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Clone Testing and Genotype x Environment Interaction in *Picea abies*

Bo Karlsson

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Akademisk avhandling som för vinnande av skoglig doktorsexamen kommer att offentligen försvaras i Aulan, Genetikcentrum, SLU, Uppsala, fredagen den 17 november 2000, kl. 10.00.

Abstract

Clones are used for accurate genetic tests of *Picea abies* (Norway spruce) in the Swedish breeding programme. This thesis deals with the efficiency of clone tests in genetic testing. Special focus is placed on the problems associated with the genotype×environment (G×E) interaction.

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For growth, clones should be selected after field-testing rather than on the basis of ortet behaviour in the nursery. However, for highly heritable traits, such as bud-break, early selection can be used.

The genotype×environment interactions for growth were statistically significant. The genetic correlations did not indicate any geographic trend. Such traits as bud-break, branch angle, and wood density were little affected by the G×E interaction.

Ecovalence estimates exhibited small differences between clones with respect to the interaction. The main cause of the G×E interaction in southern Sweden is late spring frost. In sites where late spring frosts are likely during the initial period after planting, clones with late bud-break contributed less to the interaction. Material with late bud-break is recommended for use where spring frosts are likely. In areas with low frequency of frost-prone sites, selection should be carried out only for general performance.

The G×E interaction for height increment was larger than for early height measurements, suggesting that the interaction is an increasing problem with age. Residual C-effects from the nursery could explain the relatively smaller interaction effects associated with early measurements.

No important change in the Norway spruce breeding strategy was proposed.

Keywords: *Picea abies*, provenance, clone, genotype×environment interaction, genetic correlation, variance component, ecovalence, c-effect

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Contents

Appendix

Papers I-IV

This thesis is based on the following papers, which are referred to by the corresponding Roman numerals

I. Karlsson, B. and Högberg, K-A. 1998. Genotypic parameters and clone×site interaction in clone tests of Norway spruce (*Picea abies* (L.) Karst.). *Forest Genetics* 5(1):21-30

II. Karlsson, B., Lundkvist, K., and Eriksson, G. 1998. Juvenile–mature correlations and selection effects on clone level after stratified family and individual selection of *Picea abies* (L.) Karst. Seedlings. *Silvae Genetica* 47(4):208-214

III. Karlsson, B., Wellendorf, H., Werner, M. and Roulund, H. 2000. Phenotypic performance in 11 combined provenance and clone trials with *Picea abies* in Denmark and Sweden: I. Comparison between seedlings and clone mixtures and estimation of genetic parameters within trials. *Manuscript.*

IV. Karlsson, B., Wellendorf, H., Roulund, H. and Werner, M. 2000. Phenotypic performance in 11 combined provenance and clone trials with *Picea abies* in Denmark and Sweden: II. Genotype×trial interaction and stability across sites. *Manuscript.*

Introduction

Trees are very long-lived organisms compared with other crop plants and tend to be planted in much more heterogeneous environments. It is crucial, for successful forest management, to match planting sites with the right species, and to use plants that are genetically adapted to the local climate and conditions of the planting site. Breeding programmes for most species supply large areas with considerable variation in local conditions. This necessitates sub-division of breeding populations, or seed utilisation zones, defined geographically (latitude, longitude, altitude) or climatically e.g. by temperature or precipitation. Testing of the breeding material for Norway spruce (*Picea abies* (L.) Karst) is normally conducted using progeny testing, clone testing or combinations of both.

The main objective of this work was to describe clone testing of Norway spruce and discuss the associated difficulties and possibilities, in order to estimate the occurrence of, and means to control, genotype×environment (G×E) interaction in breeding. The practical use of plant material for reforestation is also discussed. The main focus is on consequences for Scandinavia in general and southern Sweden in particular.

Distribution and migration history of *Picea abies*

Norway spruce, which is a shade tolerant secondary coloniser, currently has a wide natural distribution across Europe, from northern Italy and Greece (41-44°N) in the south to northern Norway (69°N) (Schmidt-Vogt 1977). Longitudinally, it ranges from eastern France (5°E) to eastern Siberia (155°E). The species is wind pollinated and normally regenerates by seed, but in extreme environments, e.g. in alpine conditions, it may regenerate vegetatively by layering.

It survived the last glaciation in refugia in central Russia and in southern and southeastern Europe (Huntley and Birks 1983). The migration back to Fennoscandia from the Russian refugium took place via the Baltic countries through Finland into Sweden 2000-5000 years before the present. The commonly accepted hypothesis that the migration took place only from northern Finland into Sweden has been questioned, since pollen analysis indicates that the main migration might have occurred via "the central Swedish bridgehead", from whence it spread throughout Sweden (Huntley and Birks 1983).

Norway spruce is naturally distributed across most of Sweden except high altitudes in the north and western mountains and south of approximately 56°20´N (Huntley and Birks 1983, Björkman 1996). In a historical context, Norway spruce is one of few tree species that has managed to recolonise the Scandinavian peninsula after several glaciations (Bradshaw 1995).

Norway spruce regenerates naturally in stands where it is fairly well protected from frost (Schmidt-Vogt 1977). In the majority of managed reforestation, it is

planted after clear-cutting and is thus more exposed to frost. This is one of the main problems when establishing Norway spruce plantations, especially in southern Sweden.

Approximately 45% of the standing volume in Swedish forests consists of Norway spruce, and it accounts for 56% of the annual felling (Anon. 1999). It and *Pinus sylvestris* are the most economically important species in Sweden. Planting of Norway spruce totals approximately 200 million plants annually, which is about 65% of the total tree planting in Sweden.

The Swedish breeding programme for Norway spruce

The Norway spruce breeding strategy, which was developed for Swedish conditions by Danell (1993), has been described by Karlsson and Rosvall (1993). The Swedish breeding programme follows the multiple population breeding system (MPBS) concept presented by Namkoong *et al.* (1980) (Burdon and Namkoong 1983). This breeding strategy combines genetic improvement with adequate conservation of genetic variation (Danell 1993). Within one meta-population, 22 sub-populations, with approximately 50 clones each, make up each generation. The components and activities in one sub-population in the programme are shown in Figure 1.

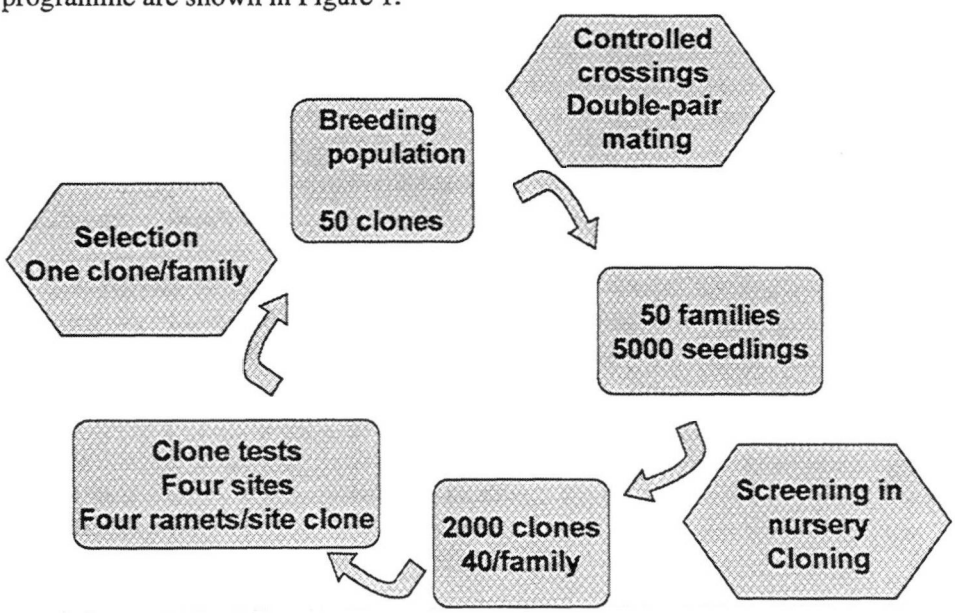

Figure 1. Schematic view of one sub-population in the Swedish breeding programme for *Picea abies*. Rounded rectangles symbolise material and hexagons symbolise activities.

The main breeding goal for Norway spruce, besides vitality, is growth (Karlsson and Rosvall 1993). In addition, quality traits such as stem straightness, branching and wood density are taken into consideration during selection for breeding and

mass propagation, either as general breeding goals or as specific goals for breeding or for mass propagation population.

The test for forward selection of individuals is carried out in two steps:

1. Screening of seedlings for highly heritable traits (e.g. bud-break) is conducted in the nursery before clone selection.
2. Field clone-tests for other traits are carried out at four sites following vegetative propagation of the cuttings from selected seedlings.

Clone testing of individuals within full-sib families (Karlsson and Rosvall 1993) has several purposes in mass propagation and breeding.

1. It can be used for the placement of tested clones in clonal forestry.
2. It is an accurate way of forward selection of clones for the next breeding generation.
3. It creates possibilities for reliable and accurate selection of parents for seed orchard establishment.

Another mass propagation method, which is not dependent on clone tests, is the use of vegetative propagation of selected families from superior parents, so-called bulk propagation. This method results in smaller gains than traditional clonal forestry with tested clones, but avoids some of the associated expenses and problems.

A review of clonal forestry, with special reference to Norway spruce

Clonal forestry in the context below is defined as the use of vegetatively propagated plants for the creation of forest plantations.

Sugi (*Cryptomeria japonica* D. Don) was one of the first species to be propagated by cuttings (Ohba 1993). Managed forests based on plants propagated vegetatively were planted in Japan more than 500 years ago. Cultivation of poplars (*Populus* sp.) and willows (*Salix* sp.) has been associated with agriculture in mid-Asia, the Near East and around the Mediterranean since antiquity (Zsuffa *et al.* 1993). Since we can assume that vegetative propagation was the most common method, these species probably have the longest history of use in clonal forestry.

The first description of cutting propagation of *Picea abies* dates back to the first half of the 19th century (Pfifferling, 1830 cited in Kleinschmidt *et al.* 1973). The first clonal tests were planted in Germany in 1947 (Bentzer, 1993), and a German cutting propagation programme aimed at practical forestry was set up in 1968 (Kleinschmit *et al.* 1973).

Bentzer (1993) described different strategies for applied clonal forestry using Norway spruce. The first German clonal forestry programme sparked other such

programmes. The German programme was based on mass selection, mostly in provenances from commercial nurseries, with a selection intensity of one clone per 3000 candidates, followed by vegetative propagation (Kleinschmit *et al.* 1973). After a nursery culling of about two thirds of the clones, the remainder were planted out in field tests.

In Denmark (Roulund, 1977) a clonal forestry programme was undertaken using primary clone selection from young progeny trials. In Sweden, there were originally two programmes in operation (Werner 1977, Bentzer 1981, Karlsson 1993) both selecting clones from eastern European seed sources in order to find late flushing material for frost prone sites in southern Sweden. Selection and testing followed the German strategy. Later, two other programmes began in central and northern Sweden (Hannerz and Wilhelmsson 1992, Karlsson and Rosvall 1993, respectively). Also, in Norway (Dietrichson and Kierulf 1982) and Finland (Lepistö 1977), minor programs were operating.

Common to all programmes was the awareness of a potentially high genetic gain through selection, testing and mass propagation of specific clones rather than cutting propagation of untested clones. A common problem was the maturation or ageing of clones, which led to decreased rooting ability and plagitrophic growth (Roulund 1981). In order to avoid the problems connected with maturation, different propagation strategies were followed. The most common methods were;

1. Clone hedge orchards, where the clones were kept less than 0.5 m tall by shearing and annual harvesting of cuttings (Kleinschmit 1992, Bentzer 1993).
2. Serial propagation, where cuttings were harvested from 3-4 year old rooted cuttings from the previous vegetative cycle (Kleinschmit *et al.* 1973).

The high cost of production of rooted cuttings compared to seedlings (approximately 50% more) has been an obstacle that has halted most programmes (Bentzer 1993). One commercial programme operated by forest industry companies in central Sweden still produces rooted cuttings of tested clones (Hannerz and Wilhelmsson 1992).

Kleinschmit (1992) lists many advantages of vegetative propagation in forestry, which include:

- Genetic gain can be exploited quickly and efficiently without loss due to recombination.
- Clones can be produced with highly specific properties, such as high basic density combined with good growth for high yielding plantations and appropriate bud-break and bud-set phenology traits for frost-prone sites.
- Clones with specific traits, e.g. late bud-break, can be used for specific environments, e.g. frost prone sites.
- Genetic gain can be exploited without the delay caused by late flowering.
- Rare and expensive seeds (e.g. full-sib families) can be used efficiently, through bulk propagation.

- By the selection of appropriate material, stands that are relatively homogeneous, with respect to certain characters, can be established.

Lundkvist (1984) highlighted the potential use of clones with specific adaptations to certain site conditions, as a major advantage of clonal forestry. Moreover, a clonal mixture can, if necessary, be tailored with respect to the genetic diversity associated with certain traits (Lindgren 1993).

An alternative way of using traditional clonal forestry, where defined clones are propagated and used in reforestation, is the bulk propagation of small seed samples with especially valuable traits (Werner and Pettersson 1981, Fletcher 1992). Bulk propagation is a method of vegetative multiplication rather than the use of defined clones. It does not utilise any specific knowledge about traits of the individual clones, but is supported by results from previous field tests on the seed sample or the parents of full- or half-sib families. One major advantage with bulk propagation is that the genetic gain in breeding populations can be utilised earlier than with tested, defined clones. Moreover, it also reduces maturation problems.

In Sweden, clonal forestry is subject to strict legal control. Legislation, for example, controls the minimum number of clones in a reforestation stock, as well as the largest number of copies that may be propagated from each clone (Anon. 1994). It also regulates the relatedness of clones in the reforestation material.

Besides the genetic effects of selection in the nursery and in subsequent tests, the possibility of a "cutting" effect has been discussed. Gemmel *et al.* (1991) found cuttings of Norway spruce to be significantly superior to seedlings from the same genetic source. In studies with other species, however, no differences between propagation methods were found for growth traits (Mason 1991, Stelzer *et al.* 1998).

Even if there are difficulties with, and failures in, commercial clonal forestry, clone tests developed for clone selection are of great value for breeding. The four different clonal forestry programmes in Sweden have resulted in 18 000 clones planted out in field tests (Hannerz and Wilhelmsson 1992, Karlsson 1993, Karlsson and Rosvall 1993). Most of these clones were selected from recommended provenances in commercial nurseries or in full-sib families with known breeding values. The clones tested were evaluated and the best genotypes included as founders of the Swedish breeding population (Karlsson and Rosvall 1993). The expected genetic gain when using the best ten per cent of clones following field tests, varies between 15 and 25% (Karlsson 1993).

Description of the phenotype

The simplified phenotype of a tree growing at a certain site can be described by the model:

$P = G + E + I_{GE}$ (Falconer 1983)

Where
P = the phenotype
G = contribution by the genotype
E = contribution by the site environment
I_{GE} = contribution by the interaction between the genotype and the environment

G can be split into $A+NA$, where A denotes additive effects and NA the non-additive, dominance and epistatic effects (Burdon and Shelbourne 1974). Thus, the following model is obtained:
$P = A + NA + E + I_{GE}$

Besides these effects, there are common-environment effects, C-effects. Such C-effects are often discussed in relation to clonal propagation (Burdon and Shelbourne 1974, Roulund 1981), but should be considered for all propagation and raising of plants (Libby 1976). C-effects may be divided into those effects common to all ramets of a clone or a family (Lerner 1958), and those affecting single propagules within clones or families (Libby and Jund 1962). The first type, **C**-effects, is often confounded with true genetic effects and can thus cause bias. The second type, **c**-effects, only increases within-entry variation (Libby 1976, Foster *et al.* 1984).

When testing genotypes after vegetative propagation aimed at forward selection for breeding populations or mass propagation, it is assumed that the genetic effect is mainly additive. Using clonal replicates in genetic tests usually increases the genetic gain relative to non-clonal tests, without increasing the test effort (Shaw and Hood 1985). Rosvall (1999) concluded that non-additive effects and C-effects could occur in clonal testing aimed at the exploitation of additive effects, without necessarily jeopardising the efficiency.

In order to maximise the genetic gain in a mass propagation population, it is important to have good control over the different components in the model. The tree-breeder's task is to test genotypes at trial sites that are representative of the real reforestation sites, in order to identify genetic entries that display superior performance. Test sites may vary depending on the purpose of the test. Nursery tests are good to predict phenological traits, such as bud break, growth termination and the probability of damage due to frost exposure in the field (Cannell and Sheppard 1982, Hannerz 1999), but less suitable for assessments of long-term growth (Mullin *et al.* 1995, Högberg and Karlsson 1998). Growth chamber tests have been used with varying, but often poor, results, to simulate the expected environmental conditions at planting sites (Jansson *et al.* 1998, Mullin and Park 1994, Danusevicius *et al.* 1999). In applied tree-breeding, however, the use of field trials is still the prevailing method for selection of superior genotypes for the breeding populations and for mass propagation.

Genotype×environment interaction

When the responses of genotypes change in relation to each other in different environments, there is a genotype×environment interaction. The interaction may be regarded as a possibility for matching certain plant material to certain sites, but it is also a disruptive factor when trying to predict the response of tested material across reforestation sites. The G×E interaction is useful in practice only when the interactions are well defined and repeatable. Furthermore, to be able to use the interaction, environmental, climatic, biotic and abiotic conditions during establishment must be repeatable (Matheson and Cotterill 1990).

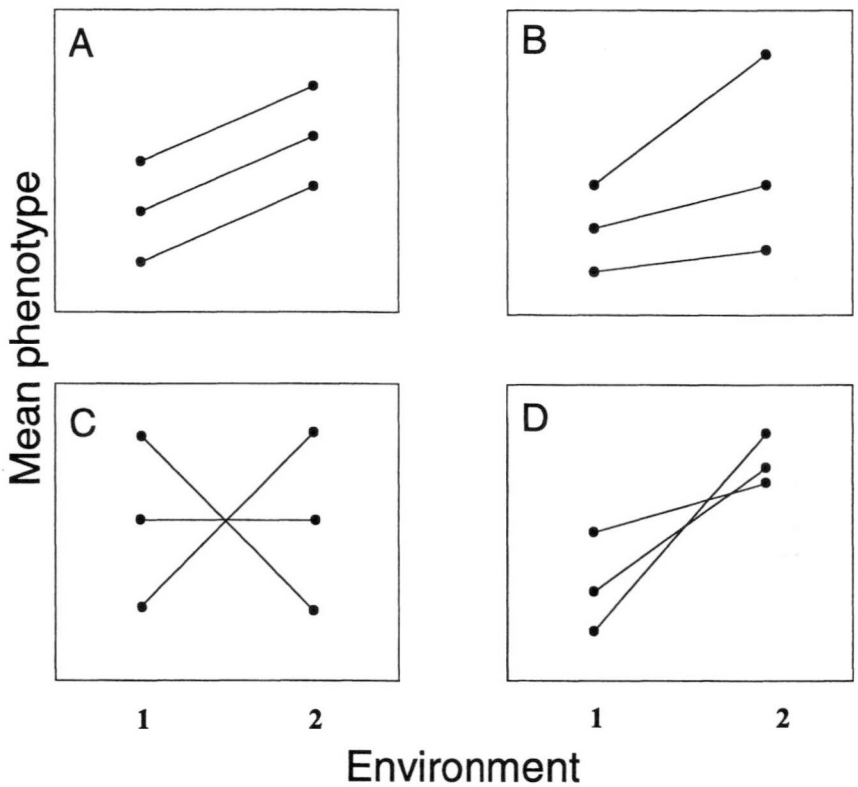

Figure 2. Reaction norms for three genotypes in response to two environments. A. No G×E interaction. B. The G×E interaction is due entirely to a change in the scale of response. C. The G×E interaction is due to a change in ranking. D. There is a change of scale as well as a change in ranking. After Lynch and Walsh (1998).

The function describing behaviour of different phenotypes over a range of environments is called the 'reaction norm' (Woltereck 1909, Schmalhausen 1949, cited in Lynch and Walsh 1998). Figure 2 shows the reaction norms for three genotypes in four situations. In case A, the reaction norms are parallel, which means that a change in the site mean for a certain trait will affect all three genotypes equally in the same direction, thus there is no G×E interaction. In B, the ranking of the genotypes is the same in the second environment, but the

increase in growth is not proportional to the increase in site mean for all three genotypes. This creates a G×E interaction due do scale effects. In C, a G×E interaction exists; created by the change in rank in the two sites. Finally in D, there is a G×E interaction due to both a change in scale and a change in rank between sites.

It is important to separate interaction into that due to change of the scale of response between environments and interaction that is a result of changes in rank. It is only the latter rank change "true interactions" that should affect breeding strategies (Burdon 1977)

Assessing genotype×environment interaction

Estimation of variance components

A common way of analysing the G×E interaction statistically, in a series of genetic trials, is an analysis of variance assuming the following general model:

$P = \mu + G + E + GE + e$

where

P = phenotypic observation

μ = mean of the series

G = genotypic effect

E = environmental effect

GE= genotype×environmental effect

e= error

The ANOVA should be the first step in an analysis of G×E interactions, since it tests the significance of genetic and interaction effects (Shelbourne 1972, Skrøppa 1984).

In order to be valid, the estimation of G×E variance components for a test series has certain prerequisites (Burdon 1977):

1. There should be homogeneous clonal variances across sites, in order to avoid G×E interaction due to scale effects (**see** also Matheson and Cotterill, 1990).
2. There should be variance homogeneity among sites.
3. The residuals should be normally distributed.

To avoid G×E interaction due to scale effects (Figure 2, case B), data must be transformed prior to analysis to ensure homogeneity of among-genotype variances in all environments (Lynch and Walsh 1998).

Using the results from the ANOVA, an estimation of variance components can be made. As a rule of thumb, gains due to selection and testing can be seriously affected if the interaction component accounts for more than 50% of the genetic variance component (Shelbourne 1972). Lindgren (1984) coined the term '*K*-statistics' for this ratio, and defined it as:

$K = \sigma^2_{G\times E} / \sigma^2_G$

Where
$\sigma^2_{G\times E}$ = the genotype×environment interaction variance
σ^2_G = the genetic variance.

Genetic correlations

Falconer (1952) proposed that one character observed in two environments could be regarded as two characters with a certain genetic correlation. The genetic correlation then expresses the extent to which the two characters have the same genetic basis. A high genetic correlation across environments implies that the same alleles or set of alleles influence the expression of the character in the same way in both environments (Via and Lande 1985). A weak genetic correlation coefficient indicates that the phenotypes in each environment are influenced either by different alleles or differently by the same alleles.

Burdon (1977) developed genetic correlations for forest tree breeding as a tool to describe the existence of G×E interactions. He stressed that the main emphasis in trees should be given to the environments rather than to the genotypes. True genetic correlations (Type A) require measurements from the same individuals. Burdon (1977) proposed the use of type B correlations, which give an approximate estimate of the genetic correlations, based on correlations between means of genetic entries from pairs of sites. In recent papers dealing with the G×E interaction in forest tree breeding, multiple-trait, mixed model equations have been used to estimate genetic correlations between traits across sites (e.g. Jansson *et al.* 1998b). This type of analysis has the benefit of simultaneous estimates of variances and covariances, which makes it more robust for unbalanced data.

Stability

Once a significant interaction is confirmed, either through statistically significant G×E interaction variance components that are large relative to the genetic component, or lack of genetic correlations across sites, it is usually of interest to find out the extent to which each genetic entry contributes to the interaction. In forest tree breeding, as well as in practical forestry, it is useful to identify plant varieties (provenances, families and clones), that are unlikely to display interactions with sites within a breeding zone or a seed utilisation region.

There are various methods of describing phenotypic stability across sites, in order to identify genotypes with low levels of interaction. Finlay and Wilkinson (1963) described the stability of genetic entries by regressing the performance of each genotype on the site performance, as described by the mean performance of all entries. Each regression coefficient b_i is a measure of stability, so that a value close to 1.0 is interpreted as average stability (the entries' performance is proportional to the site mean). Figure 3 shows a generalised interpretation of stability when regression coefficients are plotted against variety means (Li and McKeand 1989).

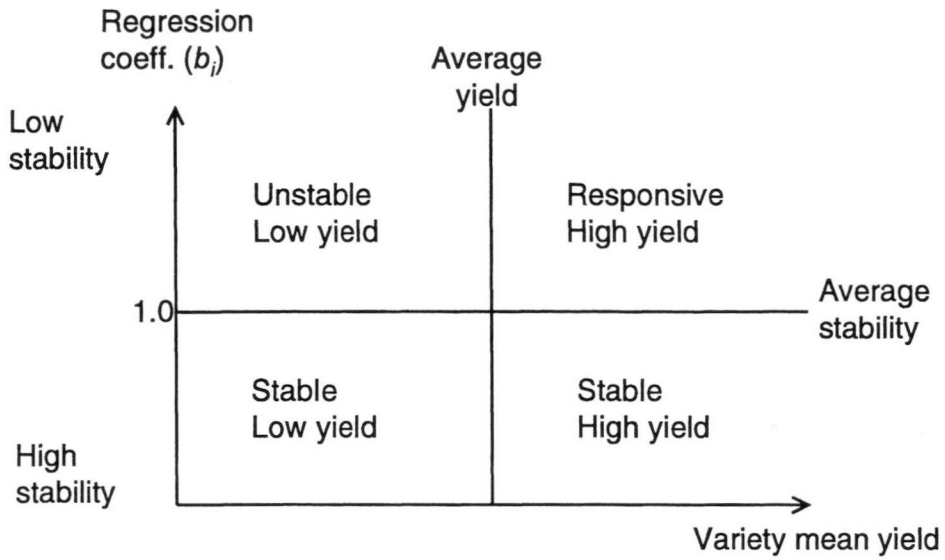

Figure 3. A generalised interpretation of the stability of genetic varieties, by plotting regression coefficients against variety mean yield across tests. After Li and McKeand (1989).

The regression coefficient only describes the tendency of a plant to respond to environmental change as a proportion of the population average. Therefore, as an additional parameter of stability, describing the deviations not accounted for by regression on the environmental index, Eberhardt and Russell (1966) proposed the inclusion of the mean square deviations from the regression line for each variety (σ^2_{di}). A stable variety should have the features b_i=1.0 and σ^2_{di}=0.0. If individual genotype regressions are used as stability indices, several environments are needed in order to give a precise estimate of regression and the deviation from regression (Shelbourne 1972).

Wricke (1962) suggested ecovalence as a means of describing genetic stability across sites. Ecovalence, which is the contribution from each genotype to the interaction sum of squares, reflects the capacity of a genotype to give consistent yields across sites. A related measure is the stability variance described by Shukla (1972), which also can be tested statistically.

Skrøppa (1984) compared mean square deviation from a regression line with ecovalence for ten parent clones in a progeny test, and reported a statistically significant rank correlation coefficient of 0.81. St Clair and Kleinschmit (1986) derived a rank correlation coefficient of 0.88 for the same pair of stability measures.

Possible reasons for the G×E interaction

Apart from identifying which genotypes are the best performers at each site and which ones are the most stable, it is important to examine which elements of the "site" and the "culture" are involved in the interaction (Shelbourne 1972).

Water

There are indications that differences in water availability may cause **a** G×E interaction in 21-year old *Picea mariana* (Johnsen *et al.* 1993). Cannell *et al.* (1978) found a family×water availability interaction for seedlings of *Pinus taeda*. Conversely Burczyck and Giertych (1991) did not find any population×drought interaction for *Picea abies*. Sonesson and Eriksson (2000) reported no significant family×water regime interaction for biomass traits in growth chamber experiments on *Pinus sylvestris*.

Damage risks

Cannell and Sheppard (1982) demonstrated differences between provenances for autumn frost damage in *Picea sitchensis*, but no differences for foliage flushing in spring and, thus, no differences in spring frost damage.

Nutrients

Jonsson *et al.* (2000) reported a strong family×nitrogen availability interaction for nitrogen concentration and utilisation, but not for growth, in families of *Picea abies* in growth chamber studies. Jonsson *et al.* (1997) found only a weak family×nitrogen availability interaction for biomass among families of *Pinus sylvestris*, also in growth chamber studies.

Interaction due to nursery differences.

If plants are handled incorrectly, a G×E interaction could be created by transferring C-effects from the nursery to the field. Wright (1973) reported several cases of such "artefact-interactions" from provenance tests in the north central United States. In one case, size differences due to different nurseries were still pronounced in field trials 11 years later. There are several causes of such nursery-induced interactions. Among the more severe are cases where plants of the same origin are grown in different nurseries and then planted out in separate field trials. Moreover, growing the plants in the same nursery without randomisation could also create considerable differences among varieties. Such differences may cause interactions in field tests, since differences are likely to be negated over different lengths of time at different sites. This is probably more likely for bare-rooted plants than for plants raised in containers. Since propagation of bare-rooted plants requires a larger area, there is likely to be more environmental variation within the nursery.

Another factor that may cause bias, which could be interpreted as an interaction, is a shortage of good quality plants in some varieties. Often the breeders' desire to

complete all replications in all trials is stronger than their drive to have plants of even size and condition. Hence, unwanted variation is likely to occur within, as well as between, varieties. The risk of creating interaction effects is obvious if the trials are generated one after another, especially if there is a large size variation within entries. Then there is the chance that the best and most vigorous plants are taken for the first trial and the smaller ones are left for the last. To avoid this problem, the same replication for all trials should be generated before the next replication is started.

G×E interaction estimates from other publications

K-statistics (Lindgren 1984) were calculated from published data relating to conifers, where both genetic variance components and G×E components were reported (Table 1). Table 2 shows correlations among trials found in published data.

From Table 1 it can be seen that most of the *K*-statistic estimates for *Picea abies* were below 0.5, which is the limit at which the interaction can be considered serious (Shelbourne 1972). In other species there are some examples of estimates that indicate serious G×E interactions. It should be noted that traits combined from two ore more single traits can display substantially greater interactions than the individual component traits (McKeand *et al.* 1997 in Table 1).

The correlation coefficients in Table 2 indicate that the estimates were quite high and correspond well to the conclusions based on the results in Table 1. The publication of more estimates of genetic correlations between sites should be encouraged.

Table 1. A sample of *K*-statistics from different published experiments. o.p. denotes open pollinated families.

Reference	Species	Material	No.of Sites/treatments	No. gen. entries	Trait (age)	*K*-statistic
Field trials						
Shaw *et al.* (1988)	*Picea abies*	clones	2	113	height(5)	0.4
Bentzer *et al.* (1988) I	- " -	- " -	6	490	height(5)	0.4
Bentzer *et al.* (1988) II	- " -	- " -	3	423	height(5)	0.2
Bentzer *et al.* (1989)	- " -	- " -	2	75	height(10)	0.1
Bentzer *et al.* (1989)	- " -	- " -	2	75	volume(10)	1.0
Kleinschmit and Svolba (1991)	- " -	- " -	6	2820	height(17)	0.5
St Clair and Kleinschmit (1986)	- " -	- " -	7	40	height(10)	0.4
Sonesson(2000)	- " -	- " -	5	476	Height(14)	0.8
Sonesson(2000)	- " -	- " -	- " -	- " -	Increment	1.1
Isik *et al.* (1995)	- " -	- " -	7	40	height(17)	0.3
Nielsen and Roulund (1996) 2 series	*Picea sitchensis*	- " -	4	151, 196	height(5)	0.5, 0.4
Gullberg and Vegefors (1987)	- " -	contr. crosses	2-3	9 ?	height(15)	2.8
- " -	- " -	- " -	- " -	- " -	height(20)	0.3
Wu *et al.* (1997)	*Pinus contorta*	- " -	4	110	height(9)	1.1
McKeand *et al.* (1997)	*Pinus taeda*	- " -	7	18	volume(12)	0.9
- " -	- " -	- " -	- " -	- " -	wood density	0.13
- " -	- " -	- " -	- " -	- " -	volume + wood density)	1.55
Matheson and Raymond (1984)	*Pinus radiata*	- " -	11[a]	30	height(9-12)	0.9
Mikola and Vakkari (1995)	*Larix sibirica*	o.p.	10	25	height(10)	3.5
Stonecypher *et al.* (1996)	*Pseudotsuga menziesii.*	families[b]			height(6-11)	0.35
Growth chamber studies						
Jonsson *et al.* (2000)	*Picea abies*	o.p.	2	15	N-utilisation	large[c]
Sonesson and Eriksson (2000) 2 sets	*Pinus sylvestris*	- " -	2water×2temp	28, 35	growth, several	0-3.5
Jonsson *et al.* (1997)	- " -	- " -	2	21	biomass	1.5
Jonsson *et al.* (1992)	- " -	- " -	2	21	biomass	low

[a] Throughout Australia [b] Full-sib, half-sib and open pollinated families [c] No family variation

Table 2. Table of the average genetic correlation coefficient estimates from different published experiments. o.p. denotes open pollinated families and f.s. denotes full-sib families.

Reference	Species	Material	No. of sites	No. gen. entries	r_G	Trait (age)	Notes
Bentzer *et al.* (1988) I	*Picea abies*	clones	6	490	0.66	height(5)	Type B
Bentzer *et al.* (1988) II	- " -	- " -	3	423	0.91	height(5)	Type B
St Clair and Kleinschmit (1986)	- " -	- " -	7	40	0.59	height(10)	[b]
Karlsson and Danell (1989)	- " -	contr. crosses	4	36	0.98	height(14)	Type B
Nielsen and Roulund (1996), 2 series	*Picea sitchensis*	clones	4	151, 161	0.67, 0.66	height(5)	Type B
Hansen and Roulund (1997)	- " -	- " -	4	191	0.70	height(11)	Type B
Haapanen (1996)	*Pinus sylvestris*	o.p.	40 pairs		0.58[a]	height(10)	Type B
Matheson and Raymond (1984)	*Pinus radiata*	- " -	11[a]	30	1.34-(-0.48)	diam(9-12)	Type B
Dieters *et al.* (1995)	*Pinus elliottii*	f.s.	142		0.61	volume(5)	Type B
- " -	- " -	- " -	21		0.88	volume(14)	Type B
Johnson(1997)	*Pseudotsuga menziesii.*	o.p.	51	25-50	0.66	height(10)	
- " -	- " -	- " -	- " -	- " -	0.72	height(15)	

[a)] Variation in estimates for the eight averages of trial series (0.38-0.73).

[b)] Correlation between clone means

Objectives

The overall objectives of the thesis were to study variation among genotypes of a few provenances of *Picea abies* for different traits, as well as to study phenotypic stability across sites.

The aims of the three clone trial series with Norway spruce were:

- To estimate genetic parameters in **a** series of clone tests (**I, II, III, IV**).
- To study effects of the selection of young ortets on subsequent clonal behaviour in field-tests (**II**).
- To study the magnitude of the G×E interaction in clone tests in southern Sweden and Denmark (**I, II, IV**).
- To study how the G×E interaction can be explained by estimates of correlations between sites (**I, II, IV**).
- To study clone stability across sites (**IV**).
- To identify traits that are good predictors of adaptational behaviour (**I, II, III, IV**).

Furthermore, the implications of the G×E interaction on the breeding strategy, and the consequences for subsequent utilisation of the production population are discussed in this thesis.

Material and methods

Plant material

The plant material used in the study was derived from breeding material from various sources for the Swedish forestry breeding population. In **I**, the 311 clones used were part of a southern Sweden clonal forestry programme (Karlsson 1993) and selected from 39 commercial seedling stocks, each 3-4 years old, from different nurseries. The clone selection process involved two stages in the nursery prior to planting out in field trials, but, for the purpose of the study, selection effects were assumed to be small (Högberg and Karlsson 1998, and **II**). The clones were divided into five provenance groups, linked to their geographic origin. The plants from the second vegetative cycle clones were planted at five test sites (Figure 4). The trials were planted as randomised blocks with single tree plots and nine replications.

In **II**, clones from five selected full-sib families were used. Selection at the seedling (ortet) level was from a broad range of material, with the aim of finding contrasting families. After two vegetative propagation cycles, selection of clones within families was carried out in order to represent the within family variation and to obtain sufficient ramets per clone. Three field trials (Figure 4) were planted in a randomised block design with plots of four trees and four replications (blocks).

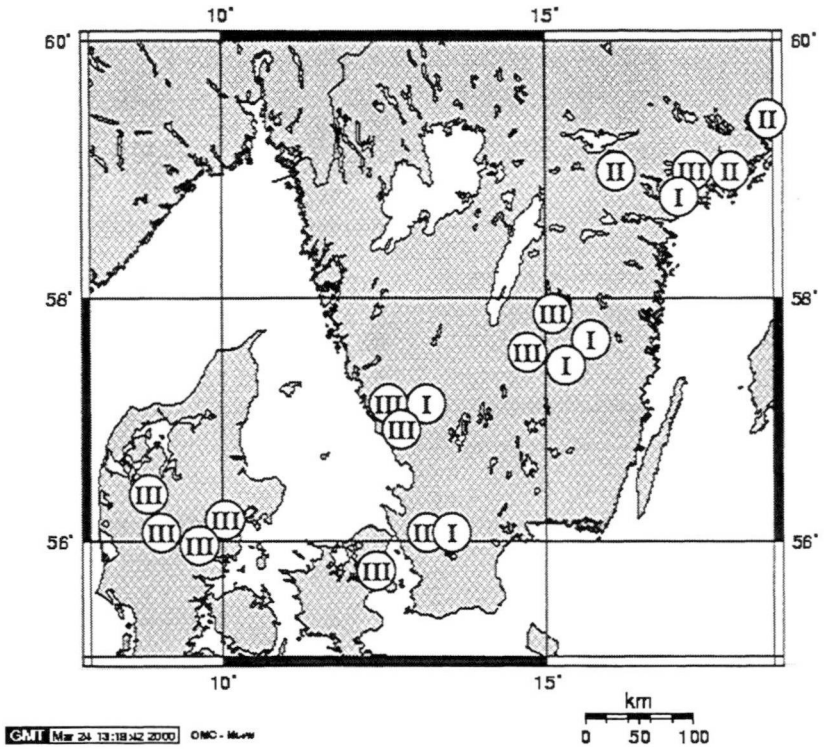

Figure 4. Trial sites in Denmark and southern Scandinavia. The sites used for **III** and **IV** were identical and are only marked '**III**'. The map was produced using 'The Generic Mapping Tools', http://imina.soest.hawaii.edu/gmt/.

The plant material used in **III** and **IV** was also derived from the south Swedish clonal forestry programme (Karlsson 1993). Four provenances were selected with the aim of representing seed sources used in Denmark and southern Sweden at the time. Within provenances, clones were randomly selected with the restriction that there had to be a sufficient number of ramets per clone for the field trials. Plants of the four provenances were produced both as seedlings and as rooted cuttings, with the intention of ensuring equal size. The trials involved two series, planted in consecutive vegetative cycles, ranging from western Denmark to eastern parts of central Sweden (Figure 4). The first series included eight sites and the second series three sites. The design, which was identical for the two series, was a split plot design with 10 replications per site. The provenances were planted in a randomised block design, and the clones were planted in single-tree plots within the provenance plots.

Assessments

In **I**, height, diameter and increment were measured along with stem and branch quality traits. In some trials, bud break, late spring frost damage and pilodyn penetration were also assessed.

In **II**, height and increment were measured and frost damage assessments were made.

In **III** and **IV**, measurements were carried out at two ages. At the first (ages 4 and 7 years), survival, height, bud break, frost damage and ramicorns were assessed, and at the second (ages 11 and 14 years), the same traits, plus diameter, were measured.

Survival rates were calculated as the ratio of the number of surviving trees at the measurement date and the number of trees originally planted.

Measurements of height and phenological behaviour were available for all nursery materials (ortets and/or ramets) or from clone archives, but these measurements were not necessarily from the actual trial plants.

Analysis

General

Most of the categorised variables that had few classes were transformed to normal score values (Gianola and Norton 1981) prior to analysis, as described by Ericsson (1994), in order to better fulfil the requirement of normal distribution.

Analysis of variance and covariance

Restricted Maximum Likelihood (REML) estimates of variance and covariance components for random sources of variation within trials were obtained using two types of software. In **I**, software developed by Harvey (1990) was used and in **II**, **III** and **IV**, SAS Proc Mixed (SAS 1996) software was used.

In **I**, **II** and **IV**, estimates of variance components for random sources of variation were made for the whole series of trials. In order to avoid bias in the estimates of interaction effects caused by heterogeneous genetic variance over trials, the data in **II** and **IV** were homogenised. Each observation was multiplied by a constant derived from either the average or a chosen (typical) genotypic standard deviation of the series, divided by the genotypic standard deviation of the particular trial.

Clone effects

Predicted clone effects were calculated as BLUP (Best Linear Unbiased Predictors) with software based on Henderson's Mixed Model Equations (MME) (Henderson 1984). In **I**, OWST-BLUP software, developed by Danell (1988), was used, and in **II** and **III** SAS Proc Mixed (SAS 1996) software was used.

Correlation estimates

In **I,** genetic correlations between traits within field trials were estimated using software written by Harvey (1990). SAS Proc Corr (SAS 1996) software was used to calculate Pearson's product-moment correlation estimates between BLUP-values for trials in **I.** In **II**, **III** and **IV,** correlations were estimated within trials, between nursery and field trials (**II**) as well as among field trials. The correlation estimates between trials in **IV** were used to calculate type B genetic correlations following Burdon (1977).

For the purpose of comparisons in this thesis, average correlation estimates for growth traits in the trials in **I** and **II** were used to calculate type B genetic correlations (Burdon 1977).

Stability across sites (Paper IV)

For each of the propagation methods, provenance means were regressed on the trial means in order to estimate stability across sites, according to the method of Finlay and Wilkinson (1963).

Clone stability across sites was assessed using the ecovalence concept (Wricke 1962). Ecovalence estimates indicate each genotype's contribution to the interaction sum of squares.

Site impact

In order to study the similarity of clone performances on the different trial sites described in **IV**, a cluster analysis was performed. Type B (Burdon 1977) genetic correlation estimates between pairs of trials for the most recent height measurement were used. This is probably the most informative trait. The software procedure used was SAHN – Sequential Agglomerative Hierarical Nested cluster analysis (Sneath and Sokal 1973).

Main results

Cuttings versus seedlings

There were only small differences in growth at the final measurement between cuttings and seedlings. The seedlings were larger in one series in **III**, but the cuttings were significantly larger in the trial series that was repeated three years later.

Differences between background materials

In **I**, there were significant differences between provenance groups for growth traits, but not for most stem and branch characters. In **III**, there were significant differences between provenances in some trials but not in others.

Broad sense heritability estimates

Broad sense heritability estimates, H^2, from **I-III** were low for survival, moderately high for growth traits (height, increment, diameter, etc.) and frost damage, and high for bud-break (Table 3).

Table 3. Arithmetic means of broad sense heritability estimates from **I**, **II** and **III**. Mean values are given, with the range of estimates in parentheses.

	I	II[1]	III
Survival	0.01 (0.00-0.03)	0.00	0.03 (0.00–0.11)
Growth	0.18 (0.08–0.34)	0.24 (0.17–0.28)	0.26 (0.08–0.45)
Bud-break	0.73 (0.67–0.78)		0.74 (0.65–0.82)
Frost damage	0.25 (0.19–0.30)		0.26 (0.10–0.50)

[1] Estimates across sites.

Correlations between traits

Correlation coefficient estimates between growth traits, within trials, tended to be high in **I** and **III**, (0.73-0.96). Of these estimates, correlations of early height to late increment produced the lowest estimates: 0.76 and 0.73 in **I** and **III** respectively.

Correlation estimates between late bud-break and growth traits within and among sites were rather low in **I-IV** (0.08-0.27). High correlation estimates were found between early bud break and frost damage in **I** and **IV** (0.67-0.80). Early bud-break correlated strongly with the formation of vertical branches (ramicorns) in **I** and **IV** (0.60 and 0.81, respectively), while correlations between frost damage and vertical branches in the same trials were moderately high (0.39 resp. 0.30).

Effects of early selection in the nursery (Paper II)

Rather low correlation estimates were found between the ortets in the nursery and cuttings from the second vegetative propagation in field trials. There was a large variation between families in respect to the correlation estimates. Selection of only the 20% tallest ortets in the nursery would have resulted in an increase in growth of 4.6% compared with 31.2% for selection after clone tests in field conditions. Correlations between ortets in the nursery and ramets in field trials varied considerably among full-sib families.

Agreement between traits across sites

Variance components

Statistically significant clone×site interactions were found for growth traits in all trials. Results, expressed as *K*-statistics, $\sigma^2_{clone \times site}/\sigma^2_{clone}$ (Lindgren 1984), from **I, II** and **IV** are shown in Table 4.

Table 4. *K*-statistics derived from variance components.

Trait	I	II	IV
Early height	0.4	1.1	0.6
Later height	0.5	1.3	0.8
Increment	0.8	2.0	0.9
Diameter	0.6	-	0.9
Volume	-	-	0.7

In **IV,** the group of clones from one provenance showed generally lower *K*-values than the other provenances. This was also the case for the group of clones with intermediate bud-break values.

Among other traits in **I,** pilodyn penetration, branch angle and bud-break produced very low *K*-statistics (0.10-0.18). In contrast, the *K*-statistic associated with vertical branches had a value of 1.5.

Correlation estimates

Estimates of correlation for the same trait measured at different sites in **I, II** and **IV** are shown in Table 5.

Table 5. Correlation coefficients between measurements of the same traits in different trials. In **I** and **II** the estimates are correlations between pairs of BLUP-values and in **IV** they are type B-correlations.

Trait	I[1]	II[1]	IV[2]
Early height	0.47	–	0.62
Later height	0.46	0.44	0.52
Increment	0.40	0.38	0.54
Diameter	0.45	–	0.56
Volume	–	–	0.61

[1] Arithmetic mean estimates
[2] Median of estimates

Bud-break displayed high correlation coefficients between sites in **I** (r=0.88) and **IV** (r_G=0.79). Branch angle and pilodyn penetration produced high correlation estimates in **I,** while ramicorns displayed generally low and inconsistent estimates (**I** and **IV**).

The growth correlation estimates from **I** and **II** (Table 5) were converted into type B genetic correlations (Burdon 1977). The conversion was based on the assumption that r_{Π} values were approximately 0.75 and 0.85 (derived from H^2

estimates and the number of replications) for **I** and **II** respectively. Thus, the estimates in Table 6 were obtained.

Table 6. Type B (Burdon 1977) genetic correlation coefficient estimates between measurements of the same traits in different trials, assuming r_{II}= 0.75 in **I** and r_{II}= 0.85 in **II.**

Trait	I	II	IV
Early height	0.79	–	0.62
Later height	0.78	0.61	0.52
Increment	0.67	0.52	0.54
Diameter	0.76	–	0.56
Volume	–	–	0.61

Stability across sites (Paper IV)

There were statistically significant, but low, effects of the provenance×trial interaction for final height of both seedlings and cuttings.

Ecovalence estimates for individual clones showed rather small variations in the contribution to the interaction among the 96 clones, even though about half of the clones made a statistically significant contribution to the interaction, none contributed significantly more than any of the others. Ecovalence varied between 0.25 and 2.76% of the interaction sum of squares. The regression analysis for both total height and height increment, showed higher values for clones with early bud-break, and also, to some extent, for those with late bud-break. This pattern was much less pronounced for the Minsk provenance.

Cluster analysis (Paper IV)

Assuming that substantial differences between sites (r_G< 0.5) would suggest subdivision of breeding-populations/seed-zones, the cluster analysis of the pair-wise genetic correlations for total height indicated that the sites were divided into two groups. However, there was no obvious geographical pattern in the grouping (Figure 5).

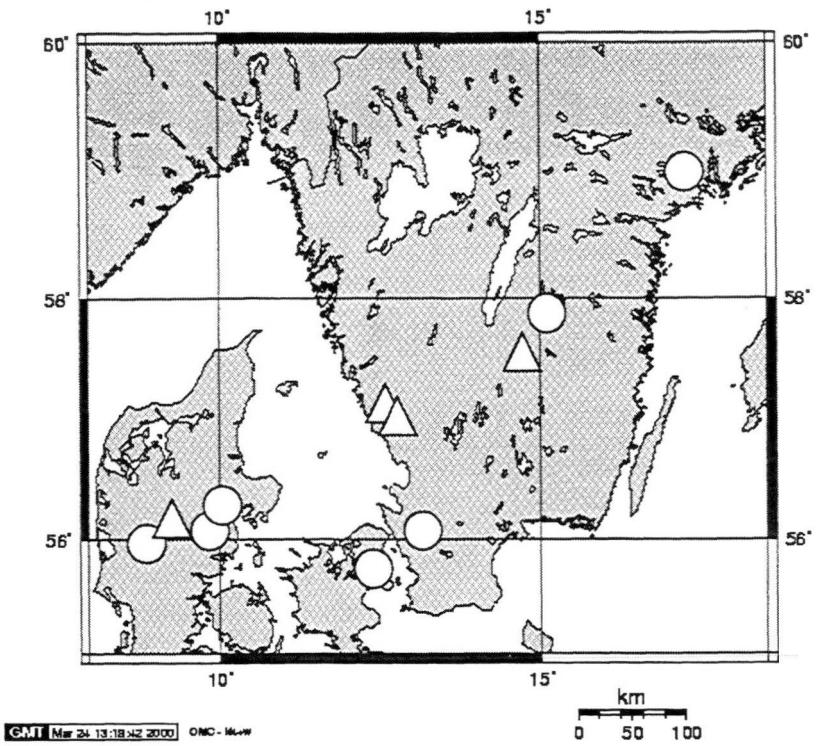

Figure 5. Map showing the results of the cluster analysis. Sites represented by circles and triangles belong to different clusters. The map was constructed using, 'The Generic Mapping Tools', http://imina.soest.hawaii.edu/gmt/.

Discussion

Picea abies tree breeders could derive great advantage from the use of clone tests. In test programmes aimed at the wide use of vegetatively propagated genotypes, it is reassuring to know that the genotype has already proved its superiority for a number of traits over a wide range of sites. In breeding programmes aimed mainly at generative mass propagation, clone tests have proved to be a highly reliable way of testing parents for breeding and seed production and for forward selection within families (Rosvall *et al.* 1998).

Broad sense heritabilities within trials

Generally, the broad sense heritabilities in the trials were medium to high, with small standard errors (where estimated) and they were stable over the series. This indicated that the quality of trials was good and the number of replications was sufficient. Survival rates however, showed low broad sense heritabilities. The reason for this is probably a combination of fairly low mortality rates and the fact that several unrelated causes of mortality were present within the same trials (drought, drowning, insects etc). Heritabilities for growth traits in **I-III,** within

trials, exhibited medium levels (Table 3), in agreement with other data on Norway spruce (e.g. Roulund *et al.* 1986, Bentzer *et al.*1989, Lepistö 1993). One of the highest H^2 values was found for bud-break (**I** and **III**), this corresponds to other studies that show a strong genetic control over bud-break (Nienstaedt 1985, Ekberg *et al.* 1994, Hannerz *et al.* 1999). There were also medium estimates for late spring frost damage in **I** and **III**. In the trial with the most frost damage in **I,** the heritabilities for early height were the second highest in that series. In the trial with the highest H^2 value for height, in **III,** there was also significant frost damage. Thus, it is likely that different alleles regulate height growth in trials with frost damage, compared to those without.

In **III,** the heritabilities for later increment were stronger than those for early height. This fact indicates that the genetic impact increases in later years. One reason could be that nursery C-effects reduce influence, while site characteristics increasingly influence the phenotype. In **I,** however, this trend is not obvious.

There were significant differences between provenances for growth characters in **I** but not between measures of quality (crooks and branch characters). This confirms the need to use plant material that is strictly defined genetically rather than using only provenance to improve wood quality traits.

Correlations among traits within and between trials

It is obvious that some phenological traits have a great impact on growth responses in field trials, and may also influence a change of response between sites for certain genotypes. In **I, II** and **III**, the general trend of the correlations between bud-break and growth traits confirmed the disadvantage for spruce trees of early bud-break (Hannerz 1999). In **IV** the median correlation indicates better growth following early flushing. There was high variability in the estimates of the median, which indicates that trees at some sites benefited from early flushing. Supposedly these trials suffered no, or only minor, late spring frosts. Increased frost damage due to early bud-break and the consequent effect on the frequency of ramicorns was obvious in those trials where ramicorns were recorded (**I, III** and **IV**).

Intra-trial correlations between early height measurement and later increment in **I** and **III** were weaker than those between early and later height. These results indicate that increment is a different trait than early height. One explanation is that nursery effects influence the plants for some time after planting out, and the lower correlation coefficient is an expression of the G×E interaction between nursery and field. This effect could have been exaggerated by variations between entries in the extent and duration of the "planting shock". These results agree with Larsen *et al.* (1997), who reported a genetic correlation estimate of 0.49 between early height (9 years of age) and later increment (between 15 and 21 years of age). The relatively strong correlation between early and final height can be

explained because it is an auto-correlation; the early height usually contributes considerably to the final height.

Selection efficiency of clones based on ortets in the nursery (II)

The 4.6% increase in growth, which results from selecting the tallest 20% of ortets, is discouraging. Compared to the gain of 31% when selecting clones after testing in field trials, ortet selection is an inefficient alternative.

Other authors have reported fairly high selection effects or high correlations between ortets in nurseries and ramets in the field (Roulund *et al.* 1986, Skrøppa and Dietrichson 1986, Larsen *et al.* 1997). However, there are also reports of low agreement between the growth of clones in nurseries and results of field tests (Mullin and Park 1994, Rautanen 1995, Högberg and Karlsson 1998). Hannerz *et al.* (1999) reported poor or inconsistent genetic correlations between height in short-term tests and height in the same families in field trials.

The genotype×environment interaction between nursery and trial environments is likely to be one reason for the variable results when studying effects of nursery selection on later responses in field trials. Isik *et al.* (1995) pointed out that early selection in field trials requires care, since nursery effects can last a long time, while the genotypes subsequently interact with the test environment. It is very hard to distinguish whether a strong correlation estimate between the nursery and field trials is a result of true genetics already expressed in the nursery combined with a lack of G×E interaction, or those cases where nursery C-effects exist and are still being manifested in field trials.

One possible reason for low nursery–field correlations is the age-dependency of free growth in Norway spruce. Since free growth is normally greatly reduced or absent after a few years in field trials (Ununger and Ekberg 1987), there will be changes in rank, as clones with high levels of free growth from the early years are progressively ranked lower in field tests. Hannerz *et al.* (1999) reported negative genetic correlations between the frequency of free growth observed in short-term tests and the final height in field tests (ages 9-14). This risk is particularly pronounced in young seedlings and ontogenetically young cuttings, which are more likely to have free growth.

If juvenile–mature correlations are sufficiently high, two-stage selection could be a good way of increasing genetic gain and reducing costs, through smaller field trials (Wu 1998). Wu *et al.* (1997) stressed the requirement that early testing should be performed under simulated field conditions. However, this requirement can be fulfilled only if the specific characteristics of the field trial sites are known, and can be accurately simulated.

It appears that nursery selection, whether it is from ortets or ramets, is efficient enough to exclude the worst material, but is not adequate for the selection of

individual genotypes (Mullin *et al.* 1995). It is confusing for the breeder to see the possibilities associated with early selection but at the same time to know the risks of unintentionally incorporating C- or nursery effects of unknown size.

Variance components across sites

There were significant G×E interaction variance components for growth traits in all of the three papers (**I**, **II** and **IV**) where analyses were carried out across sites. The ratio of the G×E variance component to the genetic component, the *K*-statistic (Lindgren 1984), exceeded 0.5 (Table 4). Above this threshold the gains from selection are seriously affected (Shelbourne 1972).

In order to avoid G×E interaction due to scale effects, data should be transformed prior to analysis so that the among-genotype variances in all environments are homogeneous (Lynch and Walsh 1998). This was carried out in **II** and **IV** but not in **I**. Thus, interaction effects might be somewhat exaggerated in **I**.

The source material of the clones used in **I** and **IV** represented material that was not expected to suffer from weather-induced damage in the trial area (Anon. 1986). In **II** some clones were selected from full-sib families where one of the parents originated in a region from which transfer is not recommended (Anon. 1986). The transfer was not extreme and, since one of the parents was of an adequate origin, the family properties should be intermediate (Ekberg *et al.* 1982). The results in **IV** showed that the *K*-statistics did not differ much when the variance components were estimated within provenances compared with the joint analysis. This suggests that the large interaction component could not be attributed to a single provenance, but is a characteristic of all the provenances. The *K*-values reported in this thesis were higher than most values for *Picea abies* reported by other authors (see Table 1).

In **I**, **II** and **IV**, there were larger genotype×trial interactions associated with increment than with early height (Table 4). This is an evidence of that the problem of interaction increases with age or developmental stage.

Correlation estimates between sites

Generally, the genetic correlations described in **I**, **II** and **IV** and in other reports related to Norway spruce (St. Clair and Kleinschmit 1986, Bentzer *et al.* 1988) were low enough to indicate that there was a significant interaction between genotypes and sites for growth traits. It is unlikely that the same trait was measured at all sites (Falconer 1952) or that different factors influenced growth at different sites.

The high correlation estimates for bud-break between the sites described in **I** and **IV**, confirm the strong genetic control over that trait. Other traits that are under strong genetic control are branch angle and pilodyn penetration (**I**). The

occurrence of ramicorns seems to be a trait that is greatly affected by the environment, since there were very weak correlations between sites, except where frost was likely to be the cause of ramicorns.

The genetic correlation estimates (Table 6) for increment were lower than the estimates for early and final height. This supports the results, from variance components, that the interactions for these trial series increase with age. One possible explanation for the decreasing correlation estimates between sites for increment, compared to early height measurement (**I**, **II** and **IV**), could be that, even though the trials were even-aged, they represented different development stages (Franklin 1979, Haapanen 1996). If the behaviour of different genotypes depends more on tree size and developmental stage than age, the large variation in trial mean heights in **IV** could explain some of the weak correlations between certain pairs of sites. The lack of correlation pattern between pairs of trials supports this hypothesis. Interestingly, trial 6, the most frost damaged site in **IV**, displayed stronger correlation estimates with other trials for increment than for early height. If the differences in development between pairs of trials influences the interactions, there should be a trend towards lower genetic correlation estimates between pairs of trials with large differences in height. However, Figure 4, which shows genetic correlation estimates against height ratios between pairs of trials, does not support this hypothesis. The correlation estimates appear to be independent of the height ratio, representing differences in development.

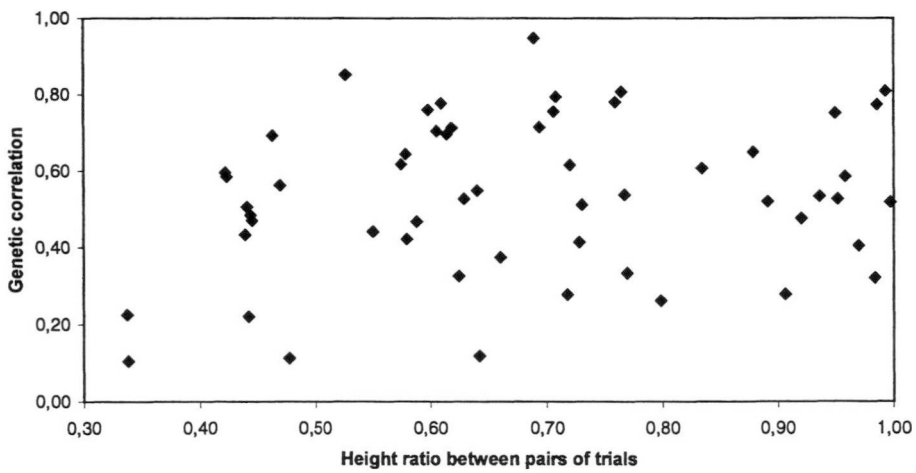

Figure 4. Plot of type B genetic correlation coefficient estimates for final height against final height ratio between pairs of trials.

The results from the cluster analysis in **IV** showed no obvious geographical division of the trials. In contrast, Wellendorf *et al.* (1986) found a defined division of zones when using cluster analysis on correlations based on provenance means for southern Sweden. The results in **IV**, however, indicated that, for

ranking clones, local conditions at each trial site were more important than regional differences.

Clone stability across trials (IV)

One important result of the ecovalence estimates was that, even though about half of the clones made a statistically significant contribution to the interaction, none contributed significantly more than any of the others. This agrees with the results of St. Clair and Kleinschmit (1986). However, reports relating to other conifer species highlighted certain varieties that were responsible for a larger part of the interaction (Nielsen and Roulund 1996, McKeand *et al.* 1997). An other fact worth noticing is that there was no obvious difference between provenances in respect to how much their clones contributed to the interaction.

Another characteristic pattern, in agreement with other reports concerning Norway spruce (Skrøppa 1984, St. Clair and Kleinschmit 1986), is that there was no association between height (yield) and stability represented by ecovalence. The higher ecovalence estimates for clones with early bud-break indicated that late spring frost damaged these clones at frost-prone sites, and that these clones behave inconsistently among sites. Thus, selection of material for breeding and mass propagation should, regardless of provenance, be carried out only for general performance when the utilisation area does not have a high frequency of sites with an increased risk of late spring frost. For frost-prone environments, material with early bud-break, and thus an increased risk of damage leading to an increased interaction, should be avoided (Shaw *et al.* 1988, Kleinschmit 1992).

There was also a tendency towards increased ecovalence estimates in clones with a late bud-break, a feature that may be interpreted in two ways:

1. Since there is a correlation between late bud-break and late growth cessation (Hannerz *et al.* 1999), these clones may have an increased risk of autumn frost damage.
2. These clones flush too late to utilise the early part of the growth season in some of the southwestern trials, where late spring frosts are rare.

Due to the absence of clear indications of autumn frost damage, the second explanation is more likely.

The unpredictable pattern of the G×E interactions suggests that Norway spruce has a large phenotypic plasticity. This is also suggested by the species' successful survival of so many glaciations and its fast recolonisation of former areas of occurrence (Schmit-Vogt 1977, Bradshaw 1995). It is easy, therefore, to see why Norway spruce is considered to be among the easiest forest tree species to cultivate.

Implications of C-effects

Nursery effects are hard to avoid and can be the result of several factors. Differences in seed weight or the condition of cuttings can be amplified by, for example, differences in germination time and rooting success. The bias caused by such C-effects may be pronounced in some field trials, thus the estimates of genetic effects will be biased (Foster *et al.* 1984). The length of time required for C-effects to disappear varies (Cannell *et al.* 1988). Since C-effects are confounded with genetic effects (Libby 1976), they are very hard to pinpoint in a trial series from a single propagation. Weak correlations between sites from a single propagation of the same clones, as found in **I**, **II** and **IV** (Table 6) create problems. The results would probably be more robust, reliable and less confusing if trials were carried out after at least two separate propagations. C-effects may, at best, remain the same, but other interaction possibilities will be added when material is repropagated (McGranahan *et al.* 1999). Such possibilities include new circumstances in the nursery and new climatic conditions in the plantation sites.

Even if results from analyses of a series of trials could be interpreted as genotype×trial interaction, the situation may be more complex. In order to aid data interpretation, it would be valuable to measure the plant height immediately after planting out in at least one trial per series. This would give a better opportunity to follow the development of variances over time (Franklin 1979).

C-effects are not necessarily detrimental, but in order to be utilised in mass-propagation they have to be consistent across propagations. If they are positively correlated with true genetic effects (Borralho and Kanowski 1994) and continue to have an impact during most of the rotation, they could make a positive contribution, at least to clone performance in clonal forestry. But, since C-effects are non-additive, they will be lost when the clones are crossed to produce the next generation.

Test strategies

The number of replications required to obtain reliable results is relatively low (Shaw and Hood 1985). Russel and Libby (1986) found, from simulation models, that for trials where $H^2<0.6$, the optimum number of ramets per clone and site is 2.6, for clone testing. Russel and Loo-Dinkins (1993) concluded that the optimal testing effort under fixed resources was approximately the same for maximising production and for breeding population gain. Their study showed that 1-2 ramets at each of 2-6 sites was an optimal distribution. The gain was significantly lower at high levels of G×E interaction, unless the clones were tested at four sites or more. By studying the increase in genetic gain after progeny testing of *Pseudotsuga menzeesii* with type B genetic correlation coefficients between sites ranging from 0.42-0.84, Johnson (1997) concluded that the optimum number of sites was 3-4. This corresponds well with the work of Lindgren (1984), who suggested 3-4 test sites for progeny-testing of *Pinus sylvestris* in Scandinavia.

Comparisons among different measures of genotype stability

For Norway spruce there are poor correlations between regression coefficient and ecovalence (Skrøppa 1984, St. Clair and Kleinschmit 1986). Unpublished results from analyses of the material in **IV** agree with this conclusion.

Implications on Norway spruce breeding in Sweden

Sub-division of populations

The breeder can roughly divide G×E interactions into two types; one which is predictable and related to one or more definable environmental variables, and the other which is more erratic and is not related to any known environmental factor (Shelbourne 1972, Matheson and Raymond 1986). In forest tree breeding the first type can, theoretically, be utilised by classifying breeding populations and seed zones into groups that contain genotypes suited to particular environments. In the second situation the interaction cannot be used, and it results in a reduction in the genetic gain produced by selection of genotypes that have good average behaviour. For Norway spruce the general recommendations for the use of provenances are based on the results of provenance trials. This is an application of the first type of interaction (Werner and Karlsson 1982, Persson and Persson 1992, Anon. 1986). The Swedish forest tree breeding strategy follows the MPBS (Multiple Population Breeding System) concept (Namkoong *et al.* 1980, Burdon and Namkoong 1983, Danell 1993) and is therefore also well suited to utilise the first type of G×E interaction. The breeding populations are defined by latitude and temperature sum target. Areas covered by the breeding populations of Norway spruce are large enough to contain the second type of interaction or rather a mix of the two types, since the reason for interaction can sometimes be pin-pointed, e.g. late spring frost, and sometimes not. However, the tools for describing the environment at the planting site, and thus predicting the behaviour of genotypes, are not sufficiently well defined to allow the forester to utilise the variation.

Whether or not it is economically viable to subdivide breeding programmes will depend on the importance of the species and the potential reduction in genetic improvement that would result from keeping only one programme (Shelbourne 1972). Matheson and Raymond (1984) proposed no division into regions of Australia's breeding population, because no homogenous regions without interaction could be identified. If environmental factors cannot be described and utilised so that the additional cost cannot be justified, there is no use in trying to divide the breeding population in order to increase genetic gain (St Clair and Kleinschmit 1986). However, sub-division could be useful for other purposes, e.g. to maintain genetic diversity in the meta-population and to emphasise specific breeding goals. In cases where environmental impact cannot be predicted, selection, both for breeding and mass propagation, should be focused on

genotypes which are stable across environments and have good average responses across sites for any desired characters (Skrøppa 1984).

Since there was neither a large variation in ecovalence estimates between clones in **IV** nor a defined geographic pattern of the interactions, there is no reason to suggest any changes to the current Swedish breeding strategy for Norway spruce.

Choice of test sites

Test results and, ultimately, the amount of genetic gain in tree breeding is, to a certain extent, a matter of choosing representative test sites. Burdon (1977) concludes that attention should primarily be given to the role of the environment rather than of individual genotypes generating interactions. It is important to identify environmental characteristics that determine a good testing site (Gullberg 1984, St. Clair and Kleinschmit 1986).

A considerable part of the annual Norway spruce regeneration area in southern Sweden consists of the type of environments where early summer frosts are likely at least once during establishment, until the trees reach a height not affected by frost (1.5–2 m). Since planting a genetic test is very expensive, there is a risk that breeders avoid sites that are obviously frost prone. Trials affected by frost damage, sometimes in combination with other damage (e.g. *Hylobius abietis* feeding), may result in weak genetic parameters and uncertain breeding values due to the large errors (Shaw *et al.* 1988). If such damaged sites are used when calculating selection indices, the index values will be reduced, compared to those of good sites, thus making them unrepresentative. In order to test genetic varieties for stability with respect to unpredictable climate, growth chambers could be used (Lundkvist 1984). Alternatively, trials at sites that are very frost-prone could be avoided by increasing the weight of the selection indices for growth traits from trials that are slightly frost-damaged. The weighting should then correspond to the frequency of frost-pronesites within the breeding zone. The breeder should, thus, not avoid frost-prone sites, but try to find one site per series with a medium frost risk.

Norway spruce is a shade tolerant, secondary coloniser in natural forest succession (Schmidt-Vogt 1977). It is likely that genetic tests only under shelter-wood would exhibit less interaction between sites, since the environment would be considerably more uniform. However, as long as the majority of replanting of Norway spruce takes place after final felling, genetic tests aimed at selecting breeding populations and mass-propagation have to be conducted in equivalent sites. Furthermore, the majority of the plus-trees entered into the breeding programme are selected from stands planted after clear-felling. If more planting is to take place in shelter-woods, testing will be required under appropriate conditions; otherwise a problem with a genotype×silviculture interaction could arise.

Deployment strategies

Bentzer *et al.* (1988) found a significant clone×site interaction in two five-year old clone test series, but no interaction between site and clonal mixtures of 42-56 clones each. This corresponds well to the results in **IV** (Figure 3) with small changes in rank over the sites for the four provenances propagated by cuttings, each represented by 24 clones. Bentzer *et al.* (1990) reported that seven-year old clone mixtures, with as few as five clones, appeared to be stable between sites after stepwise elimination of clones for inferior height growth, lack of stability or at random. Lundkvist *et al.* (1992), however, found a significant interaction between sites and clone-mixtures, which were truncated with respect to flushing time.

Conclusions and suggestions for further research

It can be concluded that genotype×environment interactions for growth traits are substantial but also very unpredictable. Different types of interactions are present in various test situations. Based on the material included in this thesis and results in other reports, the following conclusions may be drawn:

- Late spring frost seems to be the main cause of G×E interaction for growth traits in *Picea abies*. For traits that are not affected by frost e.g. bud-break, branch-angle and wood density, the G×E interaction is negligible.
- Clones are suitable and effective when testing Norway spruce for breeding and for mass propagation.
- Selection of young ortets on the basis of growth traits should not be used for subsequent clonal mass propagation. For phenological traits, such as bud-break, selection aimed at decreasing damage due to frost can be successful.
- The accuracy of selection in field trials is affected by the genotype×trial interaction. In order to reduce the problem, more test sites and fewer replications per site can be used at the same cost.
- When conducting field trials in southern Sweden, it is important to include test sites with late spring frost problems in relation to their frequency among actual forest regeneration sites.
- Bud-break is a highly heritable trait, with high levels of variation and strong juvenile–mature correlations. This makes it a reliable early selection trait, which, when used correctly, can also contribute to a reduction in the G×E interaction.
- Where breeding zones and seed utilisation areas experience frequent late spring frosts, the frequency of trees with ramicorns can be reduced if late flushing material is used.

- Provenances, families and mixtures of clones with late bud-break should be used in sites where there is a high risk of late spring frost. However, single clones cannot be guaranteed to be stable enough at such sites.
- When the utilisation area does not have a high frequency of sites with an increased risk of late spring frost, selection of material for breeding and mass propagation should, regardless of provenance, be carried out only for general performance
- The low variation in clone stability estimates, coupled with the lack of a defined geographic pattern of the G×E interactions for growth traits, suggest that no changes in division of sub-populations or breeding goals in the current Swedish breeding strategy for Norway spruce are required.
- To improve control over some nursery-generated C-effects, growth measurements should be made immediately after planting field trials with Norway spruce.
- The implications of the G×E interaction for calculating the gain of genetically improved Norway spruce should be further investigated.

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