because water and nitrogen are important factors determining tree growth in nature. This was done in an attempt to clarify the role that these traits, as components of tree growth, play in producing genotype-by-environment interaction. It is important to know to what extent genotype rank change over planting sites reflects adaptation, which might be managed and exploited, and to what extent it reflects random genetic factors, which can be ignored in a breeding program. The idea behind the study is that if single traits and single environmental variables can't explain patterns of adaptation, then maybe multiple traits and multiple environmental variables can. This deeper understanding of genetic variation is a prerequisite to the proper management of the genetic resource.

Genetic variation exists for mean trait expression as well as for plasticity for both water-use efficiency and nitrogen-use efficiency. Genetic variability within populations tends to be high despite pronounced differentiation among populations. This corresponds to the plants having a large 'fundamental niche' (Rehfeldt et al. 1999), where they can survive and can be considered adapted. Competitive exclusion in nature may lead to a narrow distribution ('realised niche', Rehfeldt et al. 1999) of genotypes, yet adaptation is not as closely linked to the environment as the actual 'realised niche' would have us believe. Though the findings of Rehfeldt et al. (1999) were limited to climate variables, they did include moisture level (but not nitrogen level) and their conclusions seem to be valid also for the traits considered in this study.

Correlations of adaptive traits with single environmental variables were weak and difficult to interpret because different environmental source variables co-vary. The combined evidence from different studies indicates that WUE may evolve in response to a combination of rainfall, temperature, and elevation. Apart from that, there is the possibility that WUE evolves strongly in response to nitrogen levels of the environment, or that growth rate evolves in response to temperature and WUE merely 'rides along' as a result of the sink effect. With regard to the evolution of NUE, the results from this study are much less clear. A wider sampling including more coastal genotypes from both very wet and very dry sites may be needed in order to get a clearer picture of the occurrence and importance of genetic variation for NUE.

Even when the multiple traits of this study are considered jointly, the patterns of

adaptation are not narrowly defined. Where the patterns of adaptation to source environmental variables differ for different traits, multivariate techniques which simplify the picture by reducing the number of dimensions are in fact hiding important information. It seems that there are several ways in which genotypes can manage to grow under stress. There is not one optimal provenance that the breeder must select in order to deal with stress environments, but rather a range of optima from which the decision maker can choose. The range of optima may be aligned along more than one dimension. Several provenances may conceivably be mixed in the planting site in order to achieve the breeder's goals.

Based on this study, it is not necessary nor is it possible for the breeder to select special seed sources based on resource-use efficiencies to ensure the survival and productivity of lodgepole pine on marginal sites. Neither should special seed sources be selected based on a specific combination of traits.

Water-use efficiency may still be used for indirect selection for growth, but it is an expensive trait to measure, rank order change for WUE itself occurs, and the choice of the best testing environment for WUE has to be carefully evaluated. There is the advantage of early selection, but that is not unique to resource-use efficiencies and exists for height growth as well. All these complications imply that the use of WUE for indirect selection for growth is by no means straightforward and may not be practically feasible.

Although the ability to grow well with minimal amounts of nitrogen may be important in nature, genetic variation for NUE in the interior families of this study was limited. It may be larger for coastal populations, which were not widely sampled in this study, or it may be larger on a larger geographical scale. Genetic differences in plasticity for NUE did exist, which may indicate that plasticity itself has some evolutionary significance here. However, other factors seem to indicate that NUE, expressed as C/N ratio, may be a misleading measure, reflecting different underlying phenomena at greatly different environmental N levels. Without more information then, there is no strong argument to justify selection for increased NUE, measured as C/N ratio, for interior lodgepole pine.

WUE and NUE are intricately linked by means of a plastic trade-off, such that any evolutionary force acting on one of them must also act on the other. The resulting genetic correlation varies in sign depending on the environment but indicates a trade-off at intermediate nitrogen levels. WUE and growth are positively correlated, indicating that genotypes differ in photosynthetic capacity rather than in stomatal conductance. Pair-wise correlations indicate that all traits are interrelated into a network. Environmental nitrogen levels strongly influence trait

relationships in that network, especially the correlations with NUE.

There is an integrated physiological system that differs among genotypes, that reacts as a whole to multiple environmental field variables and evolves as a whole in adaptation to those source variables. It is possible that this study hasn't considered enough factors and traits to completely clarify the patterns of adaptation. Two environmental factors, water and nitrogen, were shown to interact in the creation of environmental niches. Yet, the importance of the factors water and nitrogen in the creation of GxE interaction in the field could not be confirmed, since no similar groupings of genotypes emerged for field and nursery data.

Plasticity plays an important role in the adaptation of plants to their environments. Genecological studies should therefore take into account levels and patterns of variation, not just for trait means, but also for plasticity of traits with regard to important environmental variables. Different traits are interrelated, physiologically (determining multi-trait expression) as well as genetically (determining evolution). As a consequence, plant evolution and adaptation are extremely complex phenomena. A study like this one, where a few of the factors are considered in detail, may not lead to a comprehensive understanding of the whole. It is therefore necessary to focus on specific traits and environments of relevance to breeding and a careful balance of costs and benefits has to be carried out.

Breeding for generalist genotypes may be preferred because it results in the easiest deployment program, but care has to be taken that the multiple strategies of genotypes to deal with various environments are preserved. In this context, multiple trait selection involving physiological traits may not offer any advantages over simple selection for growth in the field, leaving it up to each genotype as to how they achieve that growth. Based on this study, no recommendations can be made with regard to how field test sites should be chosen, based on environmental factors, in order to best deal with GxE.

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APPENDICES

Appendix 1. Theory of carbon isotope discrimination during photosynthesis.
Sources: Farquhar et al. 1989; Guy 1995.

Most variations in isotopic composition are the result of isotope discrimination in chemical reactions. The major components contributing to carbon isotope fractionation during C_3 photosynthesis are (1) the differential diffusion of CO_2 containing ^{12}C and ^{13}C through the stomates, and (2) the enzymatic fractionation by Rubisco. These two factors need to be weighted by the relative limitation of CO_2 partial pressure difference imposed by the step involved. This leads to:

$$\Delta = a\ (c_a - c_i)/c_a + b\ c_i/c_a = a + [(b-a)\ c_i/c_a]$$

Where Δ is the carbon isotope discrimination, a is the fractionation occuring due to diffusion in air (0.44 ‰), b is the net fractionation caused by carboxylation (29 ‰, mainly discrimination by Rubisco), and c_a and c_i are the ambient and intercellular partial pressures of CO_2, respectively.
This can also be written in terms of carbon isotope composition ($\delta^{13}C$, relative to PD belemnite):

$$\delta^{13}C_{plant} = \delta^{13}C_{atmosphere} - a - [(b-a)\ c_i/c_a]$$

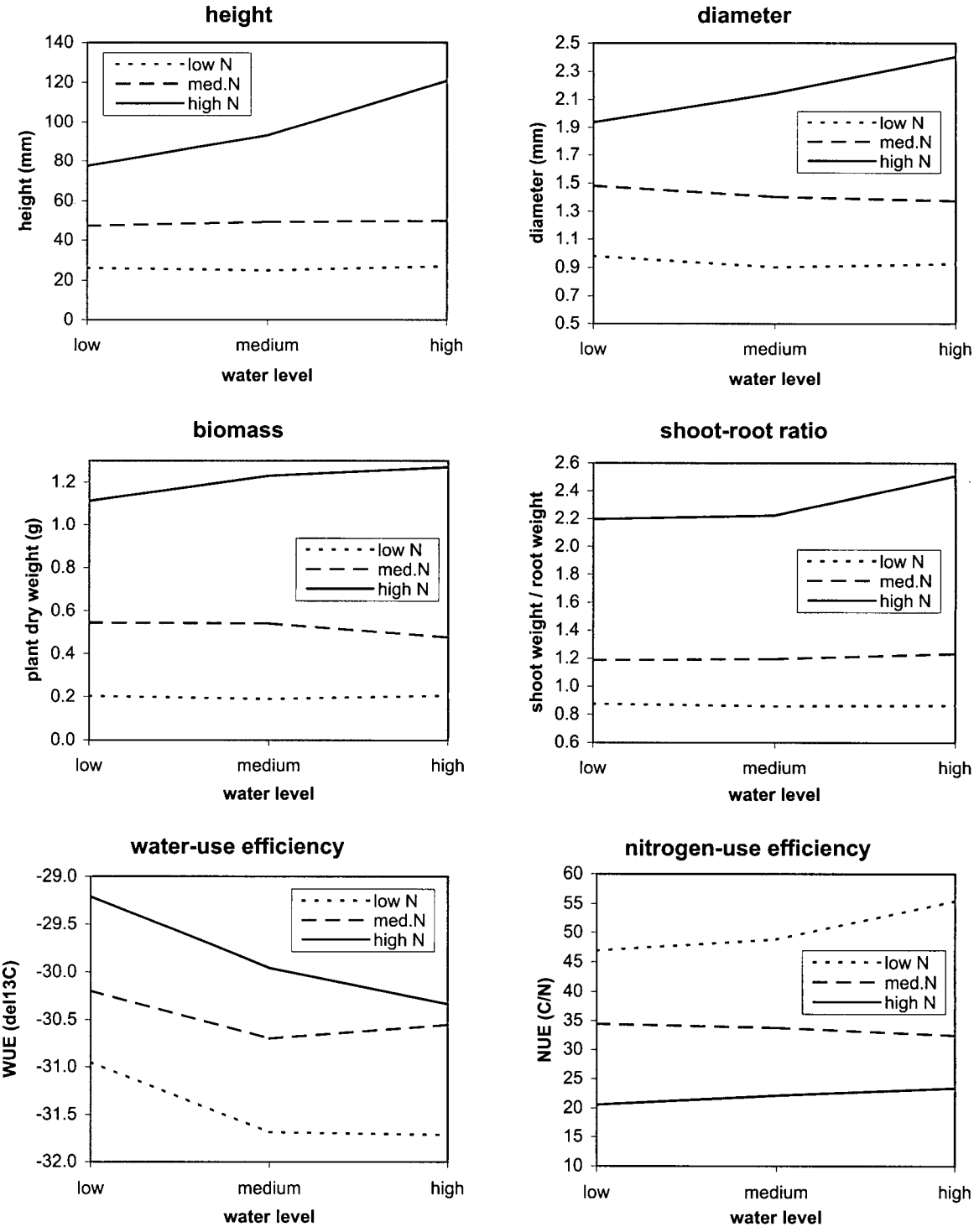
With the atmospheric isotope composition being 8 ‰ , the isotopic composition for C_3 plants can theoretically range between –12,4 ‰ (c_i/c_a=0) and –37 ‰ (c_i/c_a=1), though the range found in plants is smaller than that.
Intrinsic WUE is determined as the instantaneous ratio of CO_2 assimilation rate of a leaf, A, to its transpiration rate, E, and is given by

$$\frac{A}{E} = \frac{(c_a - c_i)\ g_{sCO_2}}{(p_i - p_a)\ g_{sH_2O}} = 0.625\ \frac{(c_a - c_i)}{(p_i - p_a)}$$

Where c_a and c_i are atmospheric and intercelular partial pressures of CO_2, p_i and p_a are the intercellular and atmospheric partial pressures of water vapour, and g_sCO_2 and g_sH_2O are the stomatal conductances of CO_2 and H_2O respectively.

Thus, WUE and Δ (or $\delta^{13}C$) are related via c_i. For plants growing in the same environment, both WUE and $\delta^{13}C$ are determined by c_i.

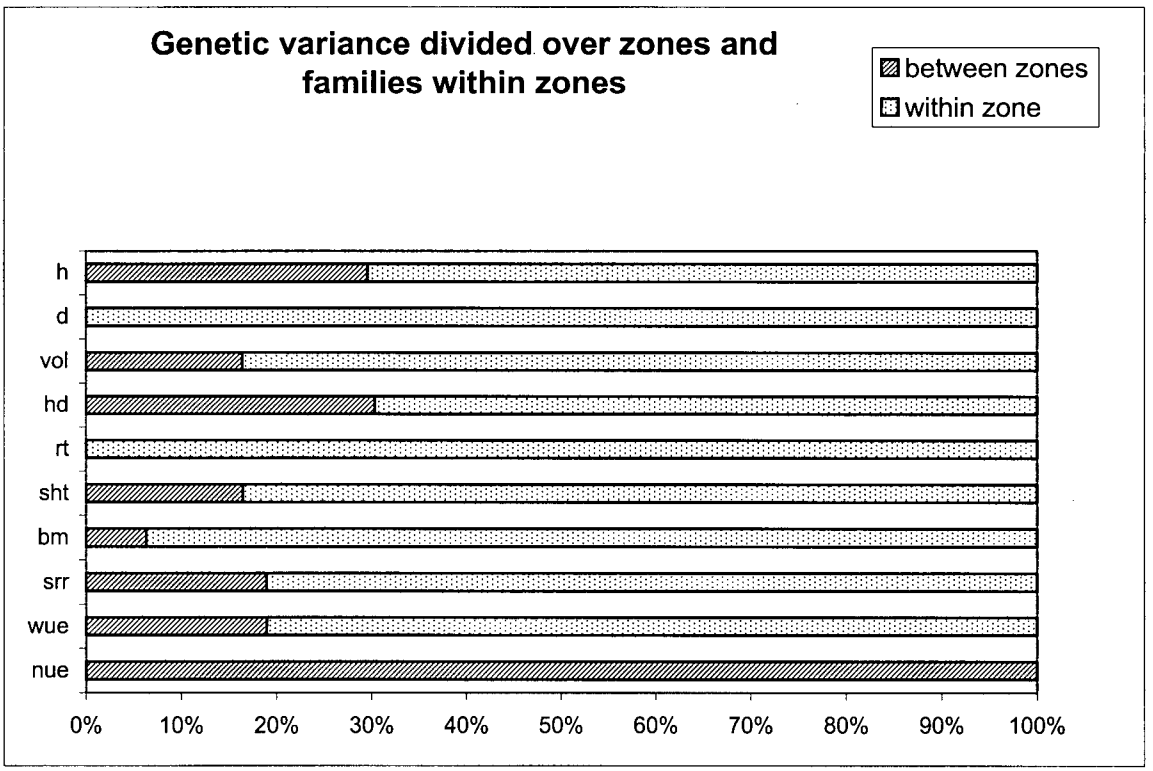
Appendix 2. General response patterns (1996 experiment). Standard errors for height range from 0.8 mm to 1.8 mm, the standard error for diameter is 0.04 mm, and standard errors for biomass range from 8 to 21 mg. Standard errors for srr range from 0.02 to 0.06, the standard error for WUE is 0.14, and standard errors for NUE range from 0.3 to 1.5.



Appendix 3. Effect of the factor 'breeding zone' (z) in the analysis of variance of the 1997 experiment.

Variance components as a percentage of total variance for ten traits.

Var.comp.	h	d	vol	hd	rt	sht	bm	srr	wue	nue
W	13.2	3.4	7.5	14.9	5.3	3.7	4.3	2.1	11.1	1.3
N	49.1	68.0	68.2	9.0	29.7	72.9	64.5	68.9	52.4	93.9
W*N	5.3	4.0	2.7	0.0	2.8	1.4	2.2	0.0	0.0	1.7
Z	2.8	0.0	0.9	5.2	0.0	0.7	0.3	1.1	1.2	0.0
F	6.6	3.4	4.6	11.9	6.2	3.6	3.8	4.6	5.0	0.0
W*Z	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N*Z	0.0	0.3	0.0	0.4	0.0	0.0	0.0	0.0	0.4	0.1
W*N*Z	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
W*F	0.3	0.4	0.3	0.1	0.2	0.0	0.0	0.1	0.7	0.0
N*F	1.0	0.6	0.7	1.9	3.7	1.3	2.2	0.7	3.7	0.4
W*N*F	0.1	0.0	0.0	1.1	0.1	0.0	0.0	0.6	0.0	0.0
e2	21.8	19.9	15.1	55.4	51.9	16.3	22.8	22.0	25.5	2.6
e1	0.7	2.6	1.3	2.4	2.0	0.4	0.5	1.3	1.1	0.5



Appendix 4. Pair-wise comparisons of treatment means, 1996 data.

Comparisons are listed in the first row, with treatments indicated by numbers. A description of treatments in terms of water and nitrogen levels follows in rows 2 and 3. For each trait, significant differences ($\alpha = 0.05$) are indicated with an asterisk.

Response to nitrogen at different water levels									
	1 2	2 3	1 3	4 5	5 6	4 6	7 8	8 9	7 9
W-level	H H	H H	H H	M M	M M	M M	L L	L L	L L
N-level	H M	M L	H L	H M	M L	H L	H M	M L	H L
h	*	*	*	*	*	*	*	*	*
d	*	*	*	*	*	*	*	*	*
bm	*	*	*	*	*	*	*	*	*
srr	*	*	*	*	*	*	*	*	*
wue		*	*	*	*	*	*	*	*
nue	*	*	*	*	*	*	*	*	*

Response to water at different nitrogen levels									
	1 4	4 7	1 7	2 5	5 8	2 8	3 6	6 9	3 9
W-level	H M	M L	H L	H M	M L	H L	H M	M L	H L
N-level	H H	H H	H H	M M	M M	M M	L L	L L	L L
h	*	*	*						
d	*	*	*						
bm				*		*			
srr	*		*						
wue		*	*					*	*
nue	*	*	*				*		*

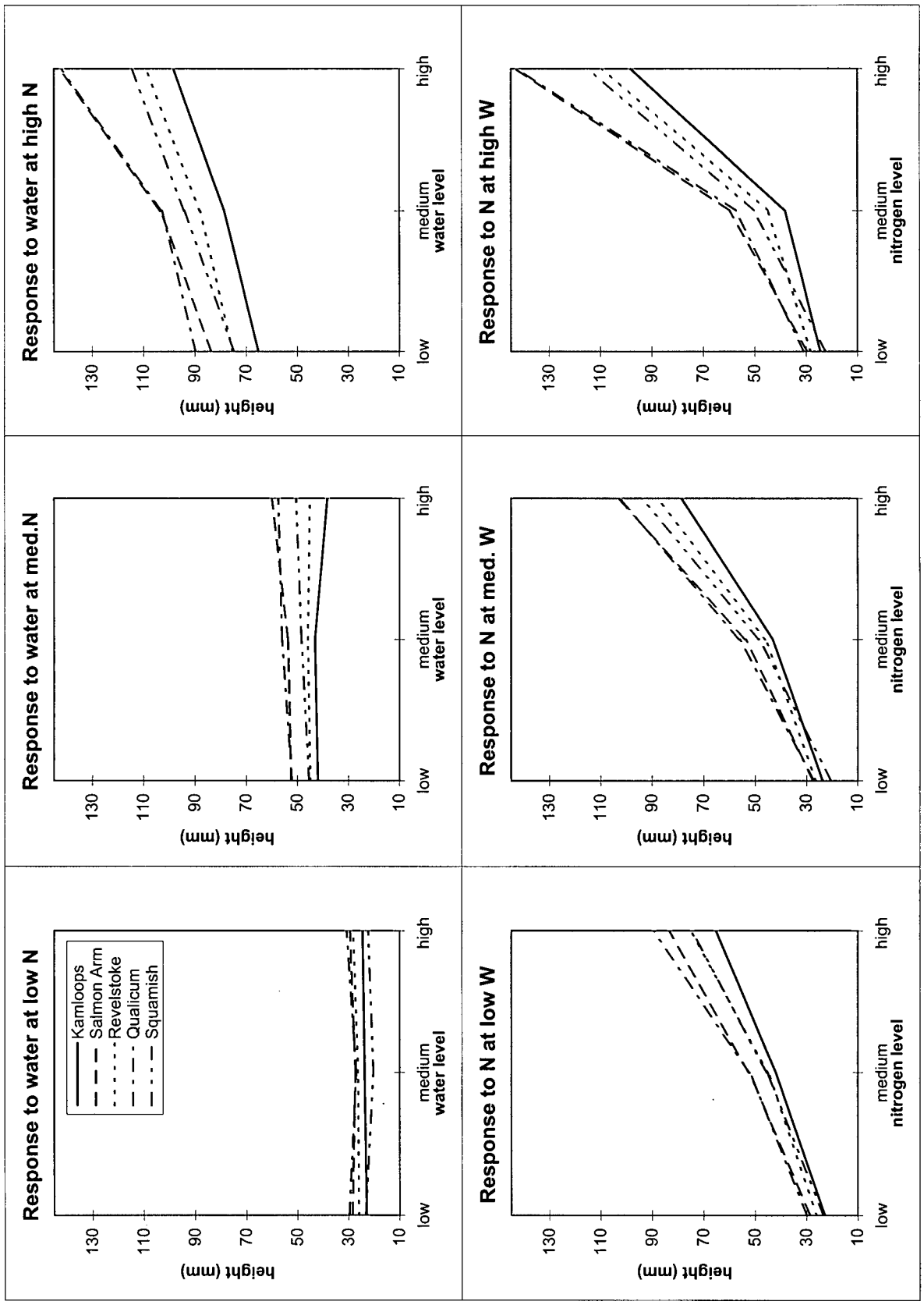
Appendix 4, ctd. Pair-wise comparisons of treatment means, 1997 data.

Comparisons are listed in the first row, with treatments indicated by numbers. A description of treatments in terms of water and nitrogen levels follows in rows 2 and 3. For each trait, significant differences ($\alpha = 0.05$) are indicated with an asterisk.

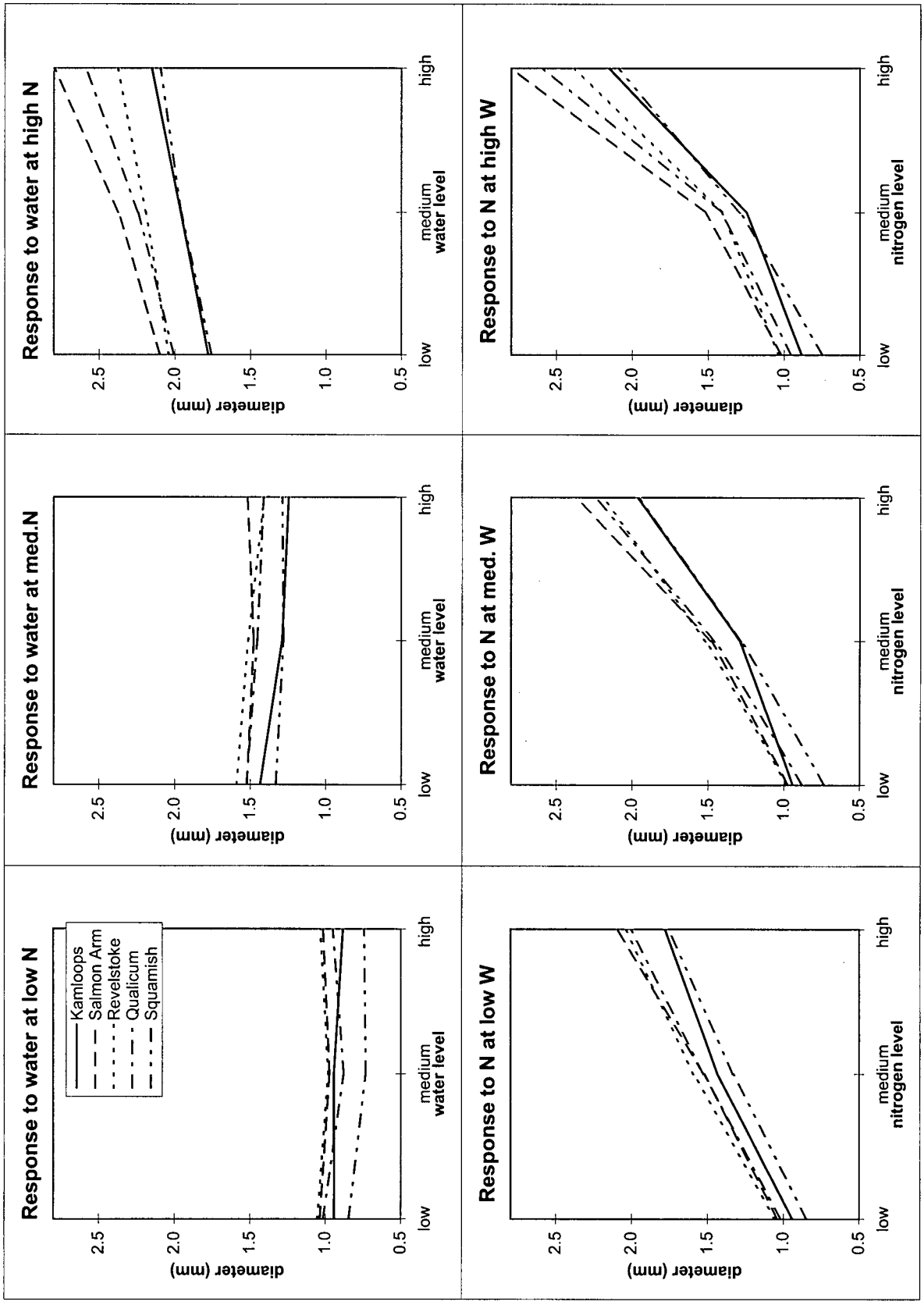
Response to nitrogen at different water levels									
	1 2	2 3	1 3	4 5	5 6	4 6	7 8	8 9	7 9
W-level	H H	H H	H H	M M	M M	M M	L L	L L	L L
N-level	H M	M L	H L	H M	M L	H L	H M	M L	H L
h	*	*	*	*	*	*	*	*	*
d	*	*	*	*	*	*	*	*	*
vol	*	*	*	*	*	*	*	*	*
hd	*	*	*	*	*	*	*	*	*
rt	*	*	*	*		*	*	*	*
sht	*	*	*	*	*	*	*	*	*
bm	*	*	*	*	*	*	*	*	*
srr		*	*		*	*		*	*
wue	*	*	*	*	*	*	*	*	*
nue	*	*	*	*	*	*	*	*	*

Response to water at different nitrogen levels									
	1 4	4 7	1 7	2 5	5 8	2 8	3 6	6 9	3 9
W-level	H M	M L	H L	H M	M L	H L	H M	M L	H L
N-level	H H	H H	H H	M M	M M	M M	L L	L L	L L
h		*	*	*	*	*	*	*	*
d					*	*	*	*	*
vol		*	*	*	*	*	*	*	*
hd		*	*		*	*	*	*	*
rt	*			*		*	*	*	*
sht		*	*		*	*	*	*	*
bm	*		*	*	*	*	*	*	*
srr	*	*	*		*	*		*	*
wue	*	*	*	*	*	*	*	*	*
nue				*	*	*	*	*	*

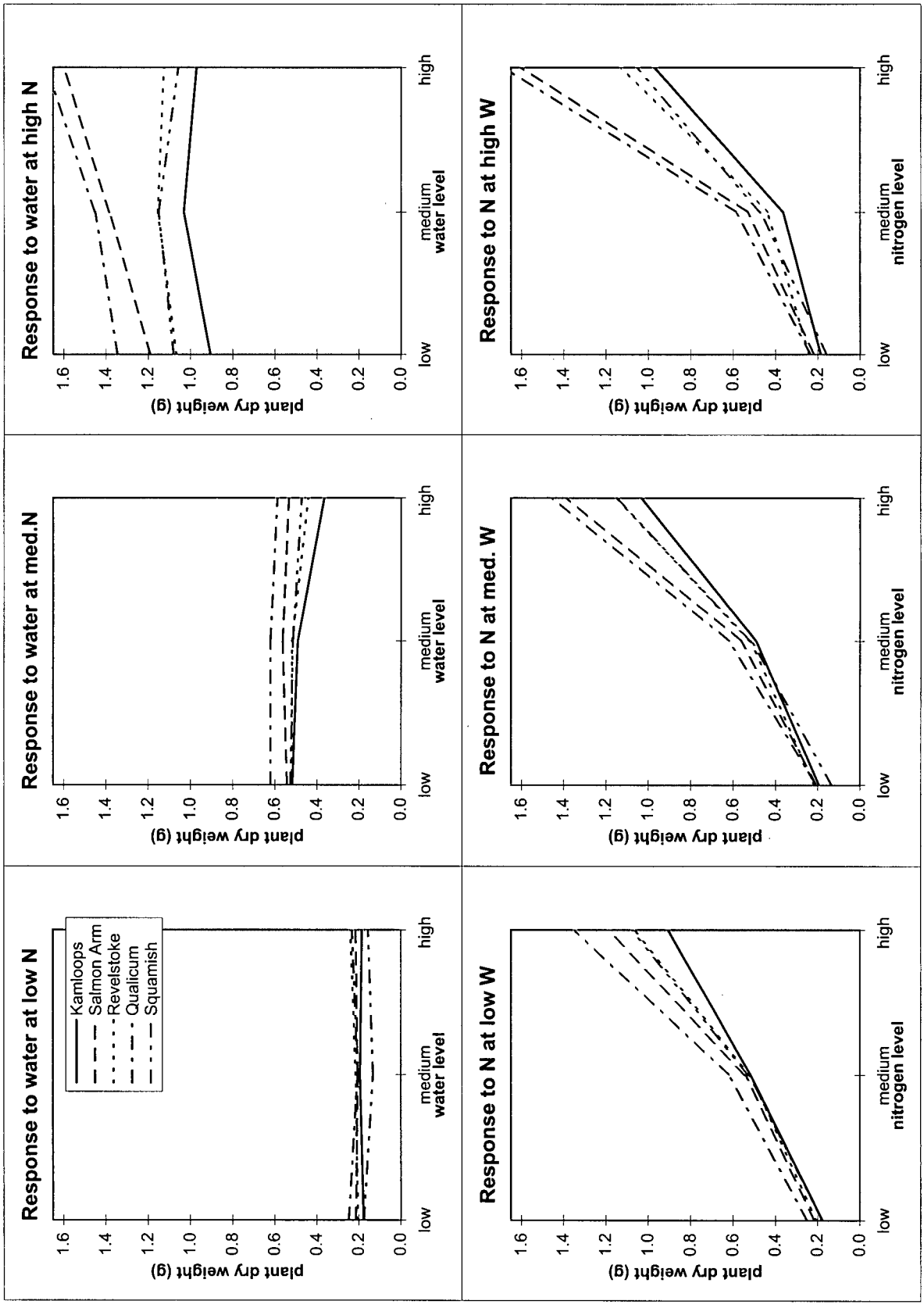
Appendix 5. Response of height for five populations to water and nitrogen levels in the environment. Standard errors range from 1.1 mm for small plants to 2.9 mm for large plants.



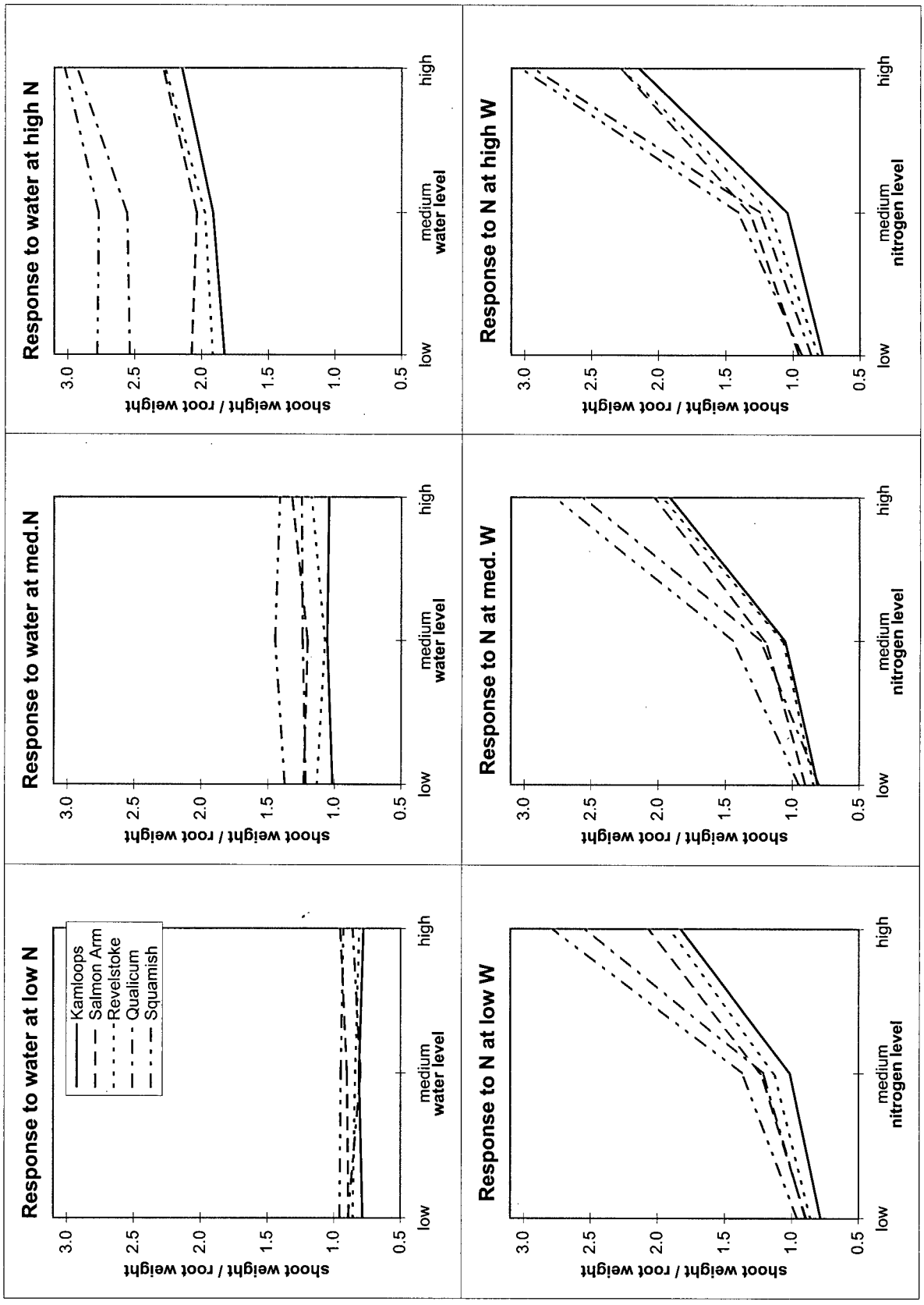
Appendix 5, ctd. Response of diameter for five populations to water and nitrogen levels in the environment. Standard error: 0.05 mm.



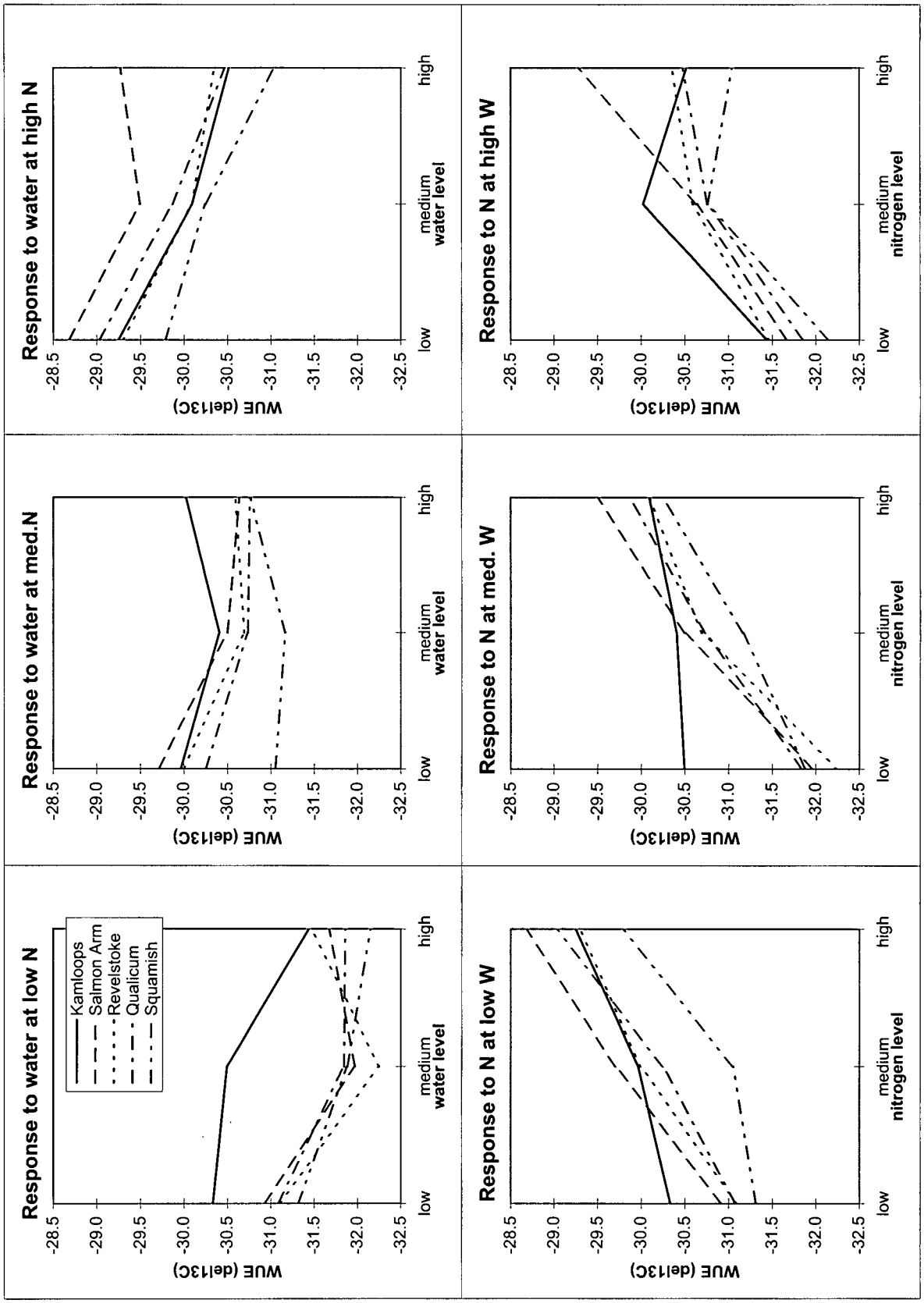
Appendix 5, ctd. Response of biomass for five populations to water and nitrogen levels in the environment. Standard errors range from 0.015 g for small plants to 0.05 g for large plants.



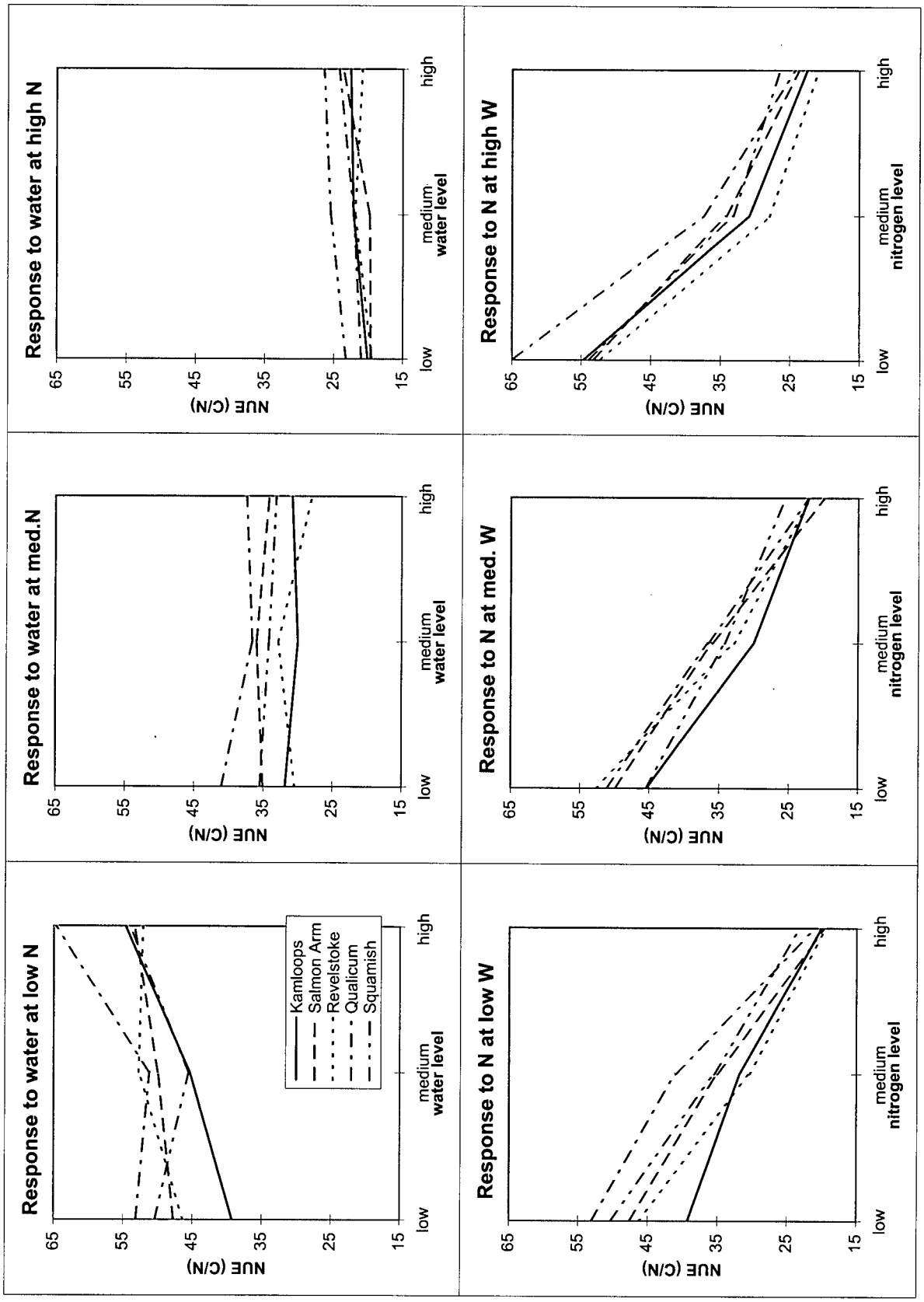
Appendix 5, ctd. Response of shoot-root ratio for five populations to water and nitrogen levels in the environment. Standard errors range from 0.03 for large ratio's to 0.10 for small ratio's.



Appendix 5, ctd. Response of water-use efficiency for five populations to water and nitrogen levels in the environment. Standard error: 0.23.



Appendix 5, ctd. Response of nitrogen-use efficiency for five populations to water and nitrogen levels in the environment. Standard errors range from 0.5 for small values of NUE to 5 for large values of NUE.



Appendix 6. Multiple range tests on provenance means within treatments for ten traits. Provenances joined by the same line are not significantly different according to the multiple range test.

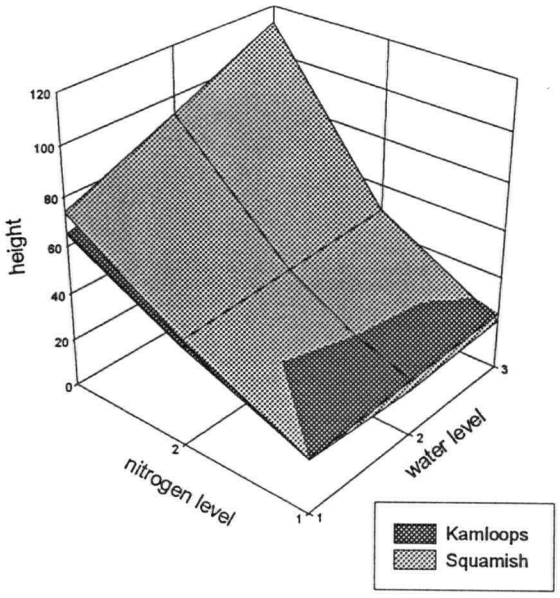
TR	h (mm)	d (mm)	vol	hd	rt (mg)	sht (mg)	bm (g)	srr	wue	nue
1	14.3 B	2.80 B	278.1 B	0.56 Q	488 B	1246 Q	1.68 Q	3.02 S	-29.27 B	26 S
1	14.2 Q	2.59 Q	236.5 Q	0.56 S	432 Q	1108 B	1.60 B	2.93 Q	-30.35 C	24 Q
1	11.5 S	2.38 C	153.0 C	0.52 B	347 C	790 S	1.12 C	2.30 B	-30.47 Q	24 B
1	10.9 C	2.15 A	125.0 S	0.47 A	311 A	775 C	1.06 S	2.27 C	-30.52 A	23 A
1	9.8 A	2.10 S	112.6 A	0.47 C	265 S	658 A	0.97 A	2.15 A	-31.04 S	21 C
2	6.0 B	1.52 B	34.3 B	0.42 Q	260 Q	325 Q	0.59 Q	1.41 S	-30.02 A	37 Q
2	5.7 Q	1.41 Q	28.3 Q	0.40 B	229 B	301 B	0.53 B	1.32 B	-30.59 C	34 B
2	5.1 S	1.41 C	22.1 C	0.40 S	203 C	277 S	0.47 S	1.25 Q	-30.64 B	33 S
2	4.5 C	1.29 S	20.8 S	0.33 C	193 S	235 C	0.44 C	1.17 C	-30.76 Q	31 A
2	3.8 A	1.25 A	14.6 A	0.32 A	178 A	185 A	0.36 A	1.04 A	-30.76 S	28 C
3	3.1 Q	1.03 C	7.4 B	0.34 Q	133 C	108 Q	0.24 C	0.95 B	-31.44 A	65 Q
3	2.9 B	1.02 B	7.3 C	0.33 S	123 Q	107 C	0.23 Q	0.93 S	-31.47 C	55 A
3	2.8 C	0.95 Q	6.8 Q	0.31 B	110 B	105 B	0.22 B	0.86 Q	-31.67 B	54 S
3	2.4 A	0.88 A	4.7 A	0.29 A	104 A	82 A	0.19 A	0.81 C	-31.86 Q	53 B
3	2.2 S	0.74 S	3.0 S	0.29 C	80 S	76 S	0.16 S	0.78 A	-32.15 S	52 C
4	10.3 B	2.38 B	145.8 B	0.49 S	457 B	1038 Q	1.45 Q	2.77 S	-29.50 B	25 S
4	10.3 Q	2.23 Q	126.4 Q	0.47 Q	410 Q	925 B	1.38 B	2.56 Q	-29.87 Q	22 A
4	9.4 S	2.19 C	104.7 C	0.44 B	389 C	843 S	1.16 C	2.03 B	-30.09 C	22 Q
4	8.8 C	1.96 S	89.7 S	0.41 A	354 A	765 C	1.15 S	1.97 C	-30.09 A	22 C
4	7.9 A	1.96 A	74.6 A	0.41 C	306 S	676 A	1.03 A	1.91 A	-30.25 S	20 B
5	5.6 Q	1.52 C	29.4 Q	0.39 Q	277 Q	342 Q	0.62 Q	1.45 S	-30.41 A	37 Q
5	5.4 B	1.48 B	29.0 B	0.39 S	256 B	305 B	0.56 B	1.24 Q	-30.50 B	36 B
5	4.8 S	1.45 Q	26.1 C	0.37 B	251 C	302 S	0.52 C	1.20 B	-30.70 C	34 S
5	4.6 C	1.30 A	19.6 S	0.34 A	239 A	265 C	0.51 S	1.07 C	-30.74 Q	33 C
5	4.3 A	1.28 S	18.1 A	0.31 C	210 S	251 A	0.49 A	1.05 A	-31.17 S	30 A
6	2.7 B	0.98 C	6.3 B	0.32 Q	117 C	97 C	0.21 C	0.95 S	-30.50 A	53 C
6	2.7 Q	0.97 B	6.2 C	0.29 S	113 Q	96 B	0.20 Q	0.90 B	-31.85 Q	51 Q
6	2.6 C	0.94 A	5.1 A	0.29 B	107 B	92 Q	0.20 B	0.84 C	-31.88 S	50 B
6	2.4 A	0.88 Q	5.1 Q	0.28 C	106 A	87 A	0.19 A	0.81 A	-31.97 B	46 S
6	2.0 S	0.73 S	2.6 S	0.27 A	66 S	65 S	0.13 S	0.80 Q	-32.25 C	45 A
7	9.0 Q	2.10 B	91.4 B	0.45 Q	387 B	963 Q	1.34 Q	2.78 S	-28.68 B	23 S
7	8.4 B	2.04 C	89.6 Q	0.43 S	379 Q	800 B	1.19 B	2.53 Q	-29.03 Q	21 Q
7	7.5 C	2.01 Q	77.7 C	0.40 B	366 C	787 S	1.08 S	2.07 B	-29.25 A	20 A
7	7.5 S	1.78 A	57.3 S	0.37 C	320 A	699 C	1.07 C	1.92 C	-29.30 C	20 B
7	6.5 A	1.76 S	51.3 A	0.37 A	285 S	583 A	0.91 A	1.83 A	-29.79 S	19 C
8	5.2 B	1.59 C	30.0 B	0.35 S	281 Q	338 Q	0.62 Q	1.37 S	-29.71 B	41 Q
8	5.2 Q	1.53 Q	29.9 Q	0.35 B	257 A	301 S	0.54 B	1.23 B	-29.97 A	35 S
8	4.5 S	1.52 B	28.1 C	0.35 Q	245 B	294 B	0.53 S	1.21 Q	-30.00 C	35 B
8	4.5 C	1.44 A	21.3 A	0.30 A	244 C	273 C	0.52 C	1.13 C	-30.25 Q	32 A
8	4.2 A	1.33 S	19.8 S	0.29 C	222 S	259 A	0.52 A	1.01 A	-31.05 S	30 C
9	3.0 Q	1.05 C	7.5 B	0.31 Q	129 Q	119 Q	0.25 Q	0.96 S	-30.33 A	53 Q
9	2.8 B	1.04 B	7.5 Q	0.29 S	113 B	100 B	0.21 B	0.89 Q	-30.92 B	50 S
9	2.6 C	1.02 Q	7.1 C	0.28 B	109 C	94 C	0.20 C	0.89 B	-31.08 Q	48 B
9	2.3 S	0.94 A	5.0 A	0.25 C	98 A	88 S	0.18 A	0.86 C	-31.10 C	46 C
9	2.3 A	0.85 S	4.1 S	0.25 A	87 S	78 A	0.18 S	0.78 A	-31.31 S	39 A

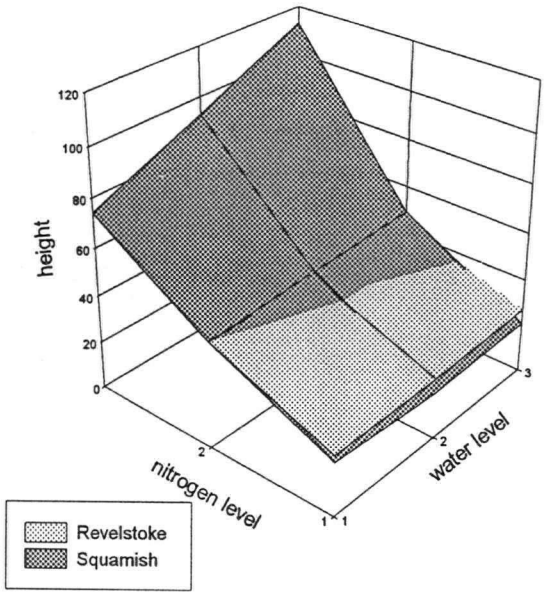
Appendix 7. Break-down of tests for provenance rank order change into quadruples for a few selected traits.

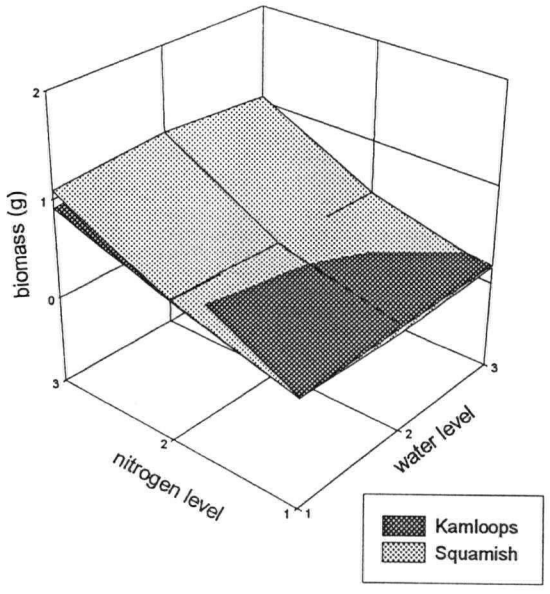
The break-down shows which genotypes change rank and over which environments they change rank. In the first row, 36 combinations of two environments are listed (one number for each environment). In the first column, ten combinations of two genotypes are listed. Thus, for each trait, 360 quadruples must be evaluated. Inside the table, the number 0 indicates no crossover at all, 1 indicates a crossover which is not significant, and 2,3 and 4 indicate crossovers significant according to the interaction-wise, the comparison-wise and the Azzalini-Cox criterion respectively.

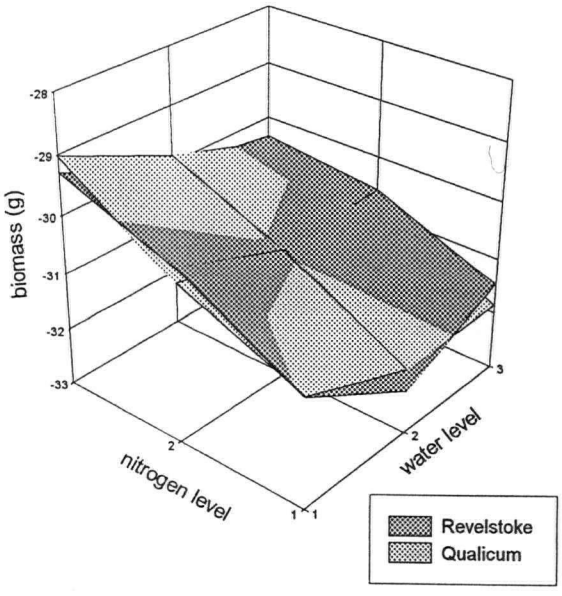
	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 3	2 4	2 5	2 6	2 7	2 8	2 9	3 4	3 5	3 6	3 7	3 8	3 9	4 5	4 6	4 7	4 8	4 9	5 6	5 7	5 8	5 9	6 7	6 8	6 9	7 8	7 9	8 9
bm																																				
AB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AQ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AS	0	2	0	0	2	0	0	1	2	0	0	4	0	0	1	2	1	0	2	1	0	0	3	0	0	1	1	0	0	1	4	1	0	0	1	1
BC	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	1	1	1	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
BQ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CQ	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	1	1	1	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
CS	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	1	0	0	1	1
QS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
srr																																				
AB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AQ	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
AS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BQ	2	3	0	0	4	0	1	0	0	2	1	0	2	0	1	3	1	0	3	0	1	0	4	0	1	0	1	0	1	0	4	0	1	1	0	1
BS	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CQ	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	2	0	0	0	2	0	0	0	2	2	1	0	0	0
CS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
QS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
wue																																				
AB	3	1	0	1	4	0	0	3	0	3	0	0	3	1	0	1	0	0	1	1	0	1	3	0	0	3	0	1	1	0	3	1	0	0	3	1
AC	1	1	0	1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
AQ	1	1	0	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	1	1	0	1	0	0	1	0	0	1	1	0
AS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BC	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BQ	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
BS	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
CQ	0	0	1	0	1	1	0	1	0	1	0	1	1	0	1	1	0	2	1	0	1	1	0	0	1	0	1	1	0	1	0	1	0	1	0	1
CS	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	1	0	0	0	2	0	0	0	2	2	1	0	0	0
QS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
nue																																				
AB	0	1	2	0	0	1	0	0	1	3	0	0	1	0	0	0	1	1	0	1	1	4	2	0	2	4	0	1	0	0	1	0	0	1	1	0
AC	0	0	0	2	3	0	0	3	0	0	2	3	0	0	3	0	1	1	0	0	1	1	1	0	0	1	0	1	1	0	1	1	0	0	1	1
AQ	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
AS	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BC	0	0	4	0	1	0	0	0	0	4	0	1	0	0	0	1	0	1	0	0	0	2	0	1	3	1	1	0	0	0	1	1	1	0	0	0
BQ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BS	1	0	0	1	2	0	0	0	1	1	0	0	1	1	1	0	1	1	0	0	0	1	2	0	0	0	0	1	1	1	2	1	1	0	0	0
CQ	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
CS	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	2	0	0	0	1	0	0	0	2	2	1	0	0	0
QS	3	3	0	2	2	0	3	1	0	3	0	0	3	0	0	3	0	0	3	0	0	2	2	0	3	1	0	2	0	0	2	0	0	3	1	0

Appendix 8. Three dimensional illustration of significant intersections between response surfaces

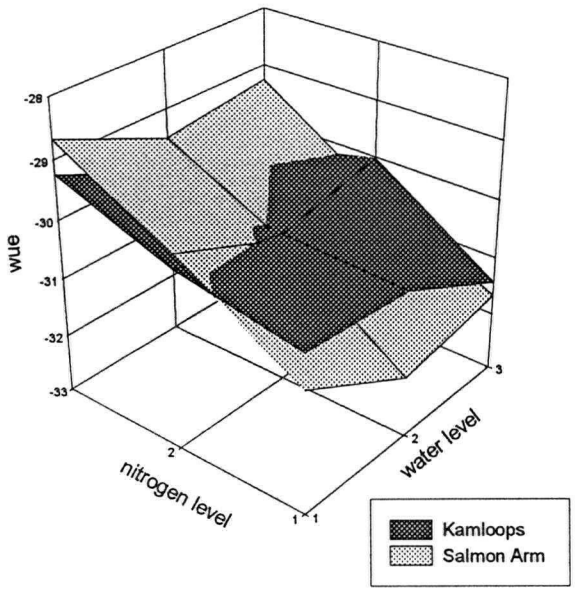


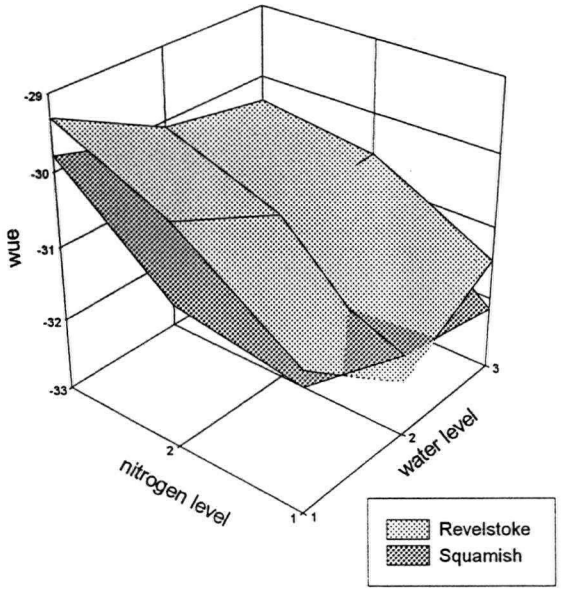




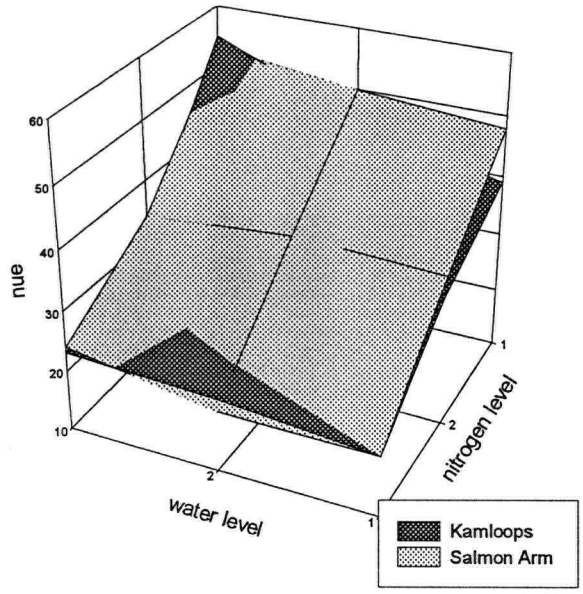


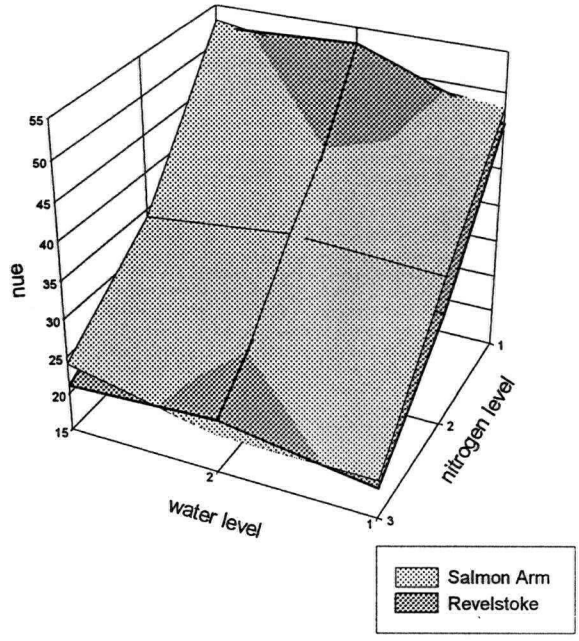
Appendix 8, ctd. Three dimensional illustration of significant intersections between response surfaces

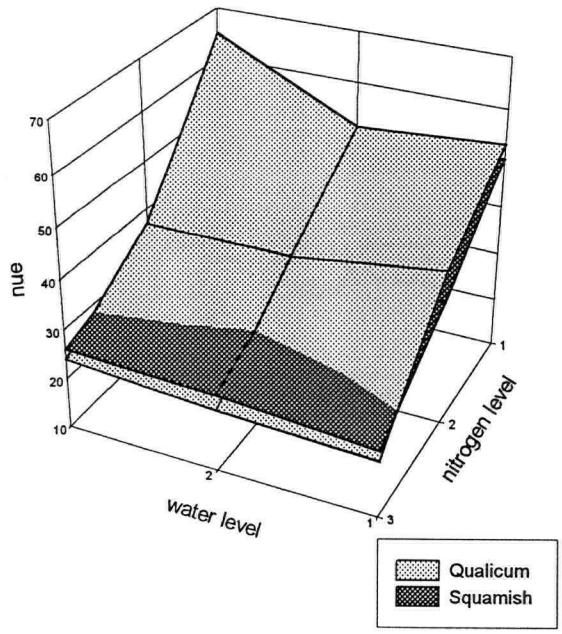




Appendix 8, ctd. Three dimensional illustration of significant intersections between response surfaces







Appendix 9. Climatic data for the source environments of all genotypes based on biogeoclimatic subzone data and the Rehfeldt et al. (1999) model. (Abbreviations and origin of data: p.60).

F	BZ	ELEV	BGC	SZMAT	SUMT	SZMAP	SUMP	MAT	MTWM	MAP	MSP
1	SA	1000	ICHmk1	2.8	10.9	665	234	4.5	16.3	405	213
2	TOD	1650	MSdm2	2.8	10.2	606	267	0.7	11.9	729	292
3	SA	1130	SBSdw1	.	.	.	.	2.9	14.5	513	244
4	SA	1210	IDFdk1	3.4	11.1	438	193	3.2	14.7	496	223
5	TOA	1295	IDFdk1	3.4	11.1	438	193	2.6	14.1	510	234
6	SA	930	ICHmw2	7.5	15.6	656	243	4.7	16.6	400	214
7	TOD	1075	IDFdk2	4.1	11.1	568	221	3.5	15.1	465	227
8	TOA	1390	MSxk	3.1	11.1	444	195	2.7	14.2	895	270
9	TOD	1075	IDFdk2	4.1	11.1	568	221	3.5	15.1	465	227
10	SA	1100	IDFdk1	3.4	11.1	438	193	3.8	15.4	479	216
11	TOD	1075	IDFdk2	4.1	11.1	568	221	3.5	15.1	465	227
12	SA	650	IDFmw2	6.7	15.6	521	207	5.9	17.8	454	217
13	TOA	1260	MSdm2	2.8	10.2	606	267	3.2	14.7	594	228
14	SA	1040	MSxk	3.1	11.1	444	195	4.1	15.6	481	214
15	SA	884	ICHmw2	7.5	15.6	656	243	4.9	16.8	399	214
16	SA	1300	ICHmk1	2.8	10.9	665	234	2.9	14.7	438	231
17	TOA	1190	MSdm2	2.8	10.2	606	267	3.7	15.2	845	251
18	SA	1290	ICHmk1	2.8	10.9	665	234	3	14.8	462	227
19	SA	620	IDFdk1	3.4	11.1	438	193	6.3	17.4	610	221
20	SA	1196	ESSFwc	.	.	.	.	2.4	14	567	264
21	SA	1550	ICHmk1	2.8	10.9	665	234	1.6	13.2	525	257
22	SA	1340	ICHmk1	2.8	10.9	665	234	2.7	14.5	442	235
23	SA	960	IDFmw1	5.8	14.1	515	224	4.3	16.2	402	220
24	SA	960	IDFmw1	5.8	14.1	515	224	4.3	16.2	403	219
25	SA	650	IDFmw2	6.7	15.6	521	207	5.9	17.8	454	217
26	WK	600	ICHmw2	7.5	15.6	656	243	7.3	19	572	194
27	BSH	925	IDFmw1	5.8	14.1	515	224	3.9	16.3	510	262
28	TOD	1650	MSdm2	2.8	10.2	606	267	0.7	11.9	729	292
29	SA	800	ICHmw2	7.5	15.6	656	243	5.1	17	410	217
30	SA	1430	ESSFxc	1.7	9.3	565	233	2.2	13.9	475	243
31	TOA	1280	IDFdk1	3.4	11.1	438	193	2.7	14.2	505	233
32	TOD	1650	MSdm2	2.8	10.2	606	267	0.7	11.9	730	292
33	SA	900	SBSmm	.	.	.	.	3.9	15.9	433	235
34	SA	1183	ICHmw2	7.5	15.6	656	243	3.3	15.1	419	229
35	SA	1189	ICHmw2	7.5	15.6	656	243	3.3	15.1	422	229
36	SA	1220	ICHmw2	7.5	15.6	656	243	3	14.8	435	235
37	TOD	1500	ICHmk1	2.8	10.9	665	234	1.7	13.2	543	255
38	SA	1122	ICHmw2	7.5	15.6	656	243	3.6	15.5	408	225
39	SA	650	IDFmw2	6.7	15.6	521	207	5.9	17.7	454	217
40	SA	1490	ICHmk1	2.8	10.9	665	234	1.9	13.5	496	250
41	SA	1645	ESSFdc1	2.0	10.2	.	261	1.1	12.6	570	269
42	TOD	1075	IDFdk2	4.1	11.1	568	221	3.5	15.1	465	227
43	SA	1250	ICHmw2	7.5	15.6	656	243	3	14.7	436	235
44	TOD	1530	MSxk	3.1	11.1	444	195	1.1	12.2	762	288
45	TOD	1372	MSdm1	3.2	10.4	638	246	2.5	14.2	489	234
46	TOD	1387	MSdm1	3.2	10.4	638	246	2.4	14	496	235
47	SA	1165	IDFdk1	3.4	11.1	438	193	3.4	14.9	510	219
48	TOA	1432	MSdm2	2.8	10.2	606	267	2	13.5	544	246
49	SA	1370	ICHmk1	2.8	10.9	665	234	2.5	14.3	450	239
A		1400	MSxk	3.1	11.1	444	195	2	13.2	621	245
B		500	IDFmw2	6.7	15.6	521	207	6.6	18.4	523	223
C		1000	ICHmw3	5.3	13.8	671	292	4	16.1	418	237
Q		20	CDFmm	.	.	.	.	9.5	16.8	1896	374
S		335	CWHds1	.	.	.	.	7.8	17.4	1188	287

Appendix 10. Derivation of summer dryness index (SDI) and annual dryness index (ADI)

Source: Guy and Holowachuk (*in press*)

$$ADI = (e_{s[MAT]} \times 1000) / MAP \quad [1]$$

$$SDI = (e_{s[MTWM]} \times 100) / MSP \quad [2]$$

Where precipitation is in mm, and e_s is the saturation vapour pressure in kPa at MAT and MTWM, respectively, calculated according to Buck (1981):

$$e_{s[T]} = 0.61121 \times (1.007 + (0.0000346 \times P)) \times \exp((17.502 \times T) / (240.97 + T)) \quad [3]$$

In equation [5], P is atmospheric pressure in kPa calculated from elevation (m) after Yin (1998):

$$P = \exp(-ELEV / 8000) \times 100 \quad [4]$$

ADI and SDI are similar to heat:moisture indices used by Rehfeldt et al. (1999), but they provide a more realistic approximation of water balance that is non-linear with temperature.

Appendix 11. Phenotypic correlations (1996) over all environments and within environments. Values in bold print are significantly different from zero. See footnote on p.70 for correlations with NUE.

PAIR	Over all E.	1 W_HN_H	2 W_HN_M	3 W_HN_L	4 W_MN_H	5 W_MN_M	6 W_MN_L	7 W_LN_H	8 W_LN_M	9 W_LN_L
d-h	**0.91**	**0.70**	**0.66**	**0.79**	**0.54**	**0.52**	**0.80**	**0.51**	**0.43**	**0.81**
vol-h	**0.97**	**0.88**	**0.89**	**0.91**	**0.82**	**0.83**	**0.92**	**0.82**	**0.78**	**0.92**
vol-d	**0.98**	**0.95**	**0.93**	**0.97**	**0.93**	**0.91**	**0.97**	**0.91**	**0.90**	**0.97**
hd-h	**0.79**	**0.62**	**0.73**	**0.35**	**0.62**	**0.73**	**0.39**	**0.74**	**0.70**	**0.43**
hd-d	**0.47**	**-0.12**	-0.01	**-0.25**	**-0.31**	**-0.19**	**-0.20**	**-0.20**	**-0.32**	**-0.15**
hd-vol	**0.62**	**0.18**	**0.34**	-0.05	0.06	**0.23**	0.01	**0.23**	0.10	0.06
rt-h	**0.80**	**0.66**	**0.68**	**0.85**	**0.47**	**0.58**	**0.83**	**0.44**	**0.40**	**0.84**
rt-d	**0.88**	**0.89**	**0.81**	**0.87**	**0.69**	**0.79**	**0.87**	**0.70**	**0.72**	**0.90**
rt-vol	**0.86**	**0.86**	**0.83**	**0.91**	**0.69**	**0.80**	**0.90**	**0.67**	**0.70**	**0.92**
rt-hd	**0.41**	-0.06	**0.18**	0.02	**-0.13**	0.05	0.04	-0.06	**-0.15**	0.03
sht-h	**0.93**	**0.77**	**0.83**	**0.91**	**0.59**	**0.75**	**0.88**	**0.59**	**0.49**	**0.89**
sht -d	**0.94**	**0.85**	**0.82**	**0.87**	**0.69**	**0.73**	**0.87**	**0.60**	**0.72**	**0.89**
sht -vol	**0.95**	**0.89**	**0.90**	**0.93**	**0.74**	**0.84**	**0.92**	**0.68**	**0.74**	**0.93**
sht -hd	**0.60**	**0.13**	**0.36**	**0.12**	0.03	**0.30**	**0.11**	**0.20**	-0.06	**0.12**
sht -rt	**0.88**	**0.86**	**0.87**	**0.95**	**0.77**	**0.84**	**0.94**	**0.61**	**0.83**	**0.94**
bm-h	**0.91**	**0.75**	**0.79**	**0.89**	**0.58**	**0.70**	**0.87**	**0.59**	**0.47**	**0.88**
bm -d	**0.94**	**0.89**	**0.84**	**0.88**	**0.73**	**0.79**	**0.88**	**0.68**	**0.75**	**0.91**
bm -vol	**0.95**	**0.90**	**0.90**	**0.93**	**0.76**	**0.86**	**0.92**	**0.74**	**0.75**	**0.94**
bm -hd	**0.56**	0.07	**0.29**	0.07	-0.02	**0.19**	0.08	**0.14**	**-0.11**	0.08
bm -rt	**0.94**	**0.93**	**0.96**	**0.99**	**0.89**	**0.96**	**0.99**	**0.80**	**0.95**	**0.99**
bm -sht	**0.99**	**0.99**	**0.98**	**0.99**	**0.98**	**0.96**	**0.98**	**0.96**	**0.96**	**0.98**
srr-h	**0.79**	0.03	**0.38**	**0.23**	**0.14**	**0.20**	**0.17**	**0.20**	**0.04**	**0.28**
srr -d	**0.70**	**-0.25**	**0.11**	0.04	-0.08	**-0.21**	0.08	-0.09	**-0.18**	**0.12**
srr -vol	**0.76**	**-0.15**	**0.24**	**0.11**	0.00	-0.05	**0.12**	0.04	-0.10	**0.18**
srr -hd	**0.65**	**0.32**	**0.41**	**0.31**	**0.26**	**0.40**	**0.14**	**0.31**	**0.18**	**0.27**
srr -rt	**0.47**	**-0.46**	**-0.15**	-0.08	**-0.42**	**-0.40**	**-0.11**	**-0.42**	**-0.49**	0.00
srr -sht	**0.80**	0.03	**0.33**	**0.22**	**0.25**	**0.15**	**0.21**	**0.45**	0.07	**0.31**
srr -bm	**0.72**	**-0.12**	**0.12**	0.06	0.04	**-0.11**	0.04	**0.20**	**-0.21**	**0.15**
wue-h	**0.59**	**0.57**	0.17	**0.61**	**0.47**	-0.07	**0.71**	**0.42**	-0.21	**0.46**
wue-d	**0.70**	**0.80**	**0.38**	**0.68**	**0.60**	**0.32**	**0.67**	**0.54**	**0.48**	**0.66**
wue-vol	**0.67**	**0.76**	**0.32**	**0.69**	**0.61**	0.16	**0.71**	**0.55**	0.29	**0.63**
wue-hd	**0.21**	-0.05	-0.08	-0.16	0.02	-0.30	0.13	0.07	**-0.39**	-0.24
wue –rt	**0.77**	**0.75**	**0.51**	**0.70**	**0.66**	**0.41**	**0.68**	**0.72**	**0.56**	**0.69**
wue –sht	**0.69**	**0.60**	0.31	**0.68**	**0.47**	0.09	**0.72**	**0.30**	**0.33**	**0.67**
wue –bm	**0.73**	**0.66**	**0.42**	**0.70**	**0.54**	0.25	**0.71**	**0.48**	**0.48**	**0.69**
wue –srr	**0.43**	**-0.40**	**-0.45**	-0.08	-0.14	**-0.53**	0.00	**-0.32**	**-0.43**	0.06
nue-h	**-0.70**	**0.57**	**0.64**	-0.24	0.21	**0.54**	-0.31	0.10	**0.34**	0.00
nue-d	**-0.71**	**0.37**	**0.47**	-0.22	-0.08	**0.58**	-0.15	-0.11	0.10	-0.23
nue-vol	**-0.72**	**0.48**	**0.61**	-0.24	0.04	**0.65**	-0.22	-0.02	0.30	-0.16
nue-hd	**-0.43**	**0.44**	**0.45**	0.01	**0.35**	0.20	-0.24	0.20	0.17	**0.30**
nue-rt	**-0.60**	**0.37**	**0.59**	**-0.25**	-0.04	**0.44**	-0.10	**-0.30**	0.26	-0.17
nue-sht	**-0.75**	**0.55**	**0.62**	**-0.28**	0.22	**0.60**	-0.23	**0.39**	**0.38**	-0.19
nue-bm	**-0.72**	**0.51**	**0.63**	**-0.27**	0.16	**0.56**	-0.16	0.23	**0.34**	-0.18
nue-srr	**-0.75**	**0.31**	-0.11	-0.11	**0.43**	0.24	**-0.47**	**0.65**	0.08	-0.13
nue-wue	**-0.68**	0.14	-0.11	**-0.64**	-0.07	-0.16	**-0.48**	**-0.31**	-0.09	**-0.59**

Appendix 11, ctd. Genetic correlations (1996) over all environments and within environments with their standard errors. For within-environment correlations, an average of the nine standard errors is given. See footnote on p.70 for correlations with NUE.

PAIR	Over all E.	s.e.	1 W_HN_H	2 W_HN_M	3 W_HN_L	4 W_MN_H	5 W_MN_M	6 W_MN_L	7 W_LN_H	8 W_LN_M	9 W_LN_L	avg.s.e.
d-h	0.69	0.07	0.62	0.68	0.81	0.59	0.55	0.80	0.36	0.62	0.85	0.16
vol-h	0.88	0.03	0.85	0.89	0.92	0.83	0.85	0.92	0.78	0.86	0.94	0.14
vol-d	0.95	0.01	0.94	0.94	0.97	0.93	0.91	0.97	0.86	0.93	0.98	0.03
hd-h	0.62	0.08	0.61	0.73	0.37	0.64	0.75	0.40	0.80	0.67	0.39	0.42
hd-d	-0.13	0.12	-0.23	0.01	-0.18	-0.24	-0.13	-0.20	-0.27	-0.13	-0.14	0.41
hd-vol	0.18	0.12	0.10	0.34	0.01	0.12	0.29	0.02	0.24	0.21	0.05	0.82
rt-h	0.66	0.07	0.63	0.69	0.86	0.51	0.59	0.82	0.36	0.56	0.84	0.20
rt-d	0.88	0.03	0.87	0.88	0.89	0.73	0.84	0.87	0.67	0.84	0.92	0.08
rt-vol	0.86	0.03	0.86	0.88	0.92	0.72	0.82	0.89	0.65	0.80	0.92	0.18
rt-hd	-0.06	0.12	-0.11	0.12	0.01	-0.09	0.04	0.04	-0.08	-0.05	-0.04	0.46
sht-h	0.86	0.03	0.67	0.79	0.92	0.55	0.73	0.88	0.38	0.73	0.90	0.20
sht -d	0.70	0.07	0.89	0.87	0.89	0.77	0.81	0.87	0.64	0.84	0.91	0.09
sht -vol	0.83	0.04	0.89	0.91	0.94	0.77	0.88	0.92	0.64	0.88	0.94	0.20
sht -hd	0.42	0.11	-0.08	0.26	0.11	-0.07	0.23	0.12	-0.03	0.14	0.09	0.61
sht -rt	0.77	0.05	0.88	0.91	0.94	0.84	0.88	0.94	0.73	0.85	0.93	0.10
bm-h	0.83	0.04	0.67	0.76	0.90	0.56	0.68	0.86	0.40	0.67	0.89	0.19
bm -d	0.82	0.04	0.91	0.89	0.91	0.79	0.85	0.88	0.69	0.87	0.93	0.07
bm -vol	0.89	0.03	0.90	0.92	0.95	0.78	0.88	0.92	0.68	0.87	0.95	0.16
bm -hd	0.24	0.12	-0.09	0.20	0.06	-0.08	0.15	0.08	-0.05	0.04	0.02	0.53
bm -rt	0.91	0.02	0.95	0.97	0.99	0.93	0.97	0.99	0.87	0.96	0.98	0.03
bm -sht	0.96	0.01	0.99	0.98	0.99	0.98	0.97	0.98	0.97	0.96	0.98	0.03
srr-h	0.23	0.12	-0.20	0.22	0.22	-0.01	0.18	0.26	0.02	0.01	0.26	0.35
srr -d	-0.30	0.11	-0.31	-0.06	0.08	-0.07	-0.18	0.13	-0.10	-0.34	0.08	0.29
srr -vol	-0.10	0.13	-0.30	0.06	0.13	-0.06	-0.03	0.19	-0.05	-0.21	0.15	0.49
srr -hd	0.64	0.08	0.09	0.34	0.31	0.08	0.37	0.20	0.09	0.30	0.34	0.41
srr -rt	-0.42	0.10	-0.62	-0.24	-0.09	-0.45	-0.37	-0.08	-0.45	-0.62	-0.08	0.29
srr -sht	0.24	0.12	-0.20	0.18	0.22	0.09	0.11	0.26	0.27	-0.12	0.29	0.41
srr -bm	-0.03	0.13	-0.35	-0.01	0.06	-0.09	-0.12	0.08	0.05	-0.39	0.10	0.37
wue-h	0.18	0.39	0.62	0.45	0.68	0.65	0.11	0.76	0.14	0.03	0.79	0.47
wue-d	0.65	0.20	0.78	0.55	0.80	0.68	0.35	0.50	0.52	0.31	0.81	0.44
wue-vol	0.50	0.26	0.76	0.55	0.78	0.71	0.28	0.65	0.50	0.26	0.82	0.35
wue-hd	-0.33	0.34	-0.14	0.07	-0.26	0.29	-0.18	0.62	-0.26	-0.21	-0.33	0.84
wue –rt	0.71	0.18	0.75	0.48	0.72	0.65	0.38	0.49	0.72	0.53	0.81	0.62
wue –sht	0.27	0.35	0.57	0.38	0.74	0.49	0.33	0.58	0.26	0.26	0.87	0.48
wue –bm	0.46	0.29	0.64	0.43	0.73	0.55	0.36	0.54	0.46	0.45	0.85	0.48
wue –srr	-0.47	.	-0.58	-0.14	0.31	-0.17	-0.01	0.16	-0.34	-0.41	0.50	0.52
nue-h	0.55	-0.29	0.50	0.67	-0.46	0.16	0.38	-0.42	0.30	0.38	-0.22	-0.98
nue-d	0.16	-0.34	0.62	0.62	-0.50	-0.23	0.54	-0.13	0.22	0.57	-0.23	-1.02
nue-vol	0.37	-0.31	0.61	0.69	-0.50	-0.06	0.52	-0.28	0.34	0.60	-0.24	-1.22
nue-hd	0.55	-0.28	-0.19	0.34	0.09	0.47	0.08	-0.50	0.05	-0.05	0.22	-2.48
nue-rt	0.05	-0.37	0.51	0.64	-0.47	0.31	0.53	-0.05	-0.24	0.35	-0.19	-1.93
nue-sht	0.64	-0.23	0.61	0.62	-0.52	0.35	0.52	-0.21	0.43	0.43	-0.30	-1.00
nue-bm	0.46	-0.30	0.59	0.63	-0.49	0.35	0.54	-0.13	0.29	0.44	-0.25	-1.03
nue-srr	0.68	.	0.11	0.00	-0.38	0.28	0.06	-0.53	0.58	0.01	-0.48	-0.96
nue-wue	-0.45	-0.30	0.36	0.12	-0.74	0.19	-0.07	-0.66	-0.10	-0.04	-0.64	-1.04

Appendix 11, ctd. Phenotypic correlations (1997) over all environments and within environments. Values in bold print are significantly different from zero. See footnote on p.70 for correlations with NUE.

PAIR	Over all E.	1 W_HN_H	2 W_HN_M	3 W_HN_L	4 W_MN_H	5 W_MN_M	6 W_MN_L	7 W_LN_H	8 W_LN_M	9 W_LN_L
d-h	**0.75**	**0.45**	**0.38**	**0.50**	**0.46**	**0.36**	**0.38**	**0.22**	**0.38**	**0.31**
vol-h	**0.91**	**0.82**	**0.82**	**0.86**	**0.81**	**0.80**	**0.83**	**0.73**	**0.81**	**0.78**
vol-d	**0.94**	**0.87**	**0.84**	**0.87**	**0.88**	**0.84**	**0.82**	**0.83**	**0.84**	**0.83**
hd-h	**0.76**	**0.79**	**0.85**	**0.85**	**0.77**	**0.84**	**0.87**	**0.81**	**0.84**	**0.82**
hd-d	**0.17**	**-0.19**	**-0.14**	-0.01	**-0.20**	**-0.19**	**-0.11**	**-0.37**	**-0.17**	**-0.25**
hd-vol	**0.47**	**0.30**	**0.41**	**0.48**	**0.26**	**0.36**	**0.47**	**0.20**	**0.39**	**0.30**
rt-h	**0.40**	**0.21**	-0.01	**0.19**	**0.26**	0.06	0.08	0.09	**0.11**	0.02
rt-d	**0.51**	**0.39**	**0.33**	**0.51**	**0.28**	**0.40**	**0.36**	**0.19**	**0.29**	**0.40**
rt-vol	**0.50**	**0.36**	**0.21**	**0.40**	**0.31**	**0.29**	**0.27**	**0.20**	**0.26**	**0.27**
rt-hd	**0.10**	-0.03	**-0.20**	-0.07	0.09	**-0.17**	**-0.11**	-0.03	-0.04	**-0.20**
sht-h	**0.77**	**0.43**	**0.42**	**0.57**	**0.38**	**0.42**	**0.53**	**0.39**	**0.49**	**0.47**
sht -d	**0.83**	**0.51**	**0.54**	**0.67**	**0.45**	**0.54**	**0.55**	**0.28**	**0.45**	**0.47**
sht -vol	**0.86**	**0.55**	**0.58**	**0.72**	**0.48**	**0.58**	**0.66**	**0.43**	**0.57**	**0.58**
sht -hd	**0.36**	**0.13**	**0.15**	**0.25**	0.10	**0.12**	**0.28**	**0.19**	**0.27**	**0.21**
sht -rt	**0.57**	**0.61**	**0.48**	**0.55**	**0.43**	**0.52**	**0.51**	**0.52**	**0.46**	**0.53**
bm-h	**0.72**	**0.40**	**0.25**	**0.45**	**0.40**	**0.29**	**0.34**	**0.33**	**0.38**	**0.28**
bm -d	**0.81**	**0.52**	**0.50**	**0.68**	**0.46**	**0.54**	**0.53**	**0.30**	**0.44**	**0.50**
bm -vol	**0.83**	**0.54**	**0.46**	**0.65**	**0.50**	**0.51**	**0.54**	**0.41**	**0.50**	**0.49**
bm -hd	**0.30**	0.09	-0.02	**0.12**	**0.11**	-0.01	0.10	**0.12**	**0.16**	0.00
bm -rt	**0.77**	**0.79**	**0.83**	**0.83**	**0.70**	**0.84**	**0.86**	**0.75**	**0.80**	**0.86**
bm -sht	**0.96**	**0.96**	**0.88**	**0.92**	**0.93**	**0.89**	**0.87**	**0.95**	**0.90**	**0.88**
srr-h	**0.66**	**0.28**	**0.42**	**0.44**	**0.20**	**0.36**	**0.46**	**0.34**	**0.41**	**0.46**
srr -d	**0.65**	**0.18**	**0.21**	**0.24**	**0.26**	**0.16**	**0.17**	**0.12**	**0.21**	0.05
srr -vol	**0.69**	**0.26**	**0.36**	**0.39**	**0.27**	**0.30**	**0.37**	**0.28**	**0.37**	**0.30**
srr -hd	**0.37**	**0.17**	**0.33**	**0.36**	0.02	**0.28**	**0.40**	**0.25**	**0.32**	**0.43**
srr -rt	-0.01	**-0.33**	**-0.52**	**-0.38**	**-0.33**	**-0.45**	**-0.52**	**-0.30**	**-0.38**	**-0.51**
srr -sht	**0.81**	**0.53**	**0.49**	**0.55**	**0.70**	**0.52**	**0.45**	**0.65**	**0.64**	**0.44**
srr -bm	**0.62**	**0.29**	0.02	**0.17**	**0.41**	0.07	-0.03	**0.39**	**0.24**	-0.03
wue-h	**0.56**	**0.66**	**0.57**	**0.47**	**0.64**	**0.51**	**0.36**	**0.49**	**0.58**	**0.38**
wue-d	**0.61**	**0.33**	**0.27**	**0.38**	**0.39**	**0.32**	**0.35**	**0.36**	**0.38**	**0.35**
wue-vol	**0.64**	**0.54**	**0.52**	**0.48**	**0.58**	**0.49**	**0.41**	**0.54**	**0.57**	**0.46**
wue-hd	**0.27**	**0.49**	**0.44**	**0.32**	**0.40**	**0.36**	**0.21**	**0.27**	**0.43**	**0.20**
wue –rt	**0.44**	**0.40**	**0.19**	**0.34**	**0.54**	**0.32**	**0.31**	**0.45**	**0.42**	**0.38**
wue –sht	**0.65**	**0.48**	**0.33**	**0.27**	**0.44**	**0.44**	**0.37**	**0.56**	**0.56**	**0.45**
wue –bm	**0.64**	**0.50**	**0.31**	**0.33**	**0.57**	**0.44**	**0.39**	**0.59**	**0.58**	**0.48**
wue –srr	**0.48**	**0.17**	0.13	-0.03	0.09	**0.17**	0.06	**0.25**	**0.25**	0.03
nue-h	**-0.51**	**0.32**	**0.26**	**0.15**	**0.18**	**0.34**	0.08	0.12	**0.38**	0.12
nue-d	**-0.66**	**0.32**	**0.24**	**0.21**	**0.32**	**0.24**	**0.22**	0.02	-0.05	0.07
nue-vol	**-0.64**	**0.37**	**0.33**	**0.19**	**0.32**	**0.36**	**0.18**	0.07	**0.20**	0.10
nue-hd	**-0.15**	0.12	**0.16**	0.03	-0.04	**0.24**	-0.03	0.12	**0.45**	0.09
nue-rt	**-0.21**	0.14	**0.56**	**0.49**	0.10	**0.44**	**0.46**	**0.24**	**0.34**	**0.36**
nue-sht	**-0.68**	**0.53**	**0.62**	**0.25**	**0.30**	**0.56**	**0.27**	**0.29**	**0.48**	**0.22**
nue-bm	**-0.59**	**0.46**	**0.69**	**0.39**	**0.27**	**0.58**	**0.43**	**0.30**	**0.49**	**0.35**
nue-srr	**-0.68**	**0.49**	0.02	**-0.20**	**0.26**	**0.17**	**-0.22**	**0.15**	**0.23**	**-0.18**
nue-wue	**-0.63**	**0.28**	**0.35**	-0.03	**0.17**	**0.43**	-0.11	0.08	**0.39**	**-0.21**

Appendix 11, ctd. Genetic correlations (1997) over all environments and within environments with their standard errors. For within-environment correlations, an average of the nine standard errors is given. See footnote on p.70 for correlations with NUE.

PAIR	Over all E.	s.e.	1 W_HN_H	2 W_HN_M	3 W_HN_L	4 W_MN_H	5 W_MN_M	6 W_MN_L	7 W_LN_H	8 W_LN_M	9 W_LN_L	avg.s.e.
d-h	0.63	0.08	0.38	0.63	0.60	0.64	0.46	0.49	0.54	0.57	0.48	0.12
vol-h	0.91	0.02	0.82	0.91	0.91	0.89	0.84	0.90	0.89	0.91	0.87	0.03
vol-d	0.89	0.03	0.84	0.89	0.87	0.92	0.87	0.81	0.86	0.85	0.85	0.05
hd-h	0.92	0.02	0.84	0.89	0.92	0.81	0.83	0.93	0.90	0.93	0.90	0.04
hd-d	0.29	0.12	-0.17	0.22	0.23	0.06	-0.10	0.14	0.13	0.22	0.06	0.19
hd-vol	0.69	0.06	0.38	0.64	0.67	0.45	0.41	0.69	0.61	0.70	0.57	0.11
rt-h	0.08	0.13	0.22	-0.03	0.20	0.31	0.11	-0.06	0.17	0.07	0.00	0.17
rt-d	0.44	0.12	0.49	0.35	0.47	0.34	0.56	0.40	0.23	0.25	0.43	0.18
rt-vol	0.29	0.12	0.44	0.16	0.36	0.36	0.39	0.16	0.24	0.17	0.25	0.16
rt-hd	-0.12	0.13	-0.04	-0.24	0.01	0.15	-0.22	-0.24	0.08	-0.02	-0.20	0.19
sht-h	0.59	0.08	0.42	0.52	0.72	0.41	0.50	0.61	0.53	0.60	0.58	0.11
sht -d	0.73	0.06	0.60	0.70	0.75	0.45	0.69	0.62	0.48	0.57	0.65	0.12
sht -vol	0.73	0.06	0.62	0.67	0.81	0.48	0.71	0.72	0.59	0.66	0.71	0.09
sht -hd	0.37	0.11	0.10	0.25	0.49	0.19	0.12	0.45	0.36	0.45	0.34	0.16
sht -rt	0.52	0.10	0.67	0.52	0.47	0.54	0.58	0.39	0.49	0.36	0.50	0.14
bm-h	0.46	0.10	0.39	0.31	0.58	0.44	0.37	0.36	0.48	0.49	0.34	0.13
bm -d	0.72	0.07	0.61	0.62	0.74	0.48	0.70	0.62	0.46	0.54	0.64	0.13
bm -vol	0.65	0.07	0.61	0.50	0.72	0.51	0.63	0.56	0.55	0.58	0.57	0.11
bm -hd	0.22	0.13	0.06	0.03	0.34	0.20	-0.03	0.16	0.32	0.34	0.09	0.18
bm -rt	0.77	0.06	0.82	0.82	0.79	0.77	0.85	0.80	0.71	0.70	0.85	0.08
bm -sht	0.94	0.02	0.97	0.91	0.91	0.94	0.92	0.87	0.96	0.92	0.88	0.03
srr-h	0.61	0.08	0.32	0.61	0.56	0.26	0.46	0.64	0.45	0.57	0.60	0.12
srr -d	0.48	0.10	0.27	0.44	0.38	0.28	0.28	0.26	0.36	0.43	0.25	0.17
srr -vol	0.60	0.08	0.36	0.60	0.53	0.30	0.44	0.56	0.46	0.57	0.49	0.13
srr -hd	0.53	0.09	0.19	0.52	0.49	0.12	0.34	0.64	0.34	0.48	0.54	0.15
srr -rt	-0.18	0.13	-0.17	-0.37	-0.37	-0.12	-0.27	-0.47	-0.24	-0.27	-0.46	0.18
srr -sht	0.74	0.06	0.60	0.60	0.64	0.77	0.62	0.62	0.73	0.80	0.53	0.10
srr -bm	0.48	0.10	0.41	0.21	0.26	0.52	0.27	0.15	0.51	0.50	0.07	0.16
wue-h	0.72	0.06	0.75	0.61	0.51	0.72	0.54	0.44	0.65	0.66	0.51	0.12
wue-d	0.63	0.09	0.29	0.30	0.49	0.37	0.42	0.36	0.48	0.42	0.48	0.21
wue-vol	0.76	0.06	0.59	0.55	0.55	0.60	0.57	0.48	0.68	0.64	0.60	0.14
wue-hd	0.59	0.09	0.62	0.50	0.34	0.65	0.37	0.33	0.47	0.56	0.32	0.18
wue –rt	0.42	0.12	0.34	0.15	0.35	0.59	0.38	0.34	0.57	0.42	0.43	0.22
wue –sht	0.74	0.06	0.50	0.36	0.45	0.56	0.56	0.40	0.76	0.70	0.53	0.17
wue –bm	0.70	0.07	0.49	0.30	0.47	0.68	0.54	0.42	0.78	0.68	0.54	0.17
wue –srr	0.57	0.10	0.28	0.26	0.11	0.18	0.32	0.10	0.49	0.48	0.09	0.24
nue-h	0.38	-0.15	0.21	0.32	0.08	0.18	0.36	-0.11	0.09	0.45	0.21	-0.26
nue-d	0.49	-0.15	0.18	0.45	0.15	0.17	0.21	0.12	0.29	0.04	0.20	-0.35
nue-vol	0.50	-0.14	0.26	0.47	0.11	0.22	0.37	-0.02	0.23	0.32	0.22	-0.28
nue-hd	0.23	-0.18	0.12	0.12	0.01	0.09	0.31	-0.20	-0.03	0.50	0.13	-0.33
nue-rt	0.45	-0.16	0.17	0.58	0.52	0.22	0.37	0.34	0.44	0.25	0.24	-0.33
nue-sht	0.46	-0.15	0.50	0.74	0.17	0.22	0.54	0.09	0.28	0.57	0.22	-0.27
nue-bm	0.51	-0.15	0.45	0.75	0.37	0.25	0.53	0.27	0.35	0.52	0.28	-0.28
nue-srr	0.20	-0.19	0.42	0.26	-0.30	0.10	0.28	-0.27	0.00	0.46	-0.04	-0.35
nue-wue	0.33	-0.17	0.18	0.34	-0.05	0.15	0.47	-0.17	0.17	0.53	-0.27	-0.27